The fat that matters: Soil food web analysis using fatty acids and their carbon stable isotope signature

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1. Introduction

Soils harbour an enormous diversity of organisms in multi-trophic food webs that are central to nutrient cycling and ecosystem services (e.g., Bengtsson et al., 1996; Scheu and Setälä, 2002; Mulder, 2006). The soil micro- and mesofauna, in particular the microbial grazers, are key components of the food web, and thus important determinants for energy and carbon flows through terrestrial systems (Mikola and Setälä, 1999; Scheu et al., 2005). Regardless of their significance, trophic relationships in soil are still poorly understood. Most of the soil fauna appear to be generalist feeders, with frequent diet switches related to food availability, but actual resource utilisation remains obscure (Scheu, 2002).

Due to their small size and the cryptic habitat, feeding strategies of soil animals are difficult to address, either experimentally or by direct observation. Diets are commonly assessed by analysis of gut contents and faeces or based on morphological characteristics (Ponge, 2000; Addison et al., 2003; Chauvat et al., 2007). Assigned feeding guilds of the soil fauna therefore often reflect more taxonomic, rather than functional, relationships. Specific preferences may be investigated in food choice experiments (Ruess et al., 2000; Maraun et al., 2003; Newsham et al., 2004; Scheu and Simmerling, 2004). However, such data are restrictive as (i) information is on food consumed during a brief window of time, (ii) discrepancy between the ingested diet and assimilated nutrients can occur, and (iii) feeding preferences observed in the laboratory may not apply under field conditions.

Recent advances in biochemical and molecular methods offer new insights into soil food webs. Over the last decade, stable isotopes have increasingly been used as biomarkers in microbial ecology (Boschker and Middelburg, 2002; Staddon, 2004). The stable isotopic ratios of C (13C/12C) and N (15N/14N) have proven to be most valuable in investigating trophic relationships between organisms (Gannes et al., 1997, 1998; Post, 2002; Crawford et al., 2008; del Rio et al., 2009). Isotopic ratios in tissues largely reflect the weighted average ratios of the dietary constituents, plus a small amount of change known as fractionation (DeNiro and Epstein, 1978, 1981). The stable isotope composition in animal tissues has been employed to reconstruct soil food webs, with nitrogen applied to rank relative trophic levels and carbon to assign food sources (e.g., Ponsard and Arditi, 2000; Scheu and Falca, 2000; Tienov, 2007). Although useful, such techniques have restrictions, as they cannot distinguish between food sources with similar isotopic ratios, and are limited by the small number of naturally occurring biologically relevant stable isotopes.

Molecular methods may also be applied to unravel belowground food webs. A recent approach is DNA-based gut content analyses of soil invertebrates, which provide information on specific feeding
strategies and predator–prey interactions (Juen and Traugott, 2005, 2006). Primers have been developed for multi-species systems (Admassu et al., 2006), offering promising tools for further research. The ingested diet can be detected with a high level of resolution, although this does not necessarily reflect the assimilation of dietary nutrients. More advanced is therefore the combination of stable isotope and molecular techniques to perform nucleic acid-based stable isotope probing (NA-SIP). Using this method trophic connectivity between soil biota and the length of food chains can be analysed (Staddon, 2004; Whiteley et al., 2006). To date studies using NA-SIP have investigated microorganisms actively involved in specific metabolic processes (Radajweski et al., 2000; Manefield et al., 2002) or microbial food webs (Lueders et al., 2004, 2006) and as yet there has been no application to higher trophic levels. Moreover, the high 13C label necessary makes it difficult to use NA-SIP under field conditions.

The drawbacks associated with stable isotope analysis in animal tissue and in DNA do not apply to fatty acids as biochemical marker molecules. Phospholipid fatty acids (PLFAs) have long been employed as taxonomic markers for the quantification and classification of microorganisms (Tunlid and White, 1992; Frostegård and Bååth, 1996; Zelles, 1997, 1999). More recently the lipid pattern in soil biota has successfully been used to assess trophic interactions either by fatty acid profiling solely (Chamberlain et al., 2005; Ruess et al., 2004, 2005a) or in combination with stable isotope techniques, i.e. compound-specific analysis of the 13C/12C ratio in individual fatty acids (Chamberlain et al., 2004, 2006a,b; Ruess et al., 2005b). Further, PLFA based stable isotope probing (PLFA-SIP) provides quantitative and chemotaxonomic information on resource allocation in soil microbial communities (e.g. Lu et al., 2004; Evershed et al., 2006; Chen et al., 2008).

Within the framework of this review we introduce fatty acid biomarkers and their isotope signatures as tools in assigning feeding strategies to decomposer invertebrates and in determining their diets in situ. We summarize the current understanding of the factors that influence the description of trophic interactions via fatty acids. Five major fields of inquiry are identified: (1) fatty acid profiles of dominant soil fauna groups, (2) dynamics of incorporation of fatty acids into consumers, (3) metabolic modification and isotopic fractionation, (4) pool size of marker fatty acids, (5) analytical concerns. The complexities of these areas are discussed in order to review the current state of knowledge and demonstrate how fatty acids may be used to elucidate below-ground trophic interactions.

2. Fatty acids as trophic biomarkers in soil food webs

2.1. Soil organisms and their lipids

Lipids are widely distributed in all living cells (Ratledge and Wilkinson, 1988). They play a vital role in organisms, both as source of energy (i.e. neutral lipids) and as structural components of cell membranes (i.e. phospholipids). Their major components, the fatty acids, consist of carbon chains which can be fully saturated or unsaturated, with fatty acids containing 1 or 2 double bonds as the most common unsaturations. Classification of unsaturated fatty acids is usually based on the number of carbon atoms from the carboxyl group (Δ end) to the nearest double bond, denoting the systematic name (IUPAC-IUB, 1978). However, a common short hand designation is given by counting carbon atoms from the terminal methyl group (ω end, Fig. 1a). The latter is biologically meaningful as the position of the double bond is dictated by the

Fig. 1. a. Nomenclature of a dienoic fatty acid; given is linoleic acid (trivial name). Classification according to the number of carbon atoms from the carboxyl group (Δ end) denotes its systematic name cis,cis-9,12-Octadecadienoic acid, whereas counting from the terminal methyl group (ω end) assigns the common short hand designation 18:2ω6,9. b. Pathways of polyunsaturated fatty acid biosynthesis in plants and animals (modified after Weete (1980) Sprecher et al. (1995), and Bell and Tocher (2009)). Solid lines represent metabolic pathways common in animals, whereas dotted lines indicate plant specific pathways of biosynthesis. Methyl dd — methyl directed desaturation, carboxyl dd — carboxyl directed desaturation.
route of biosynthesis, resulting in characteristic “ω families”, e.g. ω3, ω6, ω9 (Fig. 1b). Generally, plants and higher fungi accomplish the desaturation of fatty acids in the methyl-direction, whereas higher animals use the carboxyl-direction (Weete, 1980). In particular vertebrates lack the Δ12 and Δ15 desaturases and so cannot form linoleic (18:2ω6,9) and α-linolenic acid (18:3ω3,6,9), respectively, from oleic acid (18:1ω9), hence linoleic and α-linolenic are essential dietary components (Bell and Tocher, 2009). However, dietary linoleic and α-linolenic acid can be further desaturated (carboxyl direction) and elongated (+ C2) to form various polyunsaturated fatty acids (Fig. 1b). Interestingly, biosynthesis of linoleic acid as well as fatty acids with ω3 double bond (18:3ω3, 20:5ω3) has been observed in soil animals such as nematodes and Collembola (Rothstein and Götz, 1968; Chamberlain and Black, 2005; Haubert et al., 2006).

The different enzymatic capabilities in fatty acid metabolism of organisms have long been recognised and employed in chemical taxonomy of microorganisms (Lechevalier and Lechevalier, 1988; Welch, 1991; White et al., 1996; Morgan and Winstanley, 1997). Detailed information on the phospholipid fatty acid (PLFA) pattern of soil microorganisms are given by Ratledge and Wilkinson (1988) and by Zelles (1997). Table 1 presents an overview of characteristic fatty acids in neutral lipid or phospholipid fractions of bacteria, fungi, plants and animals common in soil. Despite the proposed primary origin, some of these fatty acids may additionally be found, although in smaller amounts, in the lipids of other organisms. This is also the case for widely accepted biomarkers such as branched-chain fatty acids (iso, anteiso) as indicator for gram-positive bacteria, and cyclopropyl fatty acids for gram-negative bacteria (Kerger et al., 1986; Frostegård et al., 1993; Zelles, 1999). Such group-specific characteristics occur due to the enzyme complexes branched-chain and straight-chain fatty acid synthetase (Kaneda, 1991). However, as elongation reactions can be carried out by either synthetase, both bacterial groups are capable of producing branched-chain or cyclopropyl fatty acids, if the appropriate substrate is available.

Unfortunately, many fatty acids are non-specific and common across taxa. Straight-chain fatty acids, such as palmitic (16:0), stearic (18:0), palmitoleic (16:1ω7) or oleic (18:1ω9) acid, frequently occur in lipid samples of diverse origins. This similarity is caused by uniform mechanisms of fatty acid biosynthesis in animals, plants and microorganisms, in that acetyl-CoA is used as primer and the carbon chain is elongated by the repeated condensation of malonyl-CoA to the primer, yielding palmitic acid as major lipid pool (Shapiro, 1967; Weete, 1980; Ratledge and Wilkinson, 1988). As a result oleic acid, which is sometimes used as biomarker for fungi (Joergensen and Wichern, 2008), is also found in considerable amounts in plant tissue (Table 1). And even for approved biomarkers group specific differences in fatty acid content exists, e.g. in fungi Ascomycetes comprise 36–61 mol% and Basidiomycetes 45–57 mol% of linoleic acid (18:2ω6,9) whereas in Zygomycetes it makes up only 12–22 mol% of total PLFAs (Klammer and Bååth, 2004).

Regardless of the complexity of fatty acid occurrence and biosynthesis, lipids as marker molecules have been used widely in terrestrial ecology (Tunlid and White, 1990; White et al., 1996). PLFAs have been employed as tools to assess microbial biomass and community structure in soil (e.g. Tunlid and White, 1992; Frostegård and Bååth, 1996; Olsson, 1999; Zelles, 1999; Joergensen and Emminger, 2006). Recent applications have used field scale isotopic labelling and PLFA-SIP to monitor the carbon flow from plants into soil microorganisms (Pombo et al., 2002, 2005; Olsson and Johnson, 2005; Evershed et al., 2006; Leake et al., 2006; Williams et al., 2006).

Table 1

<table>
<thead>
<tr>
<th>Fatty acid type</th>
<th>Frequently found</th>
<th>Lipid fraction</th>
<th>Predominant origin</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Saturated</strong></td>
<td></td>
<td></td>
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<tr>
<td>&gt;C20, straight</td>
<td>22:0, 24:0</td>
<td>PLFA, NLFA</td>
<td>Plants</td>
<td>Zelles (1999), Ruess et al. (2007)</td>
</tr>
<tr>
<td>Iso/anteiso methyl-branched</td>
<td>i, a in C14-C18</td>
<td>PLFA</td>
<td>Gram-positive bacteria</td>
<td>Zelles (1999), Ruess et al. (2007)</td>
</tr>
<tr>
<td>10-methyl-branched</td>
<td>10ME in C15-C18</td>
<td>PLFA</td>
<td>Sulphate reducing bacteria</td>
<td>Dowling et al. (1986), Kerger et al. (1986)</td>
</tr>
<tr>
<td>Cyclopropyl ring</td>
<td>cy17:0, cy19:0</td>
<td>PLFA</td>
<td>Gram-negative bacteria</td>
<td>Zelles (1997, 1999)</td>
</tr>
<tr>
<td>Hydroxy substituted</td>
<td>1H in C10-C18</td>
<td>PLFA</td>
<td>Gram-negative bacteria</td>
<td>Wakeham et al. (2003), Lee et al. (2004)</td>
</tr>
<tr>
<td><strong>Monounsaturated</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Double bond C5</td>
<td>16:1ω5</td>
<td>PLFA</td>
<td>AM fungi</td>
<td>Zelles (1999)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Bacteria</td>
<td>Nichols et al. (1986), Zelles (1997)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PLFA</td>
<td>AM fungi</td>
<td>Zelles (1999)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PLFA</td>
<td>AM fungi</td>
<td>Guckert et al. (1991), Zelles (1999)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PLFA</td>
<td>Baceteria</td>
<td>Zelles (1999)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PLFA</td>
<td>AM fungi</td>
<td>Zelles (1999)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PLFA</td>
<td>Methane-oxidising bacteria</td>
<td>Ringelberg et al. (1989)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PLFA</td>
<td>Fungi</td>
<td>Bååth (2003), Vestal and White (1989)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PLFA</td>
<td>Gram-positive bacteria</td>
<td>Zelles (1999)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PLFA, NLFA</td>
<td>Plants</td>
<td>Harwood and Russell (1984), Ruess et al. (2007)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NLFA</td>
<td>Nematodes</td>
<td>Chen et al. (2001)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PLFA</td>
<td>AM fungi (Gigaspora)</td>
<td>Sakamoto et al. (2004)</td>
</tr>
<tr>
<td><strong>Polyunsaturated</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ω6 family</td>
<td>18:2ω6,9</td>
<td>PLFA</td>
<td>Fungi</td>
<td>Frostegård and Bååth (1996), Zelles (1999)</td>
</tr>
<tr>
<td></td>
<td>NLFA</td>
<td>Animals</td>
<td>Ruess et al. (2000, 2005a), Haubert et al. (2006)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PLFA, NLFA</td>
<td>Plants</td>
<td>Miliar et al. (2000), Ruess et al. (2007)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>18:3ω6,9,12,15</td>
<td>PLFA, NLFA</td>
<td>Zygomycetes</td>
<td>van der Westhuizen et al. (1994)</td>
</tr>
<tr>
<td></td>
<td>20:4ω6,9,12,15</td>
<td>PLFA, NLFA</td>
<td>Animals widespread</td>
<td>Lechevalier and Lechevalier (1988), Stanley-Samuelson and Nelson (1993), Chen et al. (2001)</td>
</tr>
<tr>
<td>ω3 family</td>
<td>18:3ω3,9,12</td>
<td>PLFA, NLFA</td>
<td>Plants</td>
<td>Vestal and White (1989), van der Westhuizen et al. (1994)</td>
</tr>
<tr>
<td></td>
<td>20:5ω3,6,9,12,15</td>
<td>PLFA, NLFA</td>
<td>Algae</td>
<td>Lechevalier and Lechevalier (1988), Dunstan et al. (1994)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Collembola</td>
<td>Chamberlain and Black (2005)</td>
</tr>
</tbody>
</table>
2.2. Trophic transfer of fatty acids

Diet greatly influences the fatty acid composition of animals since it is energetically more efficient to incorporate dietary fatty acids into body tissue without modification, a process termed dietary routing (Blem, 1976; Pond, 1981). The mechanisms of transport have been known in mammals from the earliest days of physiological science. About 30 mols of dietary triacylglycerides are readily taken up by adipose tissue and not resorbed in cellular maintenance, and particularly medium-to long-chain fatty acids appear in chylomicra of mammal blood (Shapiro, 1967). More recently, this trophic transfer of dietary lipids to body fat has been demonstrated by stable isotope \( ^{13}C/^{12}C \) measurements in vertebrates (Stott et al., 1997). As a consequence the lipid pattern of a consumer is affected by the lipid profile of its food source. A vivid example is milk derived by organic farming, which contains more \( \omega 3 \) fatty acids than conventionally produced milk, due to the higher bypass of dietary \( \omega 2 \) linolenic acid through the rumen of cows grazing on pasture (Molkenst and Goesemann, 2007).

Much more is known about the lipid composition and trophic markers in marine food webs than in terrestrial ecosystems (e.g. Kattner et al., 2003; Stübing et al., 2003; Stevens et al., 2004a, b). The marine food chain is rich in long-chain polyunsaturated fatty acids, and microalgae contain 20–50% of their dry weight as \( \omega 3 \) forms of such molecules (Dwyer et al., 2003). Trophic transfer of long-chain fatty acids from phytoplankton to zooplankton can be used to monitor predator-prey interactions and to predict carbon flows (Ederington et al., 1995; Müller-Navarra et al., 2000; Pond et al., 2006). Several indices have been developed to infer feeding strategies of the zooplankton, for example an increase of the polyunsaturated/saturated fatty acid ratios or of 18:1n9:18:1n7 indicates enhanced carnivory and reduced omnivory (Stevens et al., 2004a, b). Dietary routing has also been shown for higher levels of the food web, i.e. in many fish species their tissues resemble the lipid composition of the prey (Dwyer et al., 2003).

In belowground systems the effects of substrate on the fatty acid composition of microorganisms has long been recognized. Erwin (1973) found de novo synthesis of fatty acids from \(^{14}\)C acetate by fungi to be almost completely suppressed in the presence of fatty acids in the culture media. Resources comprising hydrocarbons with odd numbered chains increased the proportion of odd numbered fatty acids in bacteria (Tunlid and White, 1990). Moreover, bacteria were reported to translocate substrate-derived \( n \)-alkenes into cellular lipids (Dunlap and Perry, 1967, 1968) and fatty acids into membrane lipids (Heipieper, 2001) without degradation to the acetate level.

Trophic transfer of fatty acids from primary decomposers to higher levels of the soil food web has only recently been confirmed (Table 2). Dietary routing has now been demonstrated for the soil microfauna (nematodes), mesofauna (Collembola) and macrofauna (earthworms) (Ruess et al., 2002; Chamberlain et al., 2005; Sampedro et al., 2006). These assimilated fatty acids were preferentially directed into the neutral lipid fraction of consumers (Ruess et al., 2004; Haubert et al., 2006), where they are stored as triacylglycerols in eukaryotic cells under conditions of excess carbon. In contrast, among soil prokaryotes only some actinomycetes accumulate neutral lipids to a significant extent (Alvarez and Steinbüchel, 2002). Hence, viable microbes in the gut or on the body surface of an animal consumer are part of the PLFA fraction, whereas the presence of microbial marker fatty acids in the consumer NFLA fraction indicates assimilation of microbial carbon (Fig. 2).

2.3. What makes a good biomarker fatty acid?

In many contexts major requirements for a biomarker are taxonomic specificity and rapid degradation in order to picture the living biomass. However, in relation to trophic transfer the applicability of specific marker components follows different criteria. The essential prerequisite for a biomarker fatty acid is that the molecule used as marker is subject to dietary routing, i.e. is assimilated as an entire molecule without degradation. Additionally, fatty acids applied to track interactions should fulfill the following criteria:

1) uniqueness to a specific food source;
2) no biosynthesis in consumer metabolism;
3) minor metabolic modification;
4) considerable pool size in the trophic cascade.

2.3.1. Uniqueness to a specific food source

Biosynthetic pathways can produce fatty acids that are relatively group specific, a fact which is most evident in microorganisms (White et al., 1996). However, apart from these characteristic markers, the same fatty acids occur in many organism groups (see Section 2.1). Therefore many fatty acids derived from whole soil communities cannot be assigned to a specific food source and are

Table 2
Experimental systems used to investigate trophic interactions by fatty acid biomarkers in soil ecology.

<table>
<thead>
<tr>
<th>Trophic level shift</th>
<th>Organisms</th>
<th>System</th>
<th>Target</th>
<th>Approach</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Fungi, nematodes</td>
<td>Agar culture</td>
<td>Fatty acid pattern</td>
<td>Dietary routing</td>
<td>Ruess et al. (2002)</td>
</tr>
<tr>
<td>1</td>
<td>Leaves, fungi, bacteria, nematodes, Collembola</td>
<td>Microcosms with plaster</td>
<td>Fatty acid pattern</td>
<td>Biomarker identification</td>
<td>Chamberlain et al. (2005), Haubert et al. (2006)</td>
</tr>
<tr>
<td>1</td>
<td>Fungi, Collembola</td>
<td>Microcosms with plaster</td>
<td>Fatty acid pattern</td>
<td>Food quality, starvation</td>
<td>Haubert et al. (2004)</td>
</tr>
<tr>
<td>1</td>
<td>Fungi, Collembola</td>
<td>Microcosms with plaster</td>
<td>Fatty acid pattern</td>
<td>Interactions gut microbiota</td>
<td>Sampedro et al. (2006)</td>
</tr>
<tr>
<td>1</td>
<td>Bacteria, earthworms</td>
<td>Soil culture</td>
<td>Fatty acid pattern</td>
<td>Trrophic transfer</td>
<td>Ruess et al. (2004)</td>
</tr>
<tr>
<td>1</td>
<td>Fungi, nematodes, Collembola</td>
<td>Microcosms with plaster</td>
<td>Fatty acid pattern</td>
<td>Trophic transfer, carbon turnover</td>
<td>Chamberlain et al. (2004, 2006a, b), Ruess et al. (2005b)</td>
</tr>
<tr>
<td>1</td>
<td>Fungi, nematodes, Collembola</td>
<td>13C labeled food</td>
<td>Fatty acid pattern</td>
<td>Carbon translocation</td>
<td>Chamberlain et al. (2006c)</td>
</tr>
<tr>
<td>1</td>
<td>Leaf litter, Collembola</td>
<td>Microcosms with C4 soil</td>
<td>Fatty acid pattern</td>
<td>Fatty acid pattern</td>
<td>Ruess et al. (2007)</td>
</tr>
<tr>
<td>1</td>
<td>Microflora, plant litter, Collembola</td>
<td>Deciduous forest stands</td>
<td>Fatty acid pattern</td>
<td>Feeding strategy</td>
<td>Ngosong et al. (2009)</td>
</tr>
<tr>
<td>1</td>
<td>Plant roots, microflora, Collembola</td>
<td>Agroecosystem: Conventional/organic farming</td>
<td>Fatty acid pattern</td>
<td>Feeding strategy</td>
<td>Haubert et al. (2009)</td>
</tr>
<tr>
<td>1</td>
<td>Plant roots, microflora, Collembola, spiders</td>
<td>Agroecosystem: Conventional/organic farming</td>
<td>Fatty acid pattern</td>
<td>Feeding strategy</td>
<td>Haubert et al. (2009)</td>
</tr>
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</table>
Fig. 2. Assimilation of marker fatty acids from phospholipids in microbial food sources into neutral lipids of decomposer animals.

not useful in tracking trophic interactions. Further, fatty acid indicators are not available for all soil biota, and signature lipid biomarker analysis therefore cannot detect every taxon present. Good markers are lacking for major fungal groups, as the PLFAs of both saprophytic and ectomycorrhiza fungi are characterised by linoleic acid as major component (Table 1). On the other hand, most arbuscular mycorrhiza fungi contain 16:1ω5 as specific NLFA in the deposit fat of spores (Olsom et al., 1995, 2003; Madan et al., 2002), and as this fatty acid does not occur in the neutral lipids of other organisms, it can be used as a biomarker. In plant tissues and debris polysaturated fatty acids with the first double bond at the ω3 carbon are potential trophic markers (Table 1; Fig. 1b) but dietary routing in the decomposer food chain has not yet been demonstrated. Unsaturated C20 fatty acids, in particular arachidonic acid (20:4ω6,9,12,15), predominantly occur in animals (Table 1) including soil invertebrates such as Protozoa, Nematoda, Collembola and Lumbricidae (e.g. Lechevalier and Lechevalier, 1988; Petersen and Holmstrup, 2000; Ruess et al., 2002; Sampedro et al., 2006). Arachidonic acid has potential as a qualitative marker for soil invertebrates, but in quantitative analysis the intra- and interspecific variation related to its function as precursor for hormones and importance for structural integrity of membranes has to be taken into account (Stanley-Samuelson et al., 1988; Sargent et al., 1995).

Interestingly, fatty acid molecules can travel more than one food chain level without degradation. Tritrophic transport has been shown in heterotroph (bacteria – ciliates – copepods) and autotroph (phytoplankton – copepods – cod) marine food chains (Ederington et al., 1995; Budge et al., 2008). For soil decomposers similar shifts have been observed in fungal based (fungi – nema-todes – Collembola) food chains (Ruess et al., 2004, Table 2). In such cases the marker fatty acid can indicate feeding on a specific diet and/or predation on prey also feeding on this diet (Table 3). This offers the possibility of following carbon transfer through the soil food web using the specific trophic links indicated by marker fatty acids, particularly when combining lipid analysis with stable isotope techniques (see 2.4). In summary, although the chemical taxonomy of soil microorganisms is quite advanced, and work on soil invertebrates has demonstrated the utility of fatty acid analyses, further studies screening the lipid pattern of soil decomposers are necessary to reveal unique fatty acids within food web members.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Food source</th>
<th>Biomarker type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl-branched</td>
<td>Gram-positive bacteria,</td>
<td>Absolute</td>
</tr>
<tr>
<td>(iso, anteiso)</td>
<td>bacterial-feeding prey</td>
<td></td>
</tr>
<tr>
<td>Cyclopropyl ring (cy)</td>
<td>Gram-negative bacteria,</td>
<td>Absolute</td>
</tr>
<tr>
<td></td>
<td>bacterial-feeding prey</td>
<td></td>
</tr>
<tr>
<td>Vaccenic type (ω7)</td>
<td>Bacteria in general,</td>
<td>Absolute</td>
</tr>
<tr>
<td></td>
<td>bacterial-feeding prey</td>
<td></td>
</tr>
<tr>
<td>Oleic acid (18:1ω9)</td>
<td>Plant tissue</td>
<td>Relative</td>
</tr>
<tr>
<td>Linoleic acid (18:2ω6,9)</td>
<td>Fungi</td>
<td>Relative</td>
</tr>
</tbody>
</table>

2.3.2. No biosynthesis in consumer metabolism

Ideally, marker fatty acids are absent from or only minor components of the fatty acids biosynthesised by the consumer, i.e. they are absolute markers. In the soil food web such markers are available for bacterial resources as they contain fatty acids with branched chains or alicyclic rings, which are not biosynthesised by invertebrates. Methyl-branched (iso, anteiso) fatty acids have been assigned as absolute markers for consumption of Gram-positive bacteria, and cyclic fatty acids as markers for Gram-negative bacteria in Collembola (Ruess et al., 2005a; Table 3). Additionally, some monoenoic bacterial fatty acids are potential biomarkers, particular those of vaccenic type with the double bond located at the ω7 carbon. Stevens et al. (2004b) showed dietary routing of 16:1ω7 and 18:1ω7 from marine bacteria into zooplankton, and in soil animals this has been demonstrated for Collembola consuming bacteria (Chamberlain et al., 2005; Ruess et al., 2005a). However, it should be noted that 18:1ω7 can either be directly incorporated or synthesised via chain elongation of the precursor 16:1ω7.

The general case in soil decomposer systems is that many fatty acids occur throughout diet and consumer lipid profiles due to widespread biosynthesis. To overcome this drawback, relative changes in some fatty acids can be used to identify trophic relationships. Relative markers are specific fatty acids which, although biosynthesised in consumer lipid metabolism, increase in proportion if resources rich in these components are fed upon. In Collembola fungal and plant feeding can be identified by the assimilation of 18:2ω6,9 and 18:1ω9, respectively (Table 3). Using absolute and relative marker fatty acids broad trophic groups within Collembola communities have been determined in woodland (Ruess et al., 2005a, 2007). In arable land Haubert et al. (2009) applied fatty acid analysis to investigate the feeding range of Collembola and their potential as prey for spiders. Further, Ngosong et al. (2009) showed that the fungal energy channel in the soil food web was of low importance in agricultural fields with difference in fertilizer practice. In summary, absolute and relative biomarker acids have been identified and successfully applied to assign feeding strategies in some decomposer invertebrates. However, particularly in relative biomarkers a detailed understanding of how a species responds to dietary fatty acids is needed before such compounds are widely used.

2.3.3. Minor metabolic modification

Biomarker fatty acids should not be significantly modified by consumer metabolism after assimilation. However, variation in lipid composition of both the diet and the consumer can take place due to differences in resources, metabolic activity or environmental conditions. A large body of work suggests that variation in unsaturated fatty acid composition is part of the mechanism through which organisms maintain membrane fluidity despite changes in ambient temperature. In plants α-linolenic acid plays a key role in
cold tolerance and optimal chloroplast function at low temperature (Nishida and Murata, 1996; Daligault et al., 2003). Similarly, the PLFA pattern of bacteria and fungi shows a higher degree in unsaturation with decreasing temperature (Cronan and Gelmann, 1975; Hammonds and Smith, 1986).

Homoeoviscous adaptation of membrane fluidity to temperature also alters animal lipid composition, predominantly in ectotherms such as most of the soil fauna. Collembola displayed typical changes in the degree of unsaturation in membrane lipids during acclimation to cold (Holmstrup et al., 2002), whereas variations within average environmental conditions (5–15 °C) had lower impact on lipid composition than species-specific differences (Haubert et al., 2008). These changes were not restricted to PLFAs and appeared in NLFAs as well. Likely the degree of unsaturation in membrane lipids during acclimation with decreasing temperature (Cronan and Gelmann, 1975; Hammonds and Smith, 1986). These changes were not restricted to PLFAs and appeared in NLFAs as well. Likely the degree of unsaturation in membrane lipids during acclimation with decreasing temperature (Cronan and Gelmann, 1975; Hammonds and Smith, 1986).

Alterations in the lipid composition of organisms further occur due to physiological conditions such as growth stage, nutrient status or stress response (Vestal and White, 1989; Olsson et al., 1995; Keweloh and Heipieper, 1996; Báth, 2003). Stress adaptive mechanisms in bacteria include changes in the cis-trans isomerisation of unsaturated fatty acids or the iso/anteiso ratio in methyl-branched fatty acids (Kaneda, 1991; Kieft et al., 1994; Heipieper et al., 2003). Differences in substrate quality (e.g. C/N ratio) may also alter microbial lipid composition (Dunlap and Perry, 1967, 1968; White et al., 1996), this likely plays an important role in food webs with recalcitrant plant litter as basic resource. However, other workers report reproducibility of bacterial PLFA profiles across strains and species regardless of growth conditions (Guckert et al., 1991) as well as variations of patterns within limits acceptable for species separation (Dzierzewicz et al., 1996). Generally, such alterations in fatty acid composition are observed in monocultures under laboratory conditions. However, the condition for soil microbial consortia is predominantly the stationary phase (Bartscht et al., 1999), and therefore PLFA composition can be expected to be rather stable.

The few reports available for soil animals indicate the lipid composition to be affected by metabolic state. Fatty acid patterns of nematodes are sensitive to changes in culture conditions, such as starvation and media composition (Krusberg, 1972; Abu Hatab et al., 1998; Holz et al., 1999). Similarly, Haubert et al. (2004) reported fungal food quality to alter the lipid profile in Collembola grazers. This may be attributed to compensatory responses and changes in total lipid content, as the latter is strongly correlated to the C:N ratio of an animal (Post et al., 2007). In contrast, starvation of Collembola resulted in a decline in the total amount of PLFAs with no effect on relative fatty acid composition. Thus storage fat was degraded indiscriminately and no preferential utilisation of either high-energy long-chain polyunsaturated or low-cost short-chain saturated fatty acids occurred (Haubert et al., 2004). Similar observations were made by Stübing et al. (2003) in a case study on Antarctic krill and by Canavoso et al. (1998) in Triatomoineae (Heteroptera). In summary, lipid composition is affected by environmental and physiological conditions, but although these factors will change consumer fatty acid profiles, work to date suggests that biomarker compounds will still be able to be identified and utilised.

2.3.4. Considerable pool size in the trophic cascade

In order to be usable as biomarkers, dietary fatty acids must be assimilated into consumer lipids as intact molecules to a significant extent when the diet is consumed. Identification of trophic interactions from fatty acid determinations therefore depends on the pool size of the respective acid in both diet and consumer (Fig. 3). Large fat deposits accompanied by reduced feeding and metabolic activities in consumers buffer dietary lipid supply, whereas rising lipid content in the diet decreases the activity of lipogenic enzymes and enhances dietary routing (Stübing et al., 2003; Gaye-Siesseng et al., 2004a,b). Additionally, not all tissues incorporate available pools of fatty acids at equal rates. Metabolically active tissues, such as reproductive and lipid tissue, incorporate the diet signal more rapidly than others (Gratton and Forbes, 2006). Such tissues can be a useful target for trophic studies, which is supported by the predominant dietary routing of fatty acids into storage lipids, i.e. NLFAs (Ruess et al., 2004). Due to the small size of soil animals and the limited number extracted from field soil, often whole body studies have to be performed, and this method has been shown to be robust in this context (see Ruess et al., 2005a,b, 2007).

Individual fatty acids also appear to have different incorporation and turnover rates. Diet switch experiments in Collembola indicate that fatty acids are added to or removed from the Collembolan lipid pool strictly according to their presence or absence in the diet (Chamberlain et al., 2005). Faster rates of incorporation for unsaturated compared to saturated fatty acids have been reported, and species specific differences occur (Chamberlain et al., 2004). The studies indicate that it is unlikely that any highly abundant fatty acids would have a half life longer than 6 days at 20 °C, i.e. the lipid profile of Collembola represents the dietary input and biosynthesis of recent days. At ambient temperature in the natural environment lipid patterns likely reflect the diet over a longer time period but this may still be a matter of a few weeks only.

Poole size is predominantly important when considering relative marker fatty acids which occur in more than one resource. In the soil decomposer food web this hampers the use of linoleic and oleic acid as they are components of both plants and fungi. However, plant roots are much larger (diameter in the mm scale; e.g. Reubens et al., 2007) than fungal hyphae (diameter in the μm scale; e.g. Bååth and Söderström, 1979), with a lower surface-area-to-volume ratio. Thus, regarding linoleic acid the root-derived soil PLFA pool is small compared to the fungal-derived. On the other hand, the amount of oleic acid directly assimilated by root feeders likely exceeds the soil fungal pool by several magnitudes. Based on this the ratio 18:1ω9/18:2ω6,9 has been applied to distinguish between plant and fungal feeding in Collembola in the field (Ruess et al., 2007). Further, analysis of the carbon isotope signal in relative

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**Fig. 3.** Different origin of a trophic marker fatty acid detected in a consumer. The general diet pool can be superimposed by direct and indirect pools in the consumer. Note that for an absolute marker de novo synthesis does not apply.
marker fatty acids proved to be a good tool to separate plant and microbial resources (see 2.6). Nevertheless, there is need for more laboratory experiments to gain a deeper understanding in metabolic theory and to assess the usefulness of general mixing models for dietary routing processes. Overall one should keep in mind that the trophic transfer of biomarker fatty acids should not be interpreted as absolute values but viewed in the context of the pool size of the fatty acids in question (Fig. 3).

2.4. Compound specific analysis: lipid and stable isotope techniques combined

Historically, the majority of ecological investigations using stable isotope analysis (SIA) have involved the determination of whole-tissue isotopic compositions, i.e. ‘bulk isotopic analysis’. This involves the complete combustion of organic material to CO2 and isotopic ratio determination by isotope ratio mass spectrometry (IRMS; Brenna et al., 1997). About 20 years ago instruments linking a gas chromatograph to an isotope ratio mass spectrometer via a combustion furnace became commercially available. These allow on-line isotopic analysis of individual compounds, a technique known as compound-specific isotopic analysis (CSIA) or gas-chromatography-combustion-isotope-ratio-mass-spectrometry (GC-C-IRMS). Meier-Augenstein (1999) and Staddon (2004) give useful information of the analytical equipment employed in isotopic analyses. Lipids were among the first compounds to be measured by CSIA due to the ease with which they can be chemically modified for GC analysis. Applications of lipid CSIA include the determination of sources of lipids absorbed in archaeological material (Copley et al., 2003), organic contaminants (Schmidt et al., 2004), and paleodietary reconstruction (Stott et al., 1999). Meier-Augenstein (2002) has presented a useful review of stable isotope analyses of fatty acids.

In food web studies, appropriate use of CSIA requires that prospective diets contain distinct isotopic compositions that can be traced into the consumers’ tissues. The δ13C values (i.e. differences in the 13C/12C ratio, see Enleringer and Rundel, 1989) arising due to isotopic fractionations during C3 and C4 photosynthesis have been used for CSIA studies in terrestrial and aquatic ecosystems (Hammer et al., 1998; Budge et al., 2008; Nottingham et al., 2009). Other researchers have utilised the low 13C content of microbially produced methane in marine sediments to examine the contribution of chemotrophically produced fatty acids to benthic predators (MacAvoy et al., 2003). Soil microbial and animal food webs in an agricultural system have recently been linked using CSIA determinations of animal lipids after a shift from C3 to C4 crops (Haubert et al., 2009, Table 2).

An alternative to using naturally occurring isotopic differences is to introduce a 13C label into a food web and then examine the route of the 13C-pulse through the web. This approach removes the need to consider isotopic fractionation, as the 13C pulse is generally many orders of magnitude greater than any fractionation that may occur (see Section 2.5). Stable isotope probing (SIP) of microbial PLFAs was applied to determine C flow through the microbial community using either 13C-acetate (Boschker et al., 1998), 13CH4 (Bull et al., 2000; Crossman et al., 2006; Knief et al., 2006; Maxfield et al., 2006), 13CO2 (Lu et al., 2004) or 13C-labelled plant residues (Williams et al., 2006, 2007) to determine the uptake of 13C-acetate (Boschker and Millerburg, 2002) and Evershed et al. (2006) for recent reviews. Isotopic pulse-labeling of plants has also been used to examine C transfer to soil decomposers with SIP applied to microbial PLFAs and bulk SIA to animals (Ostle et al., 2007; Ladygina et al., 2008). However, pulse-labeling studies tracing the 13C signal in fatty acids at higher trophic levels of the soil food chain are still lacking.

2.5. Isotopic fractionation in lipid metabolism

Mechanisms of isotopic fractionation are reviewed extensively elsewhere (Hayes, 1993; Schoeller, 1999; Hayes, 2001). Fractionation occurs because biomolecules are produced by enzymatic processes which preferentially utilise one isotope, and because there are multiple branch points in biochemical pathways, which lead to downstream products with different isotopic signatures. Biosynthetic fractionation leads to consistent isotopic differences between proteins, lipids and carbohydrates, the major components of organisms (Abelson and Hoering, 1961; Park and Epstein, 1961; Webb et al., 1998; Hobbie and Werner, 2004). Lipids, including fatty acids, are depleted in 13C relative to bulk tissue (and exhibit 6–8‰ lower δ13C values) due to isotopic discrimination by the key enzyme in fatty acids de novo biosynthesis, pyruvate dehydrogenase, which produces the precursor acetyl-CoA containing increased proportions of 13C (DeNiro and Epstein, 1977). The magnitude of fractionation is additionally affected by the δ13C values of sources used in acetyl-CoA production (Hayes, 1993). Biosynthesis also leads to intra-molecular differences in δ13C values (Hobbie and Werner, 2004), for example C atoms in odd and even number positions in a fatty acid chain are derived from either the carboxyl or methyl groups in acetyl-CoA, and as such reflect the differing δ13C values of these produced by enzymatic discrimination (Monson and Hayes, 1982a,b).

Besides this fractionation occurring in de novo synthesis the δ13C values of individual lipid compounds will depend upon several other factors. These include the ratios of precursor molecules used in lipid synthesis relative to other biosynthetic activities. Isotopic fractionation by other enzymes after production of the precursor palmitic acid (i.e. chain elongation, desaturation), and the balance of direct assimilation from the diet relative to de novo synthesis within an organism (Blair et al., 1985; Hayes, 2001; Abraham and Hesse, 2003). Hence, the δ13C values of a specific fatty acid can considerably vary due to the carbon pool it is derived from (Fig. 3). For example, differences in the δ13C values of linoleic acid, a relative trophic maker in Collembola, between a range of diets and consumers were 0.4–7.5‰, depending upon species and food source (Chamberlain et al., 2006b). Relative markers are therefore comprised of both molecules incorporated from the diet and molecules synthesised either de novo from acetate or via modification of fatty acid precursors (Fig. 3). On the other hand, absolute markers, i.e. not synthesised by consumers, such as bacterial iso- and anteiso fatty acids, showed similar δ13C values in Collembola and diet, which remained constant over 14d (Chamberlain et al., 2006b). This emphasises the need for more studies on lipid metabolism in soil invertebrates in order to establish reliable thresholds for diet-consumer δ13C shifts in food web analyses using CSIA.

2.6. Use of CSIA in trophic studies

In using CSIA to establish trophic preferences, it is important to differentiate between compounds which can be biosynthesised and compounds which are directly incorporated from the diet, as the isotopic ratios of the latter better reflect dietary isotopic compositions (MacAvoy et al., 2003). When food is plentiful tissue lipid composition and δ13C values are likely to be dominated by directly assimilated fatty acids (Gaye-Siessegger et al., 2004a,b; Chamberlain et al., 2006a). On the other hand, if a diet is low in lipid but abundant in energy, consumers may store the available energy in de novo synthesised neutral lipids, which will modify lipid δ13C values (Gaye-Siessegger et al., 2003).

Diet quality is a key factor for the balance between assimilation and biosynthesis and the resulting fatty acid δ13C values. It is well
known that insects feeding on low quality diets, i.e. diets with high C:N ratio, try to optimise their N assimilation by increasing feed intake (Karowe and Martin, 1989; Simpson and Simpson, 1990; Lavy and Verhoeff, 1996). This has the additional effect that the lipid pool of consumers grows in size, as they directly assimilate exogenous lipid or store the excess energy from the diet as lipid, leading to considerable changes in the overall lipid pattern (Haugbert et al., 2004) and δ13C values.

The interpretation of isotopic data usually assumes that the organism is at steady state, i.e. there has been no recent change in diet, and the organisms is not growing or starving. However such steady-state assumptions often do not correspond to physiological or ecological conditions. If from a new diet a particular fatty acid is directly assimilated into consumer tissues, associated rapid changes in the consumer δ13C values of the compound may occur due to high uptake, whilst other fatty acids will still reflect the δ13C values of the previous diet. Diet shift experiments in Collembola revealed half-lives of the rates of change in δ13C values between 29 min (linoleic acid) and 14d (vacenic acid) (Chamberlain et al., 2006b). Overall the turnover of carbon in soil invertebrates appears to be a relatively fast process, with bulk carbon and fatty acid half-lives which reflect only the diet of the last few days (Ostrom et al., 1997; Chamberlain et al., 2004).

2.7. Methodical remarks

2.7.1. Extraction of fatty acids

Microbial PLFAs in soils are generally extracted using a mix of organic solvents and aqueous buffer. Most common are modifications of the method described by Bligh and Dyer (1959), which was developed to extract lipids from frozen fish with the whole procedure taking approximately 10 min. Longer extraction periods have to be applied for soil lipids to achieve a more complete extraction in the presence of a complex organic matrix such as humic acids (Zelles, 1999). The amount of PLFAs recovered mainly depends on the choice of buffer, with citrate buffer giving a higher yield compared to phosphate or acetate buffer in organic soils (Frostegård et al., 1991; Nielsen and Petersen, 2000). In general PLFA amounts and patterns gained are well comparable to other microbial community analyses, e.g. chloroform-fugitation-extraction or DNA-based approaches (Bäath et al., 2004; Ramsey et al., 2006). In contrast to such organic solvent based extraction methods microwave-assisted extraction of sterols and fatty acids is a simpler and rapid procedure (Young, 1995; Montogmery et al., 2000; Batista et al., 2001), yet the efficiency in organic soil substrate has still to be determined. The extraction of lipids from animal tissue is simpler due to a less complex matrix, but commonly applied methods vary, most often in mix of solvents used to extract lipids.

Once the total lipids have been extracted, subsequent steps differ on the basis of what kind of analysis is desired. Total cellular fatty acids (CFA) can be determined through the use of either mild alkaline or acid hydrolysis to liberate fatty acids (Welch, 1991). CFA are often used in lipid analyses of soil animals when biomass is limiting (e.g. Ruess et al., 2002; Chamberlain et al., 2004; Sampredo et al., 2006). This is a feasible approach as in small soil invertebrates about 90% of the CFA are represented by NLFAs, i.e. the lipid fraction within which dietary routing of biomarkers occurs (Haugbert et al., 2004). Besides analysis of CFA, extracted lipid components can be separated into neutral, glyco- and phospholipid fractions using a solid phase column, with PLFAs as most sensitive measure for soil microbial biomass and community structure (e.g. Tunlid and White, 1990, 1992).

Prior to gas chromatographic analysis fatty acids are chemically converted to fatty acid methyl esters (FAMEs), either in a one-stage transesterification process (White and Ringelberg, 1998) or in a two-stage saponification and derivatisation (e.g. Ruess et al., 2004; Dungait et al., 2008). Transesterification will produce FAMES of fatty acids present in esters (O-acyl fatty acids), while saponification reduces all esters to free fatty acids which can then be derivatised. Saponification and derivatisation will therefore include FAMES derived from both esters and free fatty acids (if present in the sample), while transesterification only produces FAMES from ester-bound components. Additionally, the use of an internal standard — a related but unique compound added to the extraction process as soon as possible — enables the quantification of the extracted lipids.

These chemical extraction methods, using typical methanol-chloroform solutions to physically remove lipids from samples, generally cause insignificant fractionation of δ13C but changes in δ15N (Sotiropoulos et al., 2004; Post et al., 2007). However, the Bligh and Dyer extraction method has been reported to cause systematic errors for δ13C when applied to diatoms (Schlechtriem et al., 2003). The addition of a methyl group (i.e. one C atom) to produce the FAMES alters the δ13C values of the derivatised molecules, and the effect of this methyl group must later be taken into account when calculating the δ13C value of the original fatty acid (Rieley, 1994; Abrahamsson et al., 1998).

An alternative to lipid extraction with organic solvents was reported by Evans et al. (2003), who used a thermally assisted hydrolysis and methylation reaction to derivatise and volatile fatty acids of individual Collembola. Isotopic analysis is then performed by pyrolysis-GC-C-IRMS. Compared to the conventional “wet” chemical procedures applied to much larger numbers of Collembola, single individuals can be probed directly, thus enhancing the scope of CSIA in soil systems with low biomass of most of the fauna.

2.7.2. Gas chromatography and mass spectrometry

Modern gas chromatographic (GC) methods facilitate the separation of individual fatty acids, and with the aid of commercially available standards about 40 fatty acids can be identified by retention time and mass spectra (Brondz, 2002). Quantification of FAMES is commonly performed by GC-FID, i.e. with a GC equipped with a flame ionisation detector, due to the rapid and easy procedure (Dodds et al., 2005; Welch, 1991; White et al., 1996). Additional confirmation of analytes by gas chromatography-mass spectrometry (GC-MS) is essential in complex biological samples and further derivatisation of FAMES is sometimes necessary to produce compounds which give characteristic GC-MS fragmentations, enabling the determination of double bond positions (Rosenfeld, 2002). Using these methods and commercial libraries, the compounds present in a sample can be assigned. This results in correct identification of chromatographic peaks, determination of co-elution, and separation of targets from matrix. Recent studies revealed a higher selectivity and better sensitivity of GC-MS in selected ion monitoring (SIM) mode compared to FID (Thurnhofer and Vetter, 2005; Vetter and Thurnhofer, 2007). Of particular interest in this context will be tandem mass spectrometry (GC-MS-MS) due to the superior selectivity in analyses of sub-milligram samples (Saraf et al., 1999). Hence, combining qualification and quantification in one step, will be a promising tool for the future in fatty acid profiling.

Determination of fatty acid δ13C values by GC-C-IRMS has an error of approx. 0.3‰ associated with 13C determinations (Wong et al., 1995) but deviation of up to 3‰, depending on analytical conditions have been reported. Such variation can be minimised by careful optimisation of instrumentation (Meier-Augenstein et al., 1996; Schmitt et al., 2003). It is advisable that all determinations within an experiment are carried out with standardised methods.
on one piece of equipment. The accuracy of δ\(^{13}\)C values is predominantly reliant on the separation of chromatographic peaks such that the tail of one peak does not interfere with the front of the subsequent peak (Hayes et al., 1990; Brenna et al., 1997). Where such separations are not possible, as for closely related compounds, a composite δ\(^{13}\)C value is often reported (e.g. Boschker et al., 1998; Lu et al., 2004; Watzinger et al., 2008). Soil scientists should be aware that their lipid extractions consist of a complex matrix, which contrasts the simple model compounds used in most other studies (Moor-Kucera and Dick, 2008).

In situations where pulse-labelling is used, lipid δ\(^{13}\)C values may be high, and consideration of carry-over effects, where the δ\(^{13}\)C values of compounds eluting after enriched components or in subsequent analyses are affected by residual of 13C, should be made (Mottram and Evershed, 2003). This is particularly the case where some components are highly labelled and others not, as is common in PLFA δ\(^{13}\)C determinations with enriched isotope tracers (e.g. Bull et al., 2000). Useful reviews of GC-C-IRMS, and of potential analytical problems, are given in Sessions (2006) and Blessing et al. (2008).

3. Conclusions

Biochemical ecology has the power to resolve the trophic interactions in cryptic soil food webs. Fatty acid patterns and the δ\(^{13}\)C values of individual fatty acid biomarkers provide good indications of true diet histories of the decomposer fauna in situ, given that fatty acid biomarkers and diets with contrasting δ\(^{13}\)C values are present. The well developed analytical methods and the ubiquitous occurrence of fatty acids makes them a promising tool. Five major areas are central for the application of fatty acids as trophic markers:

(1) In some cases the lipids from a group of biologically related organisms contain particular fatty acids, which are not detected in other resident groups in the same habitat. These marker fatty acids build the base of qualitative and quantitative chemical taxonomy in soil microorganisms. However, many higher fatty acids are widespread throughout nature. There is need for a comprehensive screening of the lipid pattern of major faunal groups in order to broaden this approach to higher trophic levels of the soil food web.

(2) Known dynamics in incorporation of marker fatty acids are related to dietary routing of absolute and relative markers into consumer lipids. A vivid example is the assimilation of unique microbial PLFAs into the neutral lipid fraction of animal grazers, which links the microbial and faunal food web. Since tritrophic shifts and diet switch may complicate biomarker tracing when entire food webs are analysed, further research is necessary to establish the applicability of fatty acid biomarkers in this situation.

(3) Accurate determination of trophic links relies on minor modification of dietary marker fatty acids by consumer metabolism. More information on time scales over which amounts of fatty acids and their δ\(^{13}\)C values vary in response to biological variables (e.g. environmental conditions, diet quality, life stage) is required to determine the energy flow between resource and consumer. Simply investigating the fatty acid composition of soil animals from the field, without sufficient knowledge of the specific lipid metabolisms, is unlikely to yield usable food web information.

(4) To detect marker fatty acids in consumer tissue compounds must be incorporated to a significant extent from dietary lipids. The occurrence of marker compounds in consumers largely depends on their presence in the diet and the varying proportions of these fatty acids in combination in different consumer lipid fractions. Hence, for each trophic marker fatty acid the respective pools and their sizes have to be considered.

(5) In lipid analyses sample preparation methods are largely standardised and the challenge is in the interpretation of fatty acids pattern derived from organic soil matrix. Determination of FAMEs by GC-FID is among the most commonplace analyses in lipid research. However, in complex biological samples spectrometric confirmation is always advisable. Standard GC-MS procedures should be applied for qualitative confirmation of FAMES. Moreover, recent methodological advances allow combining spectrometric examination and quantitative determination by GC-MS.

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