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Review

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

Liliane Ruess [a,*], Paul M. Chamberlain [b]

[a] Institute of Biology, Ecology Group, Humboldt University Berlin, Philippstraße 13, 10115 Berlin, Germany
[b] Centre for Ecology and Hydrology, Lancaster Environment Centre, Library Avenue, Bailrigg, Lancaster LA1 4AP, UK

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ABSTRACT

Chemical taxonomy based upon the composition of lipids is widely applied to investigate microbial communities and fatty acids have recently been employed to connect soil microbial and faunal food webs as well as to elucidate functional groups at higher trophic levels. The additional use of compound-specific isotopic analysis of $^{13}C/^{12}C$ ratios in fatty acids allows assessing specific trophic links and belowground carbon fluxes. In this review systematic patterns and processes underlying variations in the composition of fatty acids and their $^{13}C/^{12}C$ ratio are described. The emphasis is on biomarker fatty acids, their incorporation and modification, effects of pool size, and analytical methods. Further development of the application of fatty acid profiling to soil ecology should include both advances in experimental research and growth of theory. Accordingly, areas in which future experimentation can lead to progress in soil food web analysis are identified. Overall, combining fatty acid biomarker and their isotopic ratios will allow detailed insight into belowground trophic interactions.

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1. Introduction

Soils harbour an enormous diversity of organisms in multitrophic 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 ($^{13}C/^{12}C$) and N ($^{15}N/^{14}N$) 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; Tiunov, 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

* Corresponding author. Tel.: +49 30 2093 8321; fax: +49 30 2093 8324.
E-mail address: liliane.ruess@biologie.hu-berlin.de (L. Ruess).

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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 ^{13}C 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 $^{13}C/^{12}C$ 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

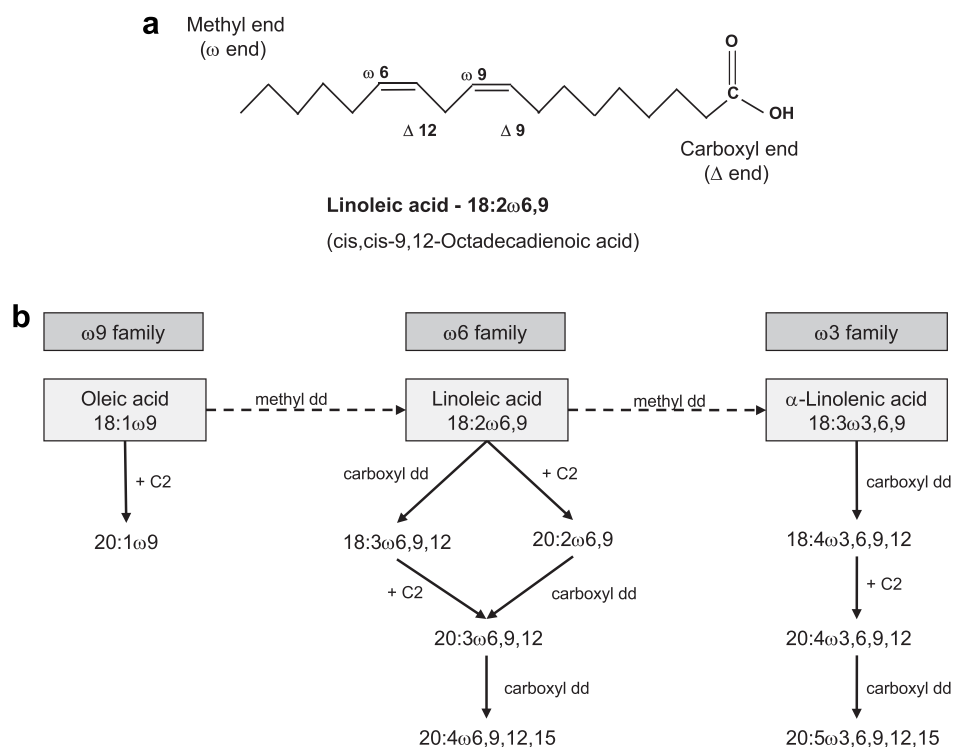

Fig. 1. a. Nomenclature of a dienoic fatty acid; given is linoleic acid (trivial name). Classification according to the number of carbon atoms from the carboxyl group (Δ end) denotes its systematic name cis,cis-9,12-Octadecadienoic acid, whereas counting from the terminal methyl group (ω end) assigns 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 (Klamer 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 Emmerling, 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
Characteristic ester-linked fatty acids in the lipids of common soil biota. NLFA – neutral lipid fatty acid, PLFA – phospholipid fatty acid.

Fatty acid type	Frequently found	Lipid fraction	Predominant origin	Reference
Saturated				
>C20, straight	22:0, 24:0	PLFA, NLFA	Plants	Zelles (1999), Ruess et al. (2007)
Iso/anteiso methyl-branched	i, a in C14-C18	PLFA	Gram-positive bacteria	Zelles (1997, 1999)
10-methyl-branched	10ME in C15-C18	PLFA	Sulphate reducing bacteria	Dowling et al. (1986), Kerger et al. (1986)
Cyclopropyl ring	cy17:0, cy19:0	PLFA	Gram-negative bacteria	Zelles (1997, 1999)
Hydroxy substituted	OH in C10-C18	PLFA	Gram-negative bacteria	Wakeham et al. (2003), Lee et al. (2004)
			Actinomycetes	Mirza et al. (1991)
Monounsaturated				
Double bond C5	16:1ω5	PLFA	AM fungi	Olsson et al. (1995, 2003), Sakamoto et al. (2004)
			Bacteria	Nichols et al. (1986), Zelles (1997)
		NLFA	AM fungi	Olsson et al. (1995, 2003), Madan et al. (2002)
Double bond C7	16:1ω7	PLFA	Bacteria widespread	Guckert et al. (1991), Zelles (1999)
	18:1ω7	PLFA	Bacteria	Zelles (1999)
			AM fungi	Olsson (1999)
		NLFA	AM fungi	Olsson (1999)
Double bond C8	18:1ω8	PLFA	Methane-oxidising bacteria	Ringelberg et al. (1989)
Double bond C9	18:1ω9	PLFA	Fungi	Bååth (2003), Vestal and White (1989)
		PLFA	Gram-positive bacteria	Zelles (1999)
		PLFA, NLFA	Plants	Harwood and Russell (1984), Ruess et al. (2007)
		NLFA	Nematodes	Chen et al. (2001)
	20:1ω9	PLFA	AM fungi (*Gigaspora*)	Sakamoto et al. (2004)
Polyunsaturated				
ω6 family	18:2ω6,9	PLFA	Fungi (saprophytic, EM)	Frostegård and Bååth (1996), Zelles (1999)
		NLFA	Animals	Ruess et al. (2000, 2005a), Haubert et al. (2006)
	18:3ω6,9,12	PLFA, NLFA	Plants	Millar et al. (2000), Ruess et al. (2007)
		PLFA	Zygomycetes	van der Westhuizen et al. (1994)
	20:4ω6,9,12,15	PLFA, NLFA	Animals widespread	Lechevalier and Lechevalier (1988), Stanley-Samuelson and Nelson (1993), Chen et al. (2001)
ω3 family	18:3ω3,9,12	PLFA	Higher fungi	Vestal and White (1989), van der Westhuizen et al. (1994)
		PLFA, NLFA	Plants	Weete (1980), Moore (1993)
	20:5ω3,6,9,12,15	PLFA	Algae	Lechevalier and Lechevalier (1988), Dunstan et al. (1994)
		PLFA, NLFA	Collembola	Chamberlain and Black (2005)

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 mol% of dietary triacylgycerides are readily taken up by adipose tissue and not respired 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 ω3 fatty acids than conventionally produced milk, due to the higher bypass of dietary α-linolenic acid through the rumen of cows grazing on pasture (Molkentin 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 ω3 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 18:1ω9/18:1ω7 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 trophic interactions should fulfil 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.

Trophic level shift	Organisms	System	Target	Approach	Reference
1	Fungi, nematodes	Agar culture	Fatty acid pattern	Dietary routing	Ruess et al. (2002)
1	Leaves, fungi, bacteria, nematodes, Collembola	Microcosms with plaster	Fatty acid pattern	Biomarker identification	Chamberlain et al. (2005), Haubert et al. (2006)
1	Fungi, Collembola	Microcosms with plaster	Fatty acid pattern	Food quality, starvation	Haubert et al. (2004)
1	Fungi, Collembola	Microcosms with plaster	Fatty acid pattern	Temperature, life stage	Haubert et al. (2008)
1	Bacteria, earthworms	Soil culture	Fatty acid pattern	Interactions gut microbiota	Sampedro et al. (2006)
2	Fungi, nematodes, Collembola	Microcosms with plaster	Fatty acid pattern	Tritrophic transfer	Ruess et al. (2004)
1	Fungi, nematodes, Collembola	Microcosms with plaster ^{13}C labeled food	Fatty acid pattern ^{13}C in fatty acids	Trophic transfer, carbon turnover	Chamberlain et al. (2004, 2006a,b), Ruess et al. (2005b)
1	Leaf litter, Collembola	Microcosms with C_4 soil	Fatty acid pattern ^{13}C in fatty acids	Carbon translocation	Chamberlain et al. (2006c)
1	Microflora, plant litter, Collembola	Deciduous forest stands	Fatty acid pattern	Feeding strategy	Ruess et al. (2007)
1	Plant roots, microflora, Collembola	Agroecosystem: Conventional/ organic farming	Fatty acid pattern	Feeding strategy	Ngosong et al. (2009)
2	Plant roots, microflora, Collembola, spiders	Agroecosystem: Conventional/ organic farming	Fatty acid pattern ^{13}C in fatty acids	Feeding strategy	Haubert et al. (2009)

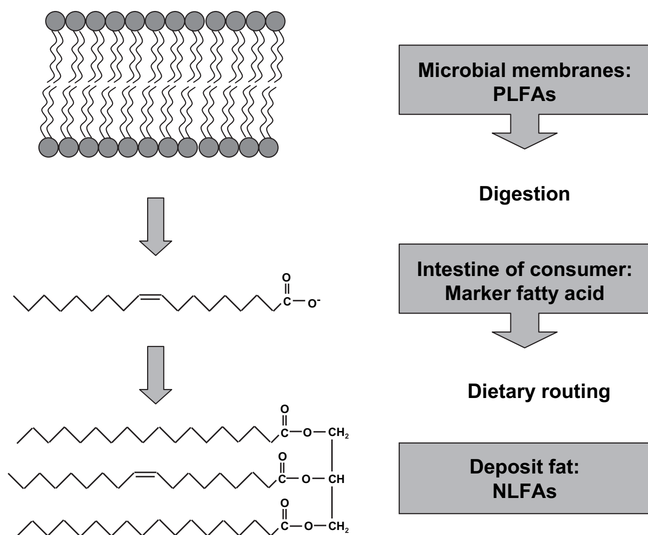

Fig. 2. Assimilation of marker fatty acids from phospholipids in microbial food sources into neutral lipids of decomposer animals.

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 (Olsson 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 polyunsaturated fatty acids with the first double bound 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 – nematodes – 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 3
Biomarker fatty acids for feeding strategies in Collembola (modified after Chamberlain et al., 2005; Ruess et al., 2005a). Note that the tritrophic transfer of marker fatty acids from microorganisms to microbial grazers and their predators can result in their occurrence in more than one trophic level. For biomarker type see chapter 2.3.2.

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

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).

Homeoviscous 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 fluidity of storage fats is modified according to ambient temperature to permit enzyme accessibility to energy reserves. Comparable effects of temperature on the composition of neutral and/or phospholipids were reported from entomopathogenic nematodes (Abu Hatab and Gaugler, 1997; Jagdale and Gordon, 1997), earthworms (Petersen and Holmstrup, 2000) and several insects (Overgaard et al., 2005). Although diet and consumer reside in the same habitat at comparable environmental conditions, organisms at different trophic levels may respond differently to temperature changes. Hence, future studies are necessary to improve and verify the applicability of fatty acids as indicators of feeding preferences under varying temperature.

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; Bååth, 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 NLFAs 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 Triatominae (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-Siessegger 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

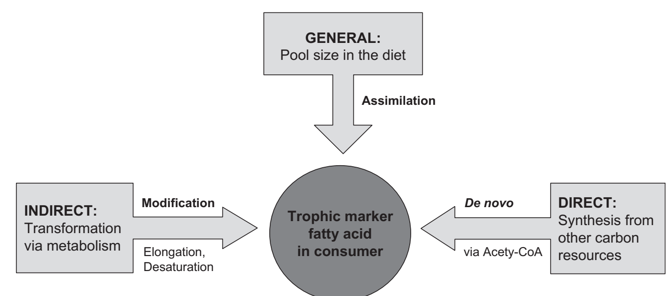

Fig. 3. Different origin of a trophic marker fatty acid detected in a consumer. The general diet pool can be superimposed by direct and indirect pools in the consumer. Note that for an absolute marker *de novo* synthesis does not apply.

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 CO_2 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 $\delta^{13}C$ differences (i.e. differences in the $^{13}C/^{12}C$ ratio, see Enleringer and Rundel, 1989) arising due to isotopic fractionations during C_3 and C_4 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 ^{13}C 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 C_3 to C_4 crops (Haubert et al., 2009, Table 2).

An alternative to using naturally occurring isotopic differences is to introduce a ^{13}C label into a food web and then examine the route of the ^{13}C-pulse through the web. This approach removes the need to consider isotopic fractionation, as the ^{13}C 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 ^{13}C-acetate (Boschker et al., 1998), $^{13}CH_4$ (Bull et al., 2000; Crossman et al., 2006; Knief et al., 2006; Maxfield et al., 2006), $^{13}CO_2$ (Lu et al., 2004) or ^{13}C-labelled plant residues (Williams et al., 2006, 2007); see Boschker and Middelburg (2002) and Evershed et al. (2006) for recent reviews. Isotopic pulse-labelling of plants has also been used to examine C transfer to soil decomposer with SIP applied to microbial PLFAs and bulk SIA to animals (Ostle et al., 2007; Ladygina et al., 2008). However, pulse-labelling studies tracing the ^{13}C 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 ^{13}C relative to bulk tissue (and exhibit 6–8‰ lower $\delta^{13}C$ 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 ^{12}C (DeNiro and Epstein, 1977). The magnitude of fractionation is additionally affected by the $\delta^{13}C$ values of sources used in acetyl-CoA production (Hayes, 1993). Biosynthesis also leads to intra-molecular differences in $\delta^{13}C$ 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 $\delta^{13}C$ values of these produced by enzymatic discrimination (Monson and Hayes, 1982a,b).

Besides this fractionation occurring in *de novo* synthesis the $\delta^{13}C$ 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 $\delta^{13}C$ 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 $\delta^{13}C$ 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 $\delta^{13}C$ 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 $\delta^{13}C$ 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 $\delta^{13}C$ 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 $\delta^{13}C$ values (Gaye-Siessegger et al., 2003).

Diet quality is a key factor for the balance between assimilation and biosynthesis and the resulting fatty acid $\delta^{13}C$ 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 Verhoef, 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 (Haubert et al., 2004) and $\delta^{13}C$ 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 $\delta^{13}C$ values of the compound may occur due to high uptake, whilst other fatty acids will still reflect the $\delta^{13}C$ values of the previous diet. Diet shift experiments in Collembola revealed half-lives of the rates of change in $\delta^{13}C$ values between 29 min (linoleic acid) and 14d (vaccenic 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-fumigation-extraction or DNA-based approaches (Leckie 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; Montogomery 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; Sampedro 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 in which dietary routing of biomarkers occurs (Haubert 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-acly 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 $\delta^{13}C$ but changes in $\delta^{15}N$ (Sotiropoulos et al., 2004; Post et al., 2007). However, the Bligh and Dyer extraction method has been reported to cause systematic errors for $\delta^{13}C$ 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 $\delta^{13}C$ values of the derivatised molecules, and the effect of this methyl group must later be taken into account when calculating the $\delta^{13}C$ value of the original fatty acid (Rieley, 1994; Abraham 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 volatilise 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 $\delta^{13}C$ values by GC-C-IRMS has an error of approx. 0.3‰ associated with ^{13}C 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 $\delta^{13}C$ 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 $\delta^{13}C$ 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 $\delta^{13}C$ values may be high, and consideration of carry-over effects, where the $\delta^{13}C$ values of compounds eluting after enriched components or in subsequent analyses are affected by residual of ^{13}C, 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 $\delta^{13}C$ 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 $\delta^{13}C$ 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 $\delta^{13}C$ 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 $\delta^{13}C$ 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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References

Abelson, P.H., Hoering, T.C., 1961. Carbon isotope fractionation in formation of amino acids by photosynthetic organisms. Proceedings of the National Academy of Sciences of the United States of America 47, 623–632.
Abraham, W.-R., Hesse, C., Pelz, O., 1998. Ratios of carbon isotopes in microbial lipids as an indicator of substrate usage. Applied Environmental Micobiology 64, 4202–4209.
Abraham, W.-R., Hesse, C., 2003. Isotope fractionations in the biosynthesis of cell components by different fungi: a basis for environmental carbon flux studies. FEMS Microbiology Ecology 46, 121–128.
Abu Hatab, M.A., Gaugler, A., 1997. Growth-mediated variations in fatty acids of *Xenorhabdus* sp. Journal of the Applied Microbiology 82, 351–358.
Abu Hatab, M.A., Gaugler, R., Ehlers, R.-U., 1998. Influence of culture method on *Steinernema glaseri* lipids. Journal of Parasitology 84, 215–221.
Addison, J.A., Trofymow, J.A., Marshall, V.G., 2003. Functional role of Collembola is successional coastal temperate forests on Vancouver Island, Canada. Applied Soil Ecology 24, 247–261.
Admassu, B., Juen, A., Traugott, M., 2006. Earthworm primers for DNA-based gut content analysis and their cross-reactivity in a multi-species system. Soil Biology & Biochemistry 38, 1308–1315.
Alvarez, H.M., Steinbüchel, A., 2002. Triacylglycerols in prokaryotic microorganisms. Applied Microbiological Biotechnology 60, 367–376.
Bååth, E., Söderström, B., 1979. The significance of hyphal diameter in calculation of fungal biovolume. Oikos 33, 11–14.
Bååth, E., 2003. The use of neutral lipid fatty acids to indicate the physiological conditions of soil fungi. Microbial Ecology 45, 373–383.
Bartscht, K., Cypriongka, H., Overmann, J., 1999. Evaluation of cell activity and of methods for the cultivation of bacteria from a natural lake community. FEMS Microbiology Ecology 28, 249–259.
Batista, A., Vetter, W., Luckas, B., 2001. Use of focused open vessel microwave-assisted extraction as prelude for the determination of the fatty acid profile of fish – a comparison with results obtained after liquid–liquid extraction according to Bligh and Dyer. European Food Research Technology 212, 377–384.
Bell, M.V., Tocher, D.R., 2009. Biosynthesis of polyunsaturated fatty acids in aquatic ecosystems: general pathways and new directions. In: Arts, M.T., Brett, M.T., Kainz, M. (Eds.), Lipids in Aquatic Ecosystems. Springer, Heidelberg, pp. 211–236.
Bengtsson, J., Setälä, H., Zheng, D.W., 1996. Food webs and nutrient cycling in soils: interactions and positive feedbacks. In: Polis, G.A., Winemiller, K.O. (Eds.), Food Webs: Integration of Patterns and Dynamics. Chapman and Hall, pp. 30–38.
Blair, N., Leu, A., Munoz, E., Olsen, J., Kwong, E., Desmarais, D., 1985. Carbon isotopic fractionation in heterotrophic microbial metabolism. Applied Environmental Microbiology 50, 996–1001.
Blem, C.R., 1976. Patterns of lipid storage and utilization in birds. American Zoologist 16, 671–684.
Blessing, M., Jochmann, M.A., Schmidt, T.C., 2008. Pitfalls in compound-specific isotope analysis of environmental samples. Analytical and Bioanalytical Chemistry 390, 591–603.
Bligh, E.G., Dyer, W.J., 1959. A rapid method for total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology 37, 911–917.
Boschker, H.T.S., Middelburg, J.J., 2002. Stable isotopes and biomarkers in microbial ecology. FEMS Microbiology Ecology 40, 85–95.

Boschker, H.T.S., Nold, S.C., Wellsbury, P., Bos, D., Graaf de, W., Pel, R., Parkes, R.J., Cappenberg, T.E., 1998. Direct linking of microbial populations to specific biogeochemical processes by ^{13}C-labelling of biomarkers. Nature 392, 801–805.

Brenna, J.T., Corso, T.N., Tobias, H.J., Caimi, R.J., 1997. High-precision continuous-flow isotope ratio mass spectrometry. Mass Spectrometry Reviews 16, 227–258.

Brondz, I., 2002. Development of fatty acid analysis by high-performance liquid chromatography, gas chromatography, and related techniques. Analytica Chimica Acta 465, 1–37.

Budge, S.M., Wooller, M.J., Springer, A.M., Iverson, S.J., McRoy, C.P., Divoky, G.J., 2008. Tracing carbon flow in an arctic marine food web using fatty acid-stable isotope analysis. Oecologia 157, 117–129.

Bull, I.D., Parekh, N.R., Hall, G.H., Ineson, P., Evershed, R.P., 2000. Detection and classification of atmospheric methane oxidizing bacteria in soil. Nature 405, 175–178.

Canavoso, L.E., Bertello, L.E., Lederkremer de, R.M., Rubiolo, E.R., 1998. Effects of fasting on the composition of the fat body lipid of *Dipetalogaster maximus, Triatoma infestans* and *Panstrongylus megistus* (Hemiptera: Reduviidae). Journal of Comparative Physiology 168, 549–554.

Chamberlain, P.M., Bull, I.D., Black, H.I.J., Ineson, P., Evershed, R.P., 2004. Lipid content and carbon assimilation in Collembola: implications for the use of compound-specific carbon isotope analysis in animal dietary studies. Oecologia 139, 325–335.

Chamberlain, P.M., Black, H.I.J., 2005. Fatty acid composition of Collembola: unusually high proportions of C20 polyunsaturated fatty acids in a terrestrial invertebrate. Comparative Biochemistry and Physiology 140, 299–307.

Chamberlain, P.M., Bull, I.D., Black, H.I.J., Ineson, P., Evershed, R.P., 2005. Fatty acid composition and change in Collembola fed differing diets: identification of trophic biomarkers. Soil Biology & Biochemistry 37, 1608–1624.

Chamberlain, P.M., Bull, I.D., Black, H.I.J., Ineson, P., Evershed, R.P., 2006a. Collembolan trophic preferences determined using fatty acid distributions and compound-specific stable carbon isotope values. Soil Biology & Biochemistry 38, 1275–1281.

Chamberlain, P.M., Bull, I.D., Black, H.I.J., Ineson, P., Evershed, R.P., 2006b. The effect of diet on isotopic turnover in Collembola examined using the stable carbon isotopic compositions of lipids. Soil Biology & Biochemistry 38, 1146–1157.

Chamberlain, P.M., McNamara, N.P., Chaplow, J., Stott, A.W., Black, H.I.J., 2006c. Translocation of surface litter carbon into soil by Collembola. Soil Biology & Biochemistry 38, 2655–2664.

Chauvat, M., Ponge, F.J., Wolters, V., 2007. Humus structure during a spruce forest rotation: quantitative changes and relationship to soil biota. European Journal of Soil Science 58, 625–631.

Chen, J., Ferris, H., Scow, K.M., Graham, K.J., 2001. Fatty acid composition and dynamics of selected fungal-feeding nematodes and fungi. Comparative Biochemistry and Physiology 130, 135–144.

Chen, Y., Dumont, M.G., McNamara, N.P., Chamberlain, P.M., Bodrossy, L., Stralis-Pavese, N., Murrell, J.C., 2008. Diversity of the active methanotropic community in acids peatlands as assessed by mRNA and SIP-PLFA analyses. Environmental Microbiology 10, 2609–2622.

Copley, M.S., Berstan, R., Dudd, S.N., Docherty, G., Mukherjee, A.J., Straker, V., Payne, S., Evershed, R.P., 2003. Direct chemical evidence for widespread dairying in prehistoric Britain. Proceedings of the National Academy of Sciences of the United States of America 100, 1524–1529.

Crawford, K., McDonald, R.A., Bearhop, S., 2008. Applications of stable isotope techniques to the ecology of mammals. Mammal Review 38, 87–107.

Cronan Jr., J.E., Gelmann, E.P., 1975. Physical properties of membrane lipids: biological relevance and regulation. Bacteriological Reviews 39, 232–256.

Crossman, Z.M., Wang, Z.P., Ineson, P., Evershed, R.P., 2006. Investigation of the effect of ammonium sulfate on populations of ambient methane oxidising bacteria by C-13-labelling and GC/C/IRMS analysis of phospholipid fatty acids. Soil Biology & Biochemistry 38, 983–990.

Daligault, F., Reed, D.W., Savile, C.K., Nugier-Chauvin, C., Patin, H., Covello, P.S., Buist, P.H., 2003. Mechanistic characterization of ω-3 desaturation in the green alga Chlorella vulgaris. Phytochemistry 63, 739–744.

DeNiro, M.J., Epstein, S., 1977. Mechanism of carbon isotope fractionation associated with lipid synthesis. Science 197, 261–263.

DeNiro, M.J., Epstein, S., 1978. Influence of diet on the distribution of carbon isotopes in animals. Geochimica et Cosmochimica Acta 42, 495–506.

DeNiro, M.J., Epstein, S., 1981. Influence of diet on the distribution of nitrogen isotopes in animals. Geochimica et Cosmochimica Acta 45, 341–351.

Dodds, E.D., McCoy, M.R., Rea, L.D., Kennish, J.M., 2005. Gas chromatographic quantification of fatty acid methyl esters: flame inonization detection vs. electron impact mass spectrometry. Lipids 40, 419–428.

Dowling, N.J.E., Widdel, F., White, D.C., 1986. Phospholipid ester-linked fatty acid biomarkers of acetate-oxidizing sulphate-reducers and other sulphide-forming bacteria. Journal of General Microbiology 132, 1815–1825.

Dungait, J.A.J., Briones, M.J.I., Bol, R., Evershed, R.P., 2008. Enhancing the understanding of earthworm feeding behaviour via the use of fatty acid δ^{13}C values determined by gas chromatography-combustion-isotope ratio mass spectrometry. Rapid Communications in Mass Spectrometry 22, 1643–1652.

Dunlap, K.R., Perry, J.J., 1967. Effect of substrate on the fatty acid composition of hydrocarbon-utilizing microorganisms. Journal of Bacteriology 94, 1919–1923.

Dunlap, K.R., Perry, J.J., 1968. Effects of substrate on the fatty acid composition of hydrocarbon- and ketone-utilizing microorganisms. Journal of Bacteriology 96, 318–321.

Dunstan, G.A., Volkman, J.K., Barrett, S.M., Leroi, J.-M., Jeffrey, S.W., 1994. Essential polyunsaturated fatty acids from 14 species of diatom (Bacillariophyceae). Phytochemistry 35, 155–161.

Dwyer, K.S., Parrish, C.C., Brown, J.A., 2003. Lipid composition of yellowtail flounder (*Limanda ferruginea*) in relation to dietary lipid intake. Marine Biology 143, 659–667.

Dzierzewicz, Z., Cwalina, B., Kurkiewicz, S., Chodurek, E., Wilczok, T., 1996. Intraspecies variability of cellular fatty acids among soil and intestinal strains of *Desulfovibrio desulfuricans*. Applied Environmental Microbiology 62, 3360–3365.

Ederington, M.C., McManus, G.B., Harvey, H.R., 1995. Trophic transfer of fatty acids, sterols, and a triterpenoid alcohol between bacteria, a ciliate, and the copepod *Acartia tonsa*. Limnology Oceanography 40, 860–867.

Enleringer, J.R., Rundel, P.W., 1989. Stable isotopes: history, units and instrumentation. In: Rundel, P.W., Enleringer, J.R., Magy, K.A. (Eds.), Ecological Studies 68: Stable Isotopes in Ecological Research. Springer Verlag, New York.

Erwin, J., 1973. Comparative biochemistry of fatty acids in eukaryotic microorganisms. In: Erwin, J.A. (Ed.), Lipids and Biomembranes of Eukaryotic Microorganisms. Academic Press, New York, pp. 42–143.

Evans, C.J., Evershed, R.P., Black, H.I.J., Ineson, P., 2003. Compound-specific stable isotope analysis of soil mesofauna using thermally assisted hydrolysis and methylation for ecological investigations. Analytical Chemistry 75, 6056–6062.

Evershed, R.P., Crossmann, Z.M., Bull, I.D., Mottram, H., Dungait, J.A.J., Maxfield, P.J., Brennand, E.L., 2006. ^{13}C-labelling of lipids to investigate microbial communities in the environment. Current Opinion in Biotechnology 17, 72–82.

Frostegård, A., Tunlid, A., Bååth, E., 1991. Microbial biomass measured as total lipid phosphate in soils of different organic content. Journal of Microbiological Methods 14, 151–163.

Frostegård, A., Bååth, E., Tunlid, A., 1993. Shifts in the structure of soil microbial communities in limed forests as revealed by phospholipid fatty acid analysis. Soil Biology & Biochemistry 25, 723–730.

Frostegård, A., Bååth, E., 1996. The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. Biology Fertility Soils 22, 59–65.

Gannes, L.Z., ÓBrien, D.M., Rio del, C.M., 1997. Stable isotopes in animal ecology: assumptions, caveats, and a call for more laboratory experiments. Ecology 78, 1271–1276.

Gannes, L.Z., Rio del, C.M., Koch, P., 1998. Natural abundance variations in stable isotopes and their potential uses in animal physiological ecology. Comparative Biochemistry and Physiology A 119, 725–737.

Gaye-Siessegger, J., Focken, U., Abel, H.J., Becker, K., 2003. Feeding level and diet quality influence trophic shift of C and N isotopes in Nile tilapia (*Oreochromis niloticus* (L.)). Isotopes Environmental Health Studies 39, 125–134.

Gaye-Siessegger, J., Focken, U., Abel, H.-J., Becker, K., 2004a. Dietary lipid content influences the activity of lipogenetic enzymes in the liver and on whole body δ^{13}C values of Nile tilapia, *Oreochromis nilaticus* (L.). Isotopes Environmental Health Studies 40, 181–190.

Gaye-Siessegger, J., Focken, U., Muetzel, S., Abel, H., Becker, K., 2004b. Feeding level and individual metabolic rate affect delta C-13 and delta N-15 values in carp: implications for food web studies. Oecologia 138, 175–183.

Gratton, C., Forbes, A.E., 2006. Changes in δ^{13}C stable isotopes in multiple tissues of insect predators fed isotopically distinct prey. Oecologia 147, 615–624.

Guckert, J.B., Ringelberg, D.B., White, D.C., Hanson, R.S., Bratina, B.J., 1991. Membrane fatty acids as phenotypic markers in the polyphasic taxonomy of methylotrophs within the Proteobacteria. Journal of General Microbiology 137, 2631–2641.

Hammer, B.T., Fogel, M.L., Hoering, T.C., 1998. Stable carbon isotope ratios of fatty acids in seagrass and redhead ducks. Chemical Geology 152, 29–41.

Hammonds, P., Smith, S.N., 1986. Lipid composition of a psychrophilic, a mesophilic and a thermophilic *Mucor* species. Transactions of the British Mycological Society 86, 551–560.

Harwood, J.L., Russell, N.J., 1984. Lipids in Plants and Microbes. George Allen and Unwin Ltd, London.

Haubert, D., Häggblom, M.M., Scheu, S., Ruess, L., 2004. Effects of fungal food quality and starvation on the fatty acid composition of *Protaphorura fimata* (Collembola). Comparative Biochemistry and Physiology B 138, 41–52.

Haubert, D., Häggblom, M.M., Langel, R., Scheu, S., Ruess, L., 2006. Trophic shift of stable isotopes and fatty acids in Collembola on bacterial diets. Soil Biology & Biochemistry 38, 2004–2007.

Haubert, D., Häggblom, M.M., Scheu, S., Ruess, L., 2008. Effects of temperature and life stage on the fatty acid composition of Collembola. European Journal of Soil Biology 44, 213–219.

Haubert, D., Birkhofer, K., Fließbach, A., Gehre, M., Scheu, S., Ruess, L., 2009. Trophic structure and major trophic links in conventional versus organic farming systems as indicated by carbon stable isotope ratios of fatty acids. Oikos 118, 1579–1589.

Hayes, J.M., 1993. Factors controlling ^{13}C contents of sedimentary organic compounds – principles and evidence. Marine Geology 113, 111–125.

Hayes, J.M., Freeman, K.H., Popp, B.N., Hoham, C.H., 1990. Compound-specific isotopic analyses – a novel tool for reconstruction of ancient biogeochemical processes. Organic Geochemistry 16, 1115–1128.

Hayes, J.M., 2001. Fractionation of carbon and hydrogen isotopes in biosynthetic processes. Stable Isotope Geochemistry 43, 225–277.

Heipieper, H.J., Waard de, P., Meer van der, P., Killian, J.A., Isken, S., Bont de, J.A.M., Eggink, G., 2001. Regiospecific effect of 1-octanol on cis-trans isomerization of unsaturated fatty acids in the solvent-tolerant strain *Pseudomonas putida* S12. Applied Microbiology Biotechnology 57, 541–547.

Heipieper, H.J., Meinhardt, F., Segura, A., 2003. The cis-trans isomerase of unsaturated fatty acids in *Pseudomonas* and *Vibrio*: biochemistry, molecular biology

and physiological function of a unique stress adaptive mechanism. FEMS Microbiology Letters 229, 1–7.
Hobbie, E.A., Werner, R.A., 2004. Intramolecular, compound-specific, and bulk carbon isotope patterns in C_3 and C_4 plants: a review and synthesis. New Phytologist 161, 371–385.
Holmstrup, M., Hedlund, K., Boriss, H., 2002. Drought acclimation and lipid composition in *Folsomia candida*: implications for cold shock, heat shock and acute desiccation stress. Journal of Insect Physiology 48, 861–970.
Holz, R.A., Troth, K., Atkinson, H.J., 1999. The influence of potato cultivar on lipid content and fecundity of Bolivian and British populations of *Globodera rostochiensis*. Journal of Nematology 31, 357–366.
IUPAC-IUB Commission on Biochemical Nomenclature, 1978. The nomenclature of lipids. Biochemistry Journal 171, 21–35.
Jagdale, G.B., Gordon, R., 1997. Effects of temperature on the composition of fatty acids in total lipids and phospholipids of entomopathogenic nematodes. Journal of Thermal Biology 22, 245–251.
Joergensen, R.G., Emmerling, C., 2006. Methods for evaluating human impact on soil microorganisms based on their activity, biomass, and diversity in agricultural soils. Journal of Plant Nutrition Soil Science 169, 295–329.
Joergensen, R.G., Wichern, F., 2008. Quantitative assessment of the fungal contribution to microbial tissue in soil. Soil Biology & Biochemistry 40, 2977–2991.
Juen, A., Traugott, M., 2005. Detecting predation and scavenging by DNA gut-content analysis: a case study using a soil insect predatory–prey system. Oecologia 142, 344–352.
Juen, A., Traugott, M., 2006. Amplification facilitators and multiplex PCR: tools to overcome PCR-inhibition in DNA-gut-content analysis of soil-living invertebrates. Soil Biology & Biochemistry 38, 1872–1879.
Kaneda, T., 1991. Iso- and anteiso-fatty acids in bacteria: biosynthesis, function, and taxonomic significance. Microbiological Reviews 55, 288–302.
Karowe, D.N., Martin, M.M., 1989. The effects of quantity and quality of diet nitrogen on the growth, efficiency of food utilization, nitrogen budget, and metabolic rate of fifth-instar *Spodoptera eridania* Larvae (Lepidoptera: Noctuidae). Journal of Insect Physiology 35, 699–708.
Kattner, G., Albers, C., Graeve, M., Schnack-Schiel, S.B., 2003. Fatty acid and alcohol composition of the small polar copepods, *Oithona* and *Oncaea*: indication of feeding modes. Polar Biology 26, 666–6671.
Kerger, B.D., Nichols, P.D., Antworth, C.P., Sand, W., Bock, E., Cox, J.C., Langworthy, T.A., White, D.C., 1986. Signature fatty acids in the polar lipids of acid-producing *Thiobacillus* spp.: methoxy, cyclopropyl, alpha-hydroxy-cyclopropyl, branched and normal monoenoic fatty acids. FEMS Microbiology Ecology 38, 67–77.
Keweloh, H., Heipieper, H.H., 1996. Trans unsaturated fatty acids in bacteria. Lipids 31, 129–137.
Kieft, T.L., Ringelberg, D.B., White, D.C., 1994. Changes in ester-linked phospholipid fatty acid profiles of subsurface bacteria during starvation and desiccation in a porous medium. Applied and Environmental Microbiology 60, 3292–3299.
Klamer, M., Bååth, E., 2004. Estimation of conversion factors for fungal biomass determination in compost using ergosterol and PLFA 18:2ω6,9. Soil Biology & Biochemistry 36, 57–65.
Knief, C., Kolb, S., Bodelier, P.L.E., Lipski, A., Dunfield, P.F., 2006. The active methanotrophic community in hydromorphic soils changes in response to changing methane concentration. Environmental Microbiology 8, 321–333.
Krusberg, L.R., 1972. Fatty acid composition of *Turbatrix aceti* and its culture medium. Comparative Biochemistry and Physiology 41, 89–98.
Ladygina, N., Caruso, T., Hedlund, K., 2008. Dietary switching of collembola in grassland soil food webs. Soil Biology & Biochemistry 40, 2898–2903.
Lavy, D., Verhoef, H.A., 1996. Effects of food quality on growth and body composition of the collembolan *Orchesella cincta*. Physiological Entomology 21, 64–70.
Leake, J.R., Ostle, N.J., Rangel-Castro, J.I., Johnson, D., 2006. Carbon fluxes from plants through soil organisms determined by field $^{13}CO_2$ pulse-labelling in an upland grassland. Applied Soil Ecology 33, 152–175.
Lechevalier, H., Lechevalier, M.P., 1988. Chemotaxonomic use of lipids – an overview. In: Ratledge, C., Wilkinson, S.G. (Eds.), Microbial Lipids. Academic Press, London.
Lee, A.K.Y., Chan, C.K., Fang, M., Lau, A.P.S., 2004. The 3-hydroxy fatty acids as biomarkers for quantification and characterization of endotoxins and Gram-negative bacteria in atmospheric aerosols in Hong Kong. Atmospheric Environment 38, 6307–6317.
Leckie, S.E., Prescott, C.E., Grayston, S.J., Neufeld, J.D., Mohn, W.W., 2004. Comparison of chloroform fumigation-extraction, phospholipid fatty acid, and DNA methods to determine microbial biomass in forest humus. Soil Biology & Biochemistry 36, 529–532.
Lu, Y., Murase, J., Watanbe, A., Sugimoto, A., Kimura, M., 2004. Linking microbial community dynamics to rhizosphere carbon flow in a wetland rice soil. FEMS Microbiology Ecology 48, 179–186.
Lueders, T., Wagner, B., Claus, P., Friedrich, M.W., 2004. Stable isotope probing of rRNA and DNA reveals a dynamic methylotroph community and trophic interactions with fungi and protozoa in oxic rice field soil. Environmental Microbiology 6, 60–72.
Lueders, T., Kindler, R., Miltner, A., Friedrich, M.W., Kaestner, M., 2006. Identification of bacterial micropredators distinctively active in a soil microbial food web. Applied Environmental Microbiology 72, 5342–5348.
MacAvoy, S., Macko, S.A., Carney, R.S., 2003. Links between chemosynthetic production and mobile predators on the Louisiana continental slope: stable carbon isotopes of specific fatty acids. Chemical Geology 201, 229–237.
Madan, R., Pankhurst, C., Hawke, B., Smith, S., 2002. Use of fatty acids for identification of AM fungi and estimation of the biomass of AM spores in soil. Soil Biology & Biochemistry 34, 125–128.
Manefield, M., Whiteley, A.S., Griffiths, R.I., Bailey, M.J., 2002. RNA stable isotope probing, a novel means of linking microbial community function to phylogeny. Applied Environmental Microbiology 68, 5367–5373.
Maraun, M., Martens, H., Migge, S., Theenhaus, A., Scheu, S., 2003. Adding to the enigma of soil animal diversity - fungal feeders and saprophagous soil invertebrates prefer similar food substrates. European Journal of Soil Biology 39, 85–95.
Maxfield, P.J., Hornibrook, E.R.C., Evershed, R.P., 2006. Estimating high-affinity methanotrophic bacterial biomass, growth, and turnover in soil by phospholipid fatty acid C-13 labeling. Applied Environmental Microbiology 72, 3901–3907.
Meier-Augenstein, W., Watt, P.W., Langhans, C.D., 1996. Influence of gas chromatographic parameters on measurement of C-13/C-12 isotope ratios by gas-liquid chromatography combustion isotope ratio mass spectrometry .1. Journal Chromatography A 752, 233–241.
Meier-Augenstein, W., 1999. Applied gas chromatography coupled to isotope ratio mass spectrometry. Journal Chromatography A 842, 351–371.
Meier-Augenstein, W., 2002. Stable isotope analysis of fatty acids by gas chromatography- isotope ratio mass spectrometry. Analytica Chimica Acta 465, 63–79.
Mikola, J., Setälä, H., 1999. Interplay of omnivory, energy channels and C availability in a microbial-based soil food web. Biology Fertility Soils 28, 212–218.
Millar, A.A., Smith, M.S., Kunst, L., 2000. All fatty acids are not equal: discrimination in plant membrane lipids. Trends in Plant Science 5, 95–101.
Mirza, M.S., Janse, J.D., Hahn, D., Akkermans, A.D.L., 1991. Identification of atypical *Frankia* strains by fatty acid analysis. FEMS Microbiology Letters 83, 91–98.
Molkentin, J., Goesemann, A., 2007. Differentiation of organically and conventionally produced milk by stable isotope and fatty acid analysis. Analytical and Bioanalytical Chemistry 388, 297–305.
Monson, K.D., Hayes, J.M., 1982a. Biosynthetic control of the natural abundance of ^{13}C at specific positions within fatty acids in *Saccharomyces cerevisiae* – isotopic fractionations in lipid synthesis as evidence for peroxisomal regulation. Journal of Biological Chemistry 257, 5568–5575.
Monson, K.D., Hayes, J.M., 1982b. Carbon isotopic fractionation in the biosynthesis of bacterial fatty acids – ozonolysis of unsaturated fatty acids as a means of determining the intramolecular distribution of carbon isotopes. Geochimica et Cosmochimica Acta 46, 139–149.
Montogomery, H.J., Monreal, C.M., Young, J.C., Seifert, K.A., 2000. Determination of soil fungal biomass from soil ergosterol analyses. Soil Biology & Biochemistry 32, 1207–1217.
Moor-Kucera, J., Dick, R.P., 2008. Application of ^{13}C-labeled litter and root materials for *in situ* decomposition studies using phospholipid fatty acids. Soil Biology & Biochemistry 40, 2485–2493.
Moore, T.S., 1993. Lipid Metabolism in Plants. CRC Press, Boca Raton.
Morgan, J.A.W., Winstanley, C., 1997. Microbial biomarkers. In: Elsas, J.D. van, Trevors, J.T., Wellington, E.M.H. (Eds.), Modern Soil Microbiology, pp. 331–352.
Mottram, H.R., Evershed, R.P., 2003. Practical considerations in the gas chromatography/combustion/isotope ratio monitoring mass spectrometry of ^{13}C-enriched compounds: detection limits and carryover effects. Rapid Communications in Mass Spectrometry 17, 2669–2674.
Mulder, C., 2006. Driving forces from soil invertebrates to ecosystem functioning: the allometric perspective. Naturwissenschaften 93, 467–479.
Müller-Navarra, D.C., Brett, M.T., Liston, A.M., Goldman, C.R., 2000. A highly unsaturated fatty acid predicts carbon transfer between primary producers and consumers. Nature 403, 74–77.
Newsham, K.K., Rolf, J., Pearce, D.A., Strachan, R.J., 2004. Differing preferences of Antarctic soil nematodes for microbial prey. European Journal of Soil Biology 40, 1–8.
Ngosong, C., Raupp, J., Scheu, S., Ruess, L., 2009. Low importance for a fungal based food web in arable soils under mineral and organic fertilization indicated by Collembola grazers. Soil Biology & Biochemistry 41, 2308–2317.
Nichols, P.D., Stulp, B.K., Jones, J.G., White, D.C., 1986. Comparison of fatty acid content and DNA homology of filamentous gliding bacteria *Vitreoscilla*, *Flexibacter* and *Filibacter*. Archives of Microbiology 146, 1–6.
Nielsen, P., Petersen, S.O., 2000. Ester-linked polar lipid fatty acid profiles of soil microbial communities: a comparison of extraction methods and evaluation of interference from humic acids. Soil Biology & Biochemistry 32, 1241–1249.
Nishida, I., Murata, N., 1996. Chilling sensitivity in plants and cyanobacteria: the crucial contribution of membrane lipids. Annual Review of Plant Physiology 47, 541–568.
Nottingham, A.T., Griffiths, H., Chamberlain, P.M., Stott, A.W., Tanner, E.V.J., 2009. Soil priming by sugar and leaf-litter substrates: a link to microbial groups. Applied Soil Ecology 42, 183–190.
Olsson, P.A., 1999. Signature fatty acids provide tools for determination of the distribution and interactions of mycorrhizal fungi in soil. FEMS Microbiology Ecology 29, 303–310.
Olsson, P.A., Larsson, L., Bago, B., Wallander, H., Arle van, I.M., 2003. Ergosterol and fatty acids for biomass estimation of mycorrhizal fungi. New Phytologist 159, 1–10.
Olsson, P.A., Bååth, E., Jakobsen, I., Söderström, B., 1995. The use of phospholipid and neutral lipid fatty acids to estimate biomass of arbuscular mycorrhizal fungi in soil. Mycological Research 99, 623–629.

Olsson, P.A., Johnson, N.C., 2005. Tracking carbon from the atmosphere to the rhizopshere. Ecology Letters 8, 1264–1270.

Ostle, N., Briones, M.J.I., Ineson, P., Cole, L., Staddon, P., Sleep, D., 2007. Isotopic detection of recent photosynthate carbon flow into grassland rhizosphere fauna. Soil Biology & Biochemistry 39, 768–777.

Ostrom, P.H., Colunga-Garcia, M., Gage, S.H., 1997. Establishing pathways of energy flow for insect predators using stable isotope ratios: field and laboratory evidence. Oecologia 109, 108–113.

Overgaard, J., Sorensen, J.G., Petersen, S.O., Loeschcke, V., Holmstrupp, M., 2005. Changes in membrane lipid composition following rapid cold hardening in *Drosophila melanogaster*. Journal of Insect Physiology 51, 1173–1182.

Park, R., Epstein, S., 1961. Metabolic fractionation of ^{13}C and ^{12}C in plants. Plant Physiology 36, 133–138.

Petersen, S., Holmstrup, M., 2000. Temperature effects on the lipid composition of the earthworms *Lumbricus rubellus* and *Eisenia nordenskioeldi*. Soil Biology & Biochemistry 32, 1787–1791.

Pombo, S.A., Pelz, O., Schroth, M.H., Zeyer, J., 2002. Field scale ^{13}C labeling of phospholipid fatty acids (PLFA) and dissolved inorganic carbon: tracing acetate assimilation and mineralization in a petroleum hydrocarbon-contaminated aquifer. FEMS Microbiology Ecology 41, 259–267.

Pombo, S.A., Kleikemper, J., Schroth, M.H., Zeyer, J., 2005. Field-scale isotopic labelling of phospholipid fatty acids from acetate-degrading sulfate-reducing bacteria. FEMS Microbiology Ecology 51, 197–207.

Pond, C.M., 1981. Storage. In: Townshend, C.R., Calow, P. (Eds.), Physiological Ecology: An Evolutionary Approach to Resource Use. Blackwell, London, pp. 190–219.

Pond, D.W., Leakey, R.J.G., Fallick, A.E., 2006. Monitoring microbial predator–prey interactions: an experimental study using fatty acid biomarker and compound-specific stable isotope techniques. Journal of Plankton Research 28, 419–427.

Ponge, J.-F., 2000. Vertical distribution of Collembola (Hexapoda) and their food resources in organic horizons of beech forests. Biology Fertility Soils 32, 508–522.

Ponsard, S., Arditi, R., 2000. What can stable isotopes (δ^{15}N and δ^{13}C) tell about the food web of soil macro-invertebrates? Ecology 81, 852–864.

Post, D.M., 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology 83, 703–718.

Post, D.M., Layman, C.A., Arrington, D.A., Takimoto, G., Quattrochi, J., Montaña, C.G., 2007. Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. Oecologia 152, 179–189.

Radajweski, S., Ineson, P., Parekh, N.R., Colin Murell, J., 2000. Stable isotope probing as a tool in microbial ecology. Nature 403, 646–649.

Ramsey, P.W., Rillig, M.C., Feris, K.P., Holben, W.E., Gannon, J.E., 2006. Choice of methods for soil microbial community analysis: PLFA maximises power compared to CLPP and PCR-based approaches. Pedobiologia 50, 275–280.

Ratledge, C., Wilkinson, S.G., 1988. Microbial Lipids, vol. 1. Academic Presss, London.

Reubens, B., Poesen, J., Danjon, F., Geudens, G., Muys, B., 2007. The role of fine and coarse roots in shallow slope stability and soil erosion control with a focus on root architecture: a review. Trees 21, 385–402.

Rieley, G., 1994. Derivatization of organic compounds prior to gas-chromatographic combustion-isotope ratio mass-spectrometric analysis – identification of isotope fractionation processes. Analyst 119, 915–919.

Ringelberg, D.B., Davis, J.D., Smith, G.A., Pfiffner, S.M., Nichols, P.D., Nickels, J.S., Henson, J.M., Wilson, J.T., Yates, M., Kampbell, D.H., Read, H.W., Stocksdale, T.T., White, D.C., 1989. Validation of signature polarlipid fatty acid biomarkers for alkane-utilizing bacteria in soils and subsurface aquifer materials. FEMS Microbiology Ecology 62, 39–50.

Rio, del, C.M., Wolf, N., Carleton, S.A., Gannes, L.Z., 2009. Isotopic ecology ten years after a call for more laboratory experiments. Biological Reviews 84, 91–111.

Rosenfeld, J.M., 2002. Application of analytical derivatizations to the quantitative and qualitative determination of fatty acids. Analytica Chimica Acta 465, 93–100.

Rothstein, M., Götz, P., 1968. Biosynthesis of fatty acids in the free-living nematode *Turbatrix aceti*. Archives of Biochemistry and Biophysics 126, 131–140.

Ruess, L., García Zapata, E.J., Dighton, J., 2000. Food preferences of a fungal-feeding *Aphelenchoides* species. Nematology 2, 223–230.

Ruess, L., Häggblom, M.M., Grazía Zapata, E.J., Dighton, J., 2002. Fatty acids of fungi and nematodes - possible biomarkers in the soil food chain? Soil Biology & Biochemistry 34, 745–756.

Ruess, L., Langel, R., Häggblom, M.M., Scheu, S., 2004. Nitrogen isotope ratios and fatty acids composition as indicators of animal diet in belowground systems. Oecologia 139, 336–346.

Ruess, L., Schütz, K., Haubert, D., Häggblom, M.M., Kandeler, E., Scheu, S., 2005a. Application of lipid analysis to understand trophic interactions in soil. Ecology 86, 2075–2082.

Ruess, L., Tiunov, A., Haubert, D., Richnow, H.H., Häggblom, M.M., Scheu, S., 2005b. Carbon stable isotope fractionation and trophic transfer of fatty acids in fungal based soil food chains. Soil Biology & Biochemistry 37, 945–954.

Ruess, L., Schütz, K., Migge-Kleian, S., Häggblom, M.M., Kandeler, E., Scheu, S., 2007. Lipid composition of Collembola and their food resources in deciduous forest stands - implications for feeding strategies. Soil Biology & Biochemistry 39, 1990–2000.

Sakamoto, K., Iijima, R., Higuchi, R., 2004. Use of specific phospholipid fatty acids for identifying and quantifying the external hyphae of the arbusbular mycorrhizal fungus *Gigaspora rosea*. Soil Biology & Biochemistry 36, 1827–1834.

Sampedro, L., Jeannotte, R., Whalne, J.K., 2006. Trophic transfer of fatty acids from gut microbiota to the earthworm *Lumbricus terrestris* L. Soil Biology & Biochemistry 38, 2188–2198.

Saraf, A., Park, J.-H., Milton, D.K., Larrson, L., 1999. Use of quadrupole GC-MS and ion trap GC-MS-MS for determining 3-hydroxy fatty acids in settled house dust: relation to endotoxin activity. Journal of Environmental Monitoring 1, 163–168.

Sargent, J.R., Bell, M.V., Bell, J.G., Henderson, R.J., Tocher, D.R., 1995. Origins and functions of (n−3) polyunsaturated fatty acids in marine organisms. In: Ceve, G., Paltauf, F. (Eds.), Phospholipids: Characterization, Metabolism and Novel Biological Applications. American Chemical Society, pp. 248–259.

Scheu, S., Falca, M., 2000. The soil food web of two beech forests (*Fagus sylvatica*) of contrasting humus type: stable isotope analysis of a macro- and mesofauna-dominated community. Oecologia 123, 285–296.

Scheu, S., 2002. The soil food web: structure and perspectives. European Journal of Soil Biology 38, 11–20.

Scheu, S., Setälä, H., 2002. Multitrophic interactions in decomposer food-webs. In: Tscharntke, T., Hawkins, B.A. (Eds.), Multitrophic Level Interactions. Cambridge University Press, Cambridge, pp. 223–264.

Scheu, S., Simmerling, F., 2004. Growth and reproduction of fungal feeding Collembola as affected by fungal species, melanin and mixed diets. Oecologia 139, 347–353.

Scheu, S., Ruess, L., Bonkowski, M., 2005. Interactions between microorganisms and soil micro- and mesofauna. In: Buscot, F., Varma, A. (Eds.), Microorganisms in Soils: Roles in Genesis and Functions. Springer, Berlin, pp. 253–275.

Schlechtriem, C., Focken, U., Becker, K., 2003. Effects of different lipid extraction methods on δ^{13}C of lipid and lipid-free fractions of fish and different fish feeds. Isotopes Environmental Health Studies 39, 135–140.

Schmidt, T.C., Zwank, L., Elsner, M., Berg, M., Meckenstock, R.U., Haderlein, S.B., 2004. Compound-specific stable isotope analysis of organic contaminants in natural environments: a critical review of the state of the art, prospects, and future challenges. Analytical and Bioanalytical Chemistry 378, 283–300.

Schmitt, J., Glaser, B., Zech, W., 2003. Amount-dependent isotopic fractionation during compound-specific isotope analysis. Rapid Communications in Mass Spectrometry 17, 970–977.

Schoeller, D.A., 1999. Isotope fractionation: why aren't we what we eat? Journal of Archaeological Science 26, 667–673.

Sessions, A.L., 2006. Isotope-ratio detection for gas chromatography. Journal of Separation Science 29, 1946–1961.

Shapiro, B., 1967. Biochemistry of triacylglycerides. In: Schettler, C. (Ed.), Lipids and Lipidoses. Springer, Berlin, pp. 40–59.

Simpson, S.J., Simpson, C.L., 1990. The mechanisms of nutritional compensation by phytophagous insects. In: Bernays, I.I.E.A. (Ed.), Insect–Plant interactions. CRC Press, Boca Raton, pp. 112–160.

Sotiropoulos, M.A., Tonn, W.M., Wassenaar, L.I., 2004. Effects of lipid extraction on stable carbon and nitrogen isotope analyses of fish tissues: potential consequences for food web studies. Ecology Freshwater Fish 13, 155–160.

Sprecher, H., Luthria, D.L., Mohammed, B.S., Baykousheva, S.P., 1995. Reevaluation of the pathways for the biosynthesis of polyunsatrurated fatty acids. Journal of Lipid Research 36, 2471–2477.

Staddon, P.L., 2004. Carbon isotopes in functional soil ecology. Trends in Ecology & Evolution 19, 148–154.

Stanley-Samuelson, D.W., Jurenka, R.A., Cripps, C., Blomquist, G.J., Derenobales, M., 1988. Fatty acids in insects – composition, metabolism, and biological significance. Archives of Insect Biochemistry Physiology 9, 1–33.

Stanley-Samuelsen, D.W., Nelson, D.R., 1993. Insect Lipids, Chemistry, Biochemistry and Biology. University of Nebraksa Press, Lincoln and London.

Stevens, C.J., Deibel, D., Parrish, C.C., 2004a. Species-specific differences in lipid composition and omnivory indices in Arctic copepods collected in deep water during autumn (North Water Polynya). Marine Biology 144, 905–915.

Stevens, C.J., Deibel, D., Parrish, C.C., 2004b. Incorporation of bacterial fatty acids and changes in a wax-ester based omnivory index during a long-term incubation experiment with *Calanus glacilis* Jaschnov. Journal of Experimental Marine Biology and Ecology 303, 135–156.

Stott, A.W., Davies, E., Evershed, R.P., 1997. Monitoring the routing of dietary and biosynthesised lipids through compound specific isotope (δ^{13}C) measurements at natural abundance. Naturwissenschaften 84, 82–86.

Stott, A.W., Evershed, R.P., Jim, S., Jones, V., Rogers, J.M., Tuross, N., Ambrose, S., 1999. Cholesterol as a new source of palaeodietary information: experimental approaches and archaeological applications. Journal of Archaeological Science 26, 705–716.

Stübing, D., Hagen, W., Schmidt, K., 2003. On the use of lipid biomarkers in marine food web analyses: An experimental case study on the Antarctic krill, *Euphausia superba*. Limnology Oceanography 48, 1685–1700.

Thurnhofer, S., Vetter, W., 2005. A gas chromatography/electron ionization-mass spectrometry-selected ion monitoring method for determining the fatty acid pattern in food after formation of fatty acid methyl esters. Journal of Agriculture Food Chemistry 53, 8896–8903.

Tiunov, A.V., 2007. Stable isotopes of carbon and nitrogen in soil ecological studies. Biology Bulletin 34, 395–407.

Tunlid, A., White, D.C., 1990. Use of lipid biomarkers in environmental samples. In: Fox, A., Morgan, S.L., Larsson, L., Odham, G. (Eds.), Analytical Microbiology Methods, Chromatography and Mass Spectrometry. Plenum Press, New York.

Tunlid, A., White, D.C., 1992. In: Stotzky, G., Bollag, J.-M. (Eds.), Biochemical analysis of biomass, community structure, nutritional status, and metabolic activity of microbial communities in soil. Soil Biochemistry 7, 229–262.

Vestal, J.R., White, D.C., 1989. Lipid analysis in microbial ecology. BioScience 39, 535–541.
Vetter, W., Thurnhofer, S., 2007. Analysis of fatty acids by mass spectrometry in selected ion monitoring mode. Lipid Technology 19, 184–186.
Wakeham, S.G., Pease, T.K., Benner, R., 2003. Hydroxy fatty acids in marine dissolved organic matter as indicators of bacterial membrane material. Organic Geochemistry 34, 857–868.
Watzinger, A., Stemer, M., Pfeffer, M., Rasche, F., Reichenauer, T.G., 2008. Methanopropic communities in a landfill cover soil as revealed by ^{13}C PLFAs and respiratory quinones: impact of high methane addition and landfill irrigation. Soil Biology & Biochemistry 40, 751–762.
Webb, S.C., Hedges, R.E.M., Simpson, S.J., 1998. Diet quality influences the δ^{13}Cand δ^{15}N of locusts and their biochemical components. Journal of Experimental Biology 201, 2903–2911.
Weete, J.D., 1980. Lipid Biochemistry of Fungi and other Organisms. Plenum Press, New York, 357 pp.
Welch, D.F., 1991. Applications of cellular fatty acid analysis. Clinical Microbiology Reviews 4, 422–438.
Westhuizenvan der, J.P.J., Kock, J.L.F., Botha, A., Botes, P.J., 1994. The distribution of the ω3 and ω6 series of cellular long-chain fatty acids in fungi. Systematic Applied Microbiology 17, 327–345.
White, D.C., Stair, J.O., Ringelberg, D.B., 1996. Quantitative comparisons of in situ microbial biodiversity by signature biomarker analysis. Journal of Industrial Microbiology 17, 185–196.
White, D.C., Ringelberg, D.B., 1998. Signature lipid biomarker analysis. In: Burlage, R.S., Atlas, R., Stahl, D., Geesey, G., Sayler, G. (Eds.), Techniques in Microbial Ecology. Oxford University Press, Oxford, pp. 255–272.
Whiteley, A.S., Manefield, M., Lueders, T., 2006. Unlocking the 'microbial black box' using RNA-based stable isotope probing technologies. Current Opinion in Biotechnology 17, 67–71.
Williams, M.A., Myrold, D.D., Bottomley, P.J., 2006. Carbon flow from C-13-labeled straw and root residues into the phospholipid fatty acids of a soil microbial community under field conditions. Soil Biology & Biochemistry 38, 759–768.
Williams, M.A., Myrold, D.D., Bottomley, P.J., 2007. Carbon flow from C-13-labeled clover and ryegrass residues into a residue-associated microbial community under field conditions. Soil Biology & Biochemistry 39, 819–822.
Wong, W.W., Hachey, D.L., Zhang, S., Clarke, L.L., 1995. Accuracy and precision of gas-chromatography combustion isotope ratio mass-spectrometry for stable carbon-isotope ratio measurements. Rapid Communications in Mass Spectrometry 9, 1007–1011.
Young, J.C., 1995. Microwave-assisted extraction of fungal metabolite ergosterol and total fatty acids. Journal of Agriculture Food Chemistry 43, 2904–2910.
Zelles, L., 1997. Phospholipid fatty acid profiles in selected members of soil microbial communities. Chemosphere 35, 275–294.
Zelles, L., 1999. Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: a review. Biology Fertility Soils 29, 111–129.