16 Empirical Methods of Identifying and Quantifying Trophic Interactions for Constructing Soil Food-Web Models

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16.1 Introduction

Food-web models, which depict the trophic relationships between organisms within a community, form a powerful and versatile approach to study the relationships between community structure and ecosystem functioning. Although food-web models have recently been applied to a wide range of ecological studies (Memmott, 2009; Sanders *et al.*, 2014), such approaches can be greatly improved by introducing high-resolution trophic information from empirical studies and experiments that realistically describe topological structure and energy flows (de Ruiter *et al.*, 2005). Over the last decades major technological advances have been made in empirically characterizing trophic networks by describing, in detail, the connectedness and flows in food webs. Existing empirical techniques, such as stable isotope probing (SIP) (Layman *et al.*, 2012), have been refined and new approaches have been created by combining methods, e.g., combining Raman spectroscopy or fatty acid analysis with SIP (Ruess *et al.*, 2005a; Li *et al.*, 2013). These empirical methods can provide insight into different aspects of food webs and together form an extensive toolbox to investigate trophic interactions. It is crucial to recognize the potential and limitations of a range of empirical approaches in order to choose the right method in the design of empirically based food-web studies.

Empirically based food webs are generally classified according to the type of input information that is required. In the following lines we will provide an overview of four types of food-web model: connectedness webs, semi-quantitative webs, energy-flow webs, and functional webs. Paine (1980) introduced three of those webs, which are widely accepted and applied in food-web studies across ecosystems. We propose to add a fourth type of empirically based food web, the semi-quantitative web. All of these food webs have the same basic structure, but the conceptual webs differ in the type of trophic information they describe and represent (Figure 16.1). *Connectedness webs* (Figure 16.1a) define the basic structure of a food web by describing the food-web connections *per se*. The food web consists of species connected by arrows, visualizing the direction of matter and energy flows. Due to the complexity of interactions, taxa are often lumped into feeding guilds, whose members have a similar trophic level and diet, and comparable function in the food web. *Semi-quantitative webs* (Figure 16.1b) differ

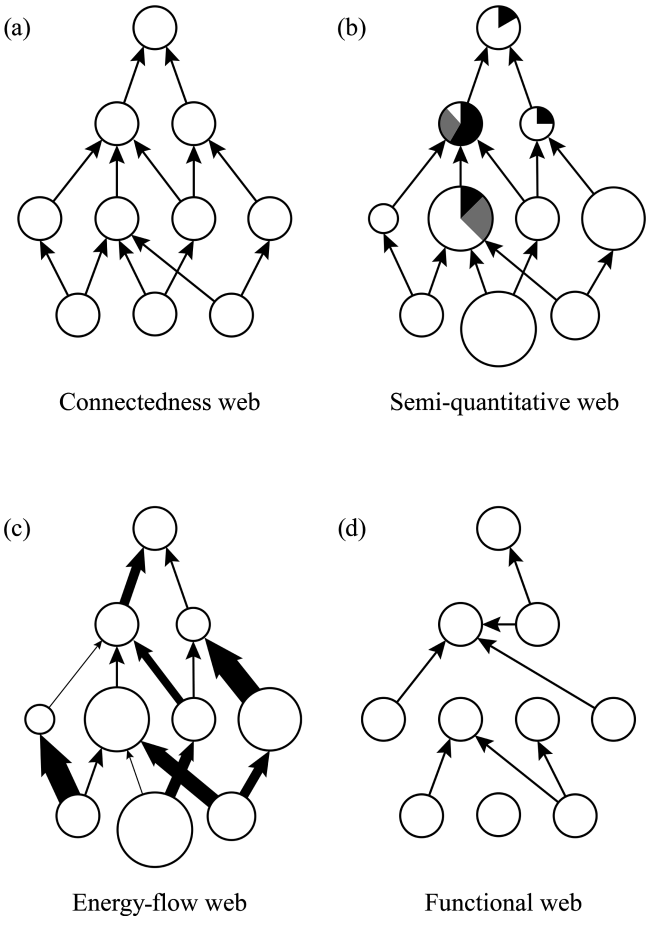

Figure 16.1 Four different types of empirically based food web, three of them (a, c, and d) as defined by Paine (1980). (a) Connectedness web visualizing qualitative feeding relationships; feeding guilds of species connected by arrows, visualizing the direction of matter and energy flows. (b) Semi-quantitative web visualizing the abundances/biomasses of species (groups) and the frequency of feeding interactions. (c) Energy-flow web visualizing the biomasses of species (groups), connected by vectors visualizing the amount of material and energy flow. (d) Functional web visualizing the effect of species manipulation on the population size of other species in the food web, highlighting the functional role of species including non-trophic effects (Figure adapted from Paine, 1980 and Selakovic *et al*., 2014).

from connectedness webs in that they contain quantitative information on the abundances or biomass of the food resources and the consumers. Additionally, they provide a semi-quantitative measure to feeding relationships such as the frequency of feeding interactions between (groups of) species, which can provide a good proxy for trophic interaction strength (Baker *et al.*, 2014). *Energy-flow webs* (Figure 16.1c) aim to assess nutrient flows quantitatively. They thus contain quantitative biomass information, and the feeding relationships are fully quantified by vectors summarizing both the direction and the amount of material and energy flows. Finally, *functional webs* (Figure 16.1d) are

characterized by experimental manipulation (e.g., species removal, resource exclosure or amendment) to assign the functional role of species. In this chapter, we examine how empirical trophic information can be used to construct such empirically based food webs.

There is an increasing demand for empirical data and experiments to improve empirically based food webs, e.g., in the fields of ecosystem engineering (Sanders *et al.*, 2014) and applied ecology (Memmott, 2009). Especially in the field of soil food-web ecology, trophic interactions are still poorly understood, since it is difficult to define the trophic roles of belowground organisms. Ideally, a food web would be described at high taxonomic resolution, representing the present species and their interspecific trophic interactions. However, species-level approaches are often difficult or impractical since the assignment of trophic interactions to specific soil organisms, and especially soil microbes, is hard due to their small size, difficult extraction from their habitat, and huge taxonomic and functional diversity (Eggers and Jones, 2000). These facts particularly hamper the determination of microbial-faunal food-web interactions. To circumvent this limitation, ecologists aggregate groups of species into feeding guilds based on diet and life-history characteristics. This approach has been especially applied to soils, the class of ecosystems on which this chapter will focus. New techniques introduced in this chapter provide better information on biological diversity and functionality within the food web, and in this way improve significantly the level of detail and realism in empirically based food webs.

Molecular and biochemical techniques that have been developed over the last decades have opened new windows in (soil) food-web ecology to study which food sources sustain specific soil heterotrophs and to assess small-scale activity and trophic links in food webs. These empirical techniques offer a great opportunity to further unravel feeding guilds and to create predictive models that are able to provide answers on specific soil organisms and their roles in trophic networks. However, a clear overview of what type of information empirical methods can offer for soil food-web modeling is missing. This chapter provides an up-to-date overview of the molecular and biochemical methods applicable in trophic soil studies. We start from a theoretical perspective of soil food webs to present the rationales and requirements of empirically based food-web modeling. Thereafter, an overview is provided on existing and upcoming empirical techniques and the research fields where they can be applied. The chapter concludes with recommendations on how to use the outcomes from empirical techniques to improve and fully exploit the use of empirically based soil food-web models as well as suggestions for future research priorities. Our goal is to provide an overview of state-of-the-art empirical approaches that can be used to create and improve food-web models described in other chapters of this book, with a special focus on food-web structure and flows.

16.2 From Empirical Data to Food-Web Models

Transferring the outcomes of empirical studies into useful data for soil food-web modeling remains one of the main challenges when combining empirical and theoretical

food-web research. This paragraph provides an overview of the types of data resulting from molecular and biochemical trophic studies that are essential to create soil food-web models. The information is categorized by the different types of food webs as introduced above: connectedness web, semi-quantitative web, energy-flow web, and functional web.

16.2.1 Connectedness Webs

Connectedness webs are the basic form of food webs, often visualized by the use of a simple box-and-arrow diagram (Figure 16.1a). The boxes and arrows represent respectively (groups of) species and the direction of their trophic interactions. This type of information can give important insights with respect to the stability and complexity of a food-web model. What does an empirically based connectedness web require? The construction of a connectedness web from empirical data requires at least information on the presence of organisms (binary) and the direction of feeding interactions: "who eats whom?" arranged per feeding guild, but preferably on a more highly resolved taxonomic level. By constructing a connectedness web, valuable information on horizontal (i.e., within a trophic level) and vertical (i.e., between trophic levels) food-web diversity is gained.

16.2.2 Semi-Quantitative Webs

Where connectedness webs only provide information on which species and feeding relationships are present, semi-quantitative webs also give information on the frequency of the trophic interactions (f_j) and the population sizes of present taxa on both the prey and consumer side (B_j) (Figure 16.1b). Frequency of interaction is based on the proportion of predators that contain remains of a specific prey, and can be calculated as:

$$f_j = \frac{F_{\text{tot}}}{N} \tag{16.1}$$

In this equation, F_{tot} refers to the total number of interactions detected and N is the total number of consumers for which the average number of interactions is calculated. This type of semi-quantification is not often used in soil ecology, but has shown great potential in aquatic food webs and host–parasitoid webs. In the past, semi-quantitative measurements of detection frequency have been criticized as a rough way of quantification, since they give no information on the biomass of prey eaten by a predator (Hyslop, 1980). Yet it gives a robust and interpretable description of the dietary composition of organisms, as reviewed by Baker *et al.* (2014). Determining the frequency of interactions makes it possible to gain quantifiable data from presence/absence data of the dietary composition that can be relatively easily gained compared to empirical methods that are necessary to quantitatively describe energy and carbon flows.

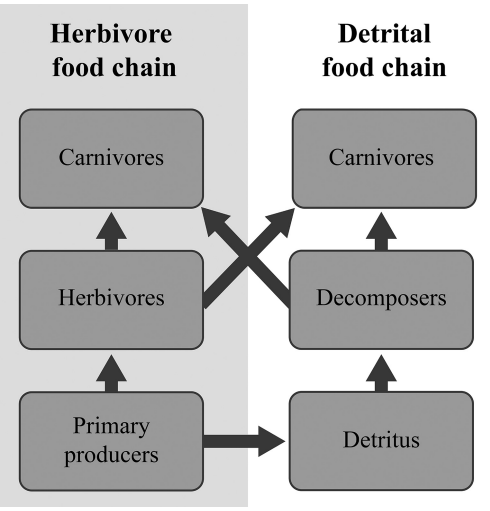

Figure 16.2 The two major soil food chains: (left) the herbivore food chain (primary-production based); (right) the detrital food chain (decomposition based).

16.2.3 Energy-Flow Webs

While the links in connectedness and semi-quantitative webs provide a measure of food-web complexity and structure, the arrows and boxes in energy-flow webs are weighted in terms of population sizes and the rate with which material is transferred from the resources to the consumers (Figure 16.1c). To construct energy-flow webs, information is therefore required on the biomass of resources and soil organisms, as well as the rates of flows of matter among the resources and species within the food web (Moore and de Ruiter, 2012).

Energy flux rates can be calculated by means of the detrital food-web model (DFWM), originally proposed by O'Neill (1969) and subsequently applied to various food webs from native (Hunt *et al.*, 1987; Berg *et al.*, 2001; Schröter *et al.*, 2003) and agricultural soils (de Ruiter *et al.*, 1993). The steady-state assumption underlies the DFWM model, i.e., that the production of a population balances the rate of loss through natural death and predation. The model was originally applied to decomposition-based (detrital) food-web models, relying on dead organic matter or detritus as a source of energy. However, the model can also be applied well to primary production-based (herbivory) food chains in soil, where carbon and nutrients are originating from living plant biomass. Figure 16.2 combines those two important food chains in soil, showing a great potential of linking them together via soil food-web modeling.

Using the steady-state assumption, the feeding rates can be calculated as:

$$F_j = \frac{d_j B_j + M_j}{e_j} \tag{16.2}$$

In this equation, F_j refers to the feeding rate of group j ($kg_{Carbon}ha^{-1}yr^{-1}$), d_j to its specific death rate (yr^{-1}), B_j to the average annual population size ($kg_{Carbon}ha^{-1}$), M_j to the death rate due to predation ($kg_{Carbon}ha^{-1}yr^{-1}$), e_j to the energy conversion efficiency. For predators feeding on more than one prey type, the feeding rate per prey type (F_{ij}) is calculated assuming that the predator feeds on a prey type according to the relative abundance of this prey type and on prey preferences:

$$F_{ij} = \frac{w_{ij}B_i}{\sum_{k=1}^{n} w_{kj}B_k} * F_j \tag{16.3}$$

In this equation, w_{ij} refers to the preference of predator j for prey i over its other prey types and n is the number of trophic groups. k is the numerator of the summation over all (n) trophic groups. The model calculates the feeding rates in a top–down sequence. It starts with the top predators, for which only natural death is assumed, i.e. $M_j = 0$. Hence in this step all necessary parameter values are available. Then the model proceeds working backwards to the lowest trophic levels. The M_j values then become available through the calculations in the former steps.

What does this DFWM approach require in terms of input data? In addition to the obtained values for population biomasses, only the preferences w_{ij} and the energy conversion efficiencies e_j are required. The energy conversion efficiency is known for most species of soil organisms, but the species diets and preferences are still largely unknown on soil-species level. New techniques (introduced below), combined with controlled laboratory experiments, will provide information on the relative preferences w_{ij} of predator j for prey i up to a higher taxonomic resolution. Such detailed information will make it possible to construct energy-flow web models with an increased level of detail and realism.

16.2.4 Functional Webs

Where energy-flow webs focus on the biomasses of feeding guilds and their interconnecting rates of energy transfer, functional webs originally describe the influence of the species manipulation (not only feeding interactions) on the population sizes of the remaining species (MacArthur, 1972; Paine, 1992) (Figure 16.1d). The relative impact of one species on another within functional webs is described by the interaction strength of a species relationship, comprising both trophic and non-trophic interactions. Where the removal of a weakly interacting species will not have large consequences for food-web structure, the removal of a strongly interacting species can have severe consequences. There are, however, multiple definitions of interaction strengths, and these vary between empiricists and theoreticians (Berlow *et al.*, 2004; Moore and de Ruiter, 2012). In this chapter, we will restrict our discussion to one of the theoretical approaches of interaction strengths by showing how the use of only trophic information can provide interaction strengths, by making use of a community (Jacobian) matrix as was first formulated by May (1974).

The strength of the trophic links is described by the interaction strength. In the theoretical approach, interaction strengths are defined as the "per capita" (or "per biomass") effects of the populations upon one another (May, 1972; Pimm, 1982). These values of interaction strengths are used as the entries of a Jacobian matrix representation of the food webs, which can be used to analyze the stability of the food webs. This procedure is based on the Lotka–Volterra approach, in which the dynamics of the trophic groups are described as:

$$\frac{dX_i}{dt} = -d_i X_i - \sum_{j=1}^{n} c_{ij} X_i X_j + \sum_{h=1}^{n} e_i c_{hi} X_h X_i \tag{16.4}$$

In this equation, X represents the population sizes of the trophic groups, c_{ij} the coefficient of interaction between group i and group j, and d and e have a similar meaning as in Eq. (16.2). Sometimes, modifications of this equation are used, for example the equation describing the dynamics of soil organic matter (Moore *et al.*, 1993). From these Lotka–Volterra equations, one can derive interaction strengths as the partial derivatives of the differential equations near equilibrium:

$$\alpha_{ij} = \left(\frac{\delta \dot{X}_i}{\delta X_j}\right)^* \tag{16.5}$$

Here α_{ij} denotes the interaction strength imposed by group j on group i, $\dot{X}_t = \frac{dX_i}{dt}$, and * denotes at equilibrium. Hence if we obtain empirically based values for α_{ij} we can build a Jacobian community matrix, analyze stability, and evaluate the importance of food-web components to food-web stability. What is required to obtain empirically based values for the interaction strengths α_{ij}?

If we take the partial derivatives of Eq. (16.2), we obtain the following formulae for interaction strength:

$$\alpha_{ij} = -c_{ij}\, X_i^* \tag{16.6}$$

for the *per capita* effect of predator j on prey i and

$$\alpha_{ji} = e_j c_{ij}\, X_j^* \tag{16.7}$$

for the *per capita* effect of prey i on predator j.

Values of c_{ij} are difficult to obtain, given the measure of this parameter expressed as "per amount per time." However, we can rewrite the formula for interaction strengths by using the following substitution, i.e., replace the terms $c_{ij}X_i^*X_j^*$ in the Lotka–Volterra equation by the feeding rates, F_{ij}, as estimated by the DFWM, and assuming that the equilibrium population sizes, X_i*, X_j*, are represented by the observed population sizes, B_i and B_j. Then we obtain:

$$\alpha_{ij} = -c_{ij}X_i^* = -\frac{F_{ij}}{B_j} \tag{16.8}$$

for the *per capita* effect of predator j on prey i

$$\alpha_{ji} = e_{ij}c_{ij}X_j^* = \frac{e_jF_{ij}}{B_i} \tag{16.9}$$

for the *per capita* effect of prey i on predator j.

From these reformulations of interaction strengths, we see that they can be directly derived from observed biomasses, calculated feeding rates, and known energy-conversion efficiencies. Then we are able to evaluate the stability of the Jacobian matrix and hence of the food web. This, however, does not provide an empirically based functional web. To fill in this gap, Neutel *et al*. (2002) proposed to look at food-web stability in terms of lengths and weights of trophic interaction loops. A trophic interaction loop describes a pathway of interactions (i.e., not feeding rates) from a species through the web back to the same species without visiting the species more than once; hence a loop is a closed chain of trophic links. An example of such a loop is the soil microbial loop (e.g., Bonkowski, 2004), where carbon is allocated from plant roots to rhizosphere bacteria, which are linked to micro-faunal predators, mainly protozoa. Grazing of protozoa on soil bacteria causes nutrients to be released, which are taken up by plants. These processes are running mainly between bacteria and protozoa with no higher trophic food-web levels involved, thereby forming a trophic interaction loop. Such loops may vary in length; the *loop length* being the number of trophic groups visited, and in weight, the *loop weight* being the geometric mean of the interaction strengths in the loop, defined as the per capita effects of the Jacobian matrices. The maximum of all loop weights is an indicator of food-web stability. Looking at the weights of trophic loops has a twofold meaning. First of all, it allows us to better understand the patterns in interaction strengths underlying stability. Second, it identifies food-web components that are key to food-web stability, which is close to the functional webs derived from manipulation experiments (sensu Paine 1980, 1992), while they can be calculated by using the obtained values for interaction strength.

To summarize, all required parameters for creating empirically based soil food-web models are compiled in Table 16.1. Not all of those parameters are necessary to measure and qualify in empirical experiments, since some of the parameters can be derived as explained above. The following paragraphs will therefore focus on those parameters (f_j, F_j, B_j, and w_{ij}) that cannot be derived from formulae (such as parameters Mj, c_{ij}, and α_{ij}) or are already known on species level (such as parameters e_j and d_j), but need real-world quantification in order to create empirically based soil food-web models.

16.3 Incorporating Empirical Information into Food-Web Models

The empirical design of food-web research largely depends on the questions addressed and, correspondingly, the type of food-web model one wishes to construct. We therefore

Table 16.1 Overview of parameters used to create a connectedness, semi-quantitative, energy-flow, or functional web. Each food-web model is described by the given parameters for that specific type of model, including all of the above.

	Name	Description	Unit
Connectedness web		Presence of organisms	[a]
		Feeding relationships: "who eats whom"	[a]
Semi-quantitative web	f_j	Frequency of interaction	[a]
	B_j	Population size	$kg_{Carbon}ha^{-1}$
Energy-flow web	F_j	Feeding rates	$kg_{Carbon}ha^{-1}yr^{-1}$
	d_j	Specific death rate	yr^{-1}
	M_j	Death rate due to predation[b]	$kg_{Carbon}ha^{-1}yr^{-1}$
	e_j	Energy conversion efficiency	[c]
	w_{ij}	Prey preferences	[a]
Functional web	c_{ij}	Coefficient of interaction[d]	$kg_{Carbon}{}^{-1}yr^{-1}$

Note: units are based on their frequent use in detrital-based food-web models. Not all parameters need to be measured empirically, since some parameters (e.g., coefficient of interactions and interaction strengths) can be derived from equations.
[a] Dimensionless; [b] Derived from Eqs. (16.1) and (16.2) (assuming $M_j = 0$ for top predators); [c] Dimensionless if units for prey and consumer population sizes are the same; [d] Derived from Eqs. (16.7) and (16.8).

provide a comprehensive overview of the available empirical techniques in function of the type of information they can provide and how they can be combined with different theoretical models.

16.3.1 Connectedness Webs

Soil food webs usually consist of diverse communities of species arranged along the subterranean herbivore and detrital food chains (Scheu, 2002). Disentangling the feeding interactions in these complex communities is not easy as the opaque habitat makes it impossible to observe who is feeding on whom directly. Furthermore, the small sizes of the interacting species, liquid feeding, and extra-oral digestion complicate the use of a morphology-based assessment of trophic interactions. Soil food-web research has thus greatly benefited from the development of techniques that overcome these hurdles, such as molecular biological methods and biomarker approaches.

16.3.1.1 DNA-Based Techniques on Dietary Samples

DNA-based approaches can identify the DNA of food remains at high specificity and sensitivity, thereby opening up new possibilities to examine feeding relationships and how organisms are trophically connected in natural communities (Pompanon *et al.*,

2012; Traugott *et al.*, 2013). In brief, food DNA is extracted from the dietary sample, specific short fragments of it are amplified using the polymerase chain reaction (PCR) technique, and the resulting PCR products are identified either by sequencing or by their length, which is indicative for a specific taxon (i.e., diagnostic PCR see below). Molecular techniques can be used for analyzing almost all of the trophic links expressed within soil food webs, including trophic interactions between mesofauna (e.g., Heidemann *et al*., 2014a) and macrofauna (Juen and Traugott, 2007; Lundgren and Fergen, 2014) – the only requirement is that amplifiable food DNA is present in the dietary sample. Moreover, these techniques are not restricted to analyzing the consumption of fresh food, but it is also possible to detect the DNA of scavenged prey (Juen and Traugott, 2005) and decaying plant material that has been consumed (Wallinger *et al.*, 2013). It is important to point out that this approach does not take into account what has been metabolized by a consumer but merely detects what has been consumed.

A variety of sample types have been used for the molecular study of feeding interaction. The simplest way is to identify food remains, either directly taken from the consumer (e.g., masticated prey from wasps; Kasper *et al.*, 2004) or collected in the environment, is by examining the DNA present within them. In most cases, however, DNA of consumed food present within either feces, the gut content in the consumer, and regurgitates is examined. Feces are usually employed to study vertebrate food choice while in invertebrates typically whole-body DNA extracts are used to retrieve food DNA from the gut content (King *et al.*, 2008). As such, gut content analysis is lethal to the consumer. In situations where these post-mortem approaches are not appropriate (i.e., in rare or protected species or when multiple feeding events of individual consumers are of interest) fecal pellets (Boyer *et al.*, 2011) and regurgitates (Waldner and Traugott, 2012) provide a means to obtain dietary samples from invertebrates non-invasively. Fecal pellets of invertebrate decomposers can also be used to assign them to their producer at the species level using PCR techniques (Seeber *et al.*, 2010), extending the possibilities for molecular profiling of soil faunal communities (Andújar *et al.*, 2015).

Aside from analyzing dietary samples for trophic information, molecular methods can also be extremely valuable to identify the consumer via diagnostic PCR or DNA barcoding (Wirta *et al.*, 2014), providing food webs that are taxonomically highly resolved on both the consumer/host and food/parasitoid sides. The molecular methods used for analyzing trophic interactions can be classified into two basic approaches of (1) diagnostic PCR and (2) sequence-based identification (Traugott *et al.*, 2013). In the former, taxon-specific primers are employed to amplify short fragments of food DNA followed by electrophoretic separation and visualization. The amplification of these specific fragments diagnoses the presence of the targeted DNA in the sample. The level of taxonomic identification can be set according to the needs of the study, i.e., earthworm prey can be identified either generally on a family level using earthworm-group specific primers (e.g., Harper *et al.*, 2005) or down to species and even lineage level (King *et al*., 2010). Primer pairs for different prey taxa can be mixed together in multiplex PCRs allowing us to test dietary samples for several targets in parallel, strongly increasing the efficiency of the analysis (Harper *et al.*, 2005; Sint *et al.*, 2012). In sequence-based food identification, primers are employed that target short DNA fragments of a wide range of

food taxa (Pompanon *et al.*, 2012). The resulting PCR products are subjected to high-throughput sequencing using next-generation sequencing (NGS) techniques. After quality checking and sorting these sequences, they can be assigned tentative identities using either public sequence databases or specific reference sequences (Pompanon *et al.*, 2012).

While diagnostic PCR is ideally suited for rapid and low-cost screening of a large numbers of samples, this approach will only allow one to detect the a-priori selected taxa targeted by the primers. Although several multiplex PCR assays can be used in parallel to detect several tens of taxa within the consumer's diet, this approach becomes inefficient when a broad range of dietary items needs to be examined. In such cases, sequence-based food detection via NGS is advantageous as it allows us to explore the diet spectrum of generalist consumers or to obtain dietary information on a population level using a pool of individual dietary samples (Deagle *et al.*, 2009). Sequenced-based diet identification, however, can be hampered by potentially poor coverage of sample sequences in public databases. Other problems include excessive co-amplification of consumer DNA, which requires the application of blocking primers (Vestheim and Jarman, 2008), the lack of suitably conserved regions for primer bindings sites to allow for amplification of fragments suitable for barcoding a broad range of target taxa (Deagle *et al.*, 2014), and the comparably high costs for testing large numbers of individual samples. The decision of which approach to use largely depends on the nature of the research project and the questions addressed: diagnostic PCR is typically employed for assessing the detection frequency via individual-based dietary analysis using larger numbers of samples, which would currently be too costly to be processed by NGS. Next-generation sequencing-based food identification, on the other hand, is most efficient for pooled dietary/consumer samples, allowing us to obtain an in-depth picture of the diet of a specific consumer on a population level. Moreover, it is important to consider that the detection of food DNA in dietary samples does not necessarily confirm that the specific food taxon was digested and metabolized into the consumer's tissue. For example, nematodes can have a short bacterial residence time in the intestine, which means that not all prokaryote cells are digested (Ghafouri and McGhee, 2007). We advise the reader to consult the latest reviews, such as King *et al.* (2008), Pompanon *et al.* (2012), Symondson (2012), Traugott *et al.* (2013), and Clare (2014), for more detailed information.

16.3.1.2 Lipid Analysis

Feeding interactions are generally drawn from primary producers via herbivores to carnivores, suggesting that population development at any given trophic level is limited by populations in the trophic level below. Such bottom–up control is widespread in soil food webs, as decomposers lack influence on the amount of organic matter, e.g., litter, feces, or necromass, available as basal resource. In soil, the bacterial decomposition pathway is predominantly resource controlled, while the fungal pathway faces greater top–down effects mainly mediated by micro-arthropods (Scheu *et al.*, 2005). A useful way to assess the carbon flux in food webs is the analysis of lipids, namely phospholipid fatty acids (PLFAs) and neutral lipid fatty acids (NLFA). This *in situ* method allows one

to assign animal diets and carbon transfer in cryptic systems such as soil food webs (Ruess and Chamberlain, 2010; Traugott *et al.*, 2013), providing information on feeding relationships.

In soil, total fatty acid analyses have successfully been used as a qualitative measure for carbon assimilation in primary and secondary decomposers. To date, most such feeding habits studies have focused on micro- and mesofauna, i.e., nematodes (Chen *et al.*, 2001; Ruess *et al.*, 2002, 2004) and Collembola (Ruess *et al.*, 2004, 2005b; Chamberlain *et al.*, 2005). Only recently have higher trophic levels of the soil food web been considered, such as by taking into account lipid patterns in centipedes and spiders (Haubert *et al.*, 2009; Pollierer *et al.*, 2010; Ferlian *et al.*, 2012). An underlying assumption related to the use of lipids as trophic biomarkers is a concept referred to as "dietary routing," which denotes the transfer of fatty acids from the diet into consumer tissue without modification. This process is well known in vertebrates and used, for example, in food chemistry to assign the origin of dairy products (Molkentin and Giesemann, 2007). Moreover, it was applied frequently in the herbivore food chain of marine ecosystems to monitor predator–prey interactions and carbon flow between phytoplankton and zooplankton (e.g., Müller-Navarra *et al.*, 2000; Stübing *et al.*, 2003; Pond *et al.*, 2006). The basic principle of this approach is that organisms at the base of the food web are capable of synthesizing specific fatty acids, which do not occur in the metabolism of organisms at higher trophic levels, and therefore can be used as biomarkers. Two general types of marker fatty acids have to be distinguished: (1) absolute markers the consumer cannot synthesize, and only appear in the lipid profile when it has fed on the respective diet, and (2) relative markers that are components of consumer metabolism but are additionally highly accumulated from the diet (Ruess *et al.*, 2005b). Ruess and Chamberlain (2010) provide a useful review on method application, advantages, and drawbacks in fatty acids as a tool in soil food-web analysis.

Table 16.2 provides an overview of fatty acid biomarkers for, respectively, the herbivore and detrital food chain (Figure 16.2) based on current knowledge. However, future research is likely to reveal additonal biomarkers. In plant and algal tissues marker fatty acids occur in both the phospholipid and neutral lipid fractions (Ruess *et al.*, 2007; Buse *et al.*, 2013), yet for soil microbes and fungi these are predominantly found in the phospholipids of membranes (White *et al.*, 1996; Zelles, 1999). One exception is the

Table 16.2 Fatty acid biomarkers useful for determination of carbon flows in herbivore and detrital food chains, respectively.

Herbivore food chain	Detrital food chain
Plants	**Bacteria**
18:1ω9	iso/anteiso – gram-positive
18:3ω3,6,9	cyclopropyl – gram-negative
18:3ω6,9,12	
Algae	**Fungi**
16:2ω6,9	16:1ω5 – arbuscular mycorrhiza
16:3ω3,6,9	18:2ω6,9 – ectomycorrhiza and saprotrophs

arbuscular mycorrhiza fungi (AMF), as the marker 16:1ω5 is common in phospholipids of bacteria and fungi, whereas in the neutral lipids it is exclusive to AMF (Ngosong *et al.*, 2012). There is one notable difference in fatty acid transfer, i.e., the direction of feeding interactions: in the classical trophic cascade of the herbivore food chain it is unidirectional, whereas marker fatty acids derived from litter or debris of primary producers also fuel the detrital food chain. An additional blur between both food chains arises from cross-feeding at higher trophic levels. Generally, tritrophic transport occurs as shown for bacteria-based (bacteria–nematodes–Collembola) and fungal-based (fungi–nematodes–Collembola) food chains (Ruess *et al.*, 2004; Chamberlain *et al.*, 2005) up to top predators such as centipedes (Pollierer *et al.*, 2010). Thus a marker fatty acid can indicate feeding on a specific diet or predation on prey also feeding on this diet. On one hand, this can hamper assignment of a binary link, but on the other hand it allows one to follow the feeding relationships across multiple trophic levels of the food web.

One additional fact makes the application of fatty acids in soil food-web studies particularly attractive: after feeding and ingestion of the diet, marker fatty acids are predominantly routed into the neutral lipids of consumers (Ruess *et al.*, 2004; Haubert *et al.*, 2006). As only some actinobacteria possess neutral lipids to a significant extent (Alvarez and Steinbüchel, 2002), the detection of microbial marker fatty acids in consumer storage fat enables distinguishing between viable microbes, in the gut or on the body surface, and the microbial tissue assimilated by the animal grazer. That goes beyond the detection of a bacterial DNA via gut content analysis, as it assigns bacterial carbon allocated in consumer biomass. This is a great advantage in decomposer systems, where bacteria form a basal resource, as it offers the possibility to link microbial and faunal food webs.

16.3.2 Semi-Quantitative Webs

Molecular prey detection, as discussed earlier in this chapter, usually provides an absence/presence matrix for the food DNA detected within a sample. With the use of these data, one can establish a detection frequency of specific food taxa (f_j) as a proxy for the strength of trophic interactions, which is necessary to construct a semi-quantitative food web. With the use of detection frequency of feeding relationships, it will allow us to assess the most important diet resources for the consumer assuming that frequently consumed foods are more important for sustaining the consumer than rarely consumed ones (King *et al.*, 2008; Heidemann *et al.*, 2014b). Moreover, it is important to consider that the analysis of a dietary sample provides a snap-shot picture of the recently consumed food. Therefore the quality and robustness of the trophic data generated is positively correlated with the number of dietary samples analyzed. Factors such as food and consumer identity (Greenstone *et al.*, 2007; Waldner *et al.*, 2013; Wallinger *et al.*, 2013) can affect post-feeding food DNA detection intervals and need to be considered when analyzing and interpreting molecularly derived trophic data (for reviews see King *et al.*, 2008; Pompanon *et al.*, 2012; Symondson, 2012; Traugott *et al.*, 2013; Greenstone *et al.*, 2014).

Although quantitative PCR (qPCR) allows one to estimate the number of food DNA molecules present within a sample (Zhang *et al.*, 2007), it is of little help for quantifying the number of prey items consumed or estimating the meal size from gut content samples. This is because a small and a big meal digested for a short and a long time, respectively, can easily provide a similar number of food DNA molecules (King *et al.*, 2008). As it is usually unknown in a field-collected consumer when a feeding event occurred before it was caught, the number of food DNA molecules cannot be used to estimate meal size or number of prey consumed. However, in feces, which are an end product of digestion, qPCR can provide a semi-quantitative estimate of diet composition (Deagle and Tollit, 2007).

16.3.3 Energy-Flow and Functional Webs

Biomarker and molecular-based techniques are not only useful to qualify feeding interactions, but also to quantify those interactions in terms of energy flow. The following paragraphs give an overview of state-of-the-art techniques that are currently used to gather energy-flow data for soil ecosystems, complemented with future perspectives on cutting-edge techniques to study energy food-web models empirically. Table 16.1 shows that the construction of functional webs does not require additional empirical measurements compared to energy-flow webs, since the necessary parameters can be derived from equations as introduced above in Section 16.2. Techniques discussed in this subsection will therefore provide data for both the construction of energy-flow and functional webs.

16.3.3.1 Stable Isotope Probing

Stable isotope probing (SIP) is one of the empirical approaches that has been upcoming in food-web ecology over the past decade. It combines the use of molecular techniques with the detection of stable isotopes (e.g., ^{13}C and ^{15}N), making it possible to trace flows of matter in food webs on the smallest scale in all trophic levels of the food web. The main idea of SIP is that organisms feeding on a specific stable isotope-enriched substrate can be traced by probing the fate of these stable isotopes into cellular biomarkers of active consumers. The big advantage of SIP is the possibility to observe the link between identity and (metabolic) functioning *in situ*, which can give important information about both the structure and flows in food webs.

The term "stable isotope probing" was used for the first time by Radajewski *et al.* (2000), describing the tracing of a ^{13}C-enriched carbon source into microbial DNA. However, the labeling of metabolically active organisms can be followed by tracing labeled biomarkers such as DNA, RNA, and fatty acids (i.e., DNA–, RNA–, and FA–SIP). Lipids were among the first compounds to be measured after labeling due to the ease in their GC analysis. First approaches using stable isotope-labeled substrates were done by the use of microbial lipid analysis; Boschker *et al.* (1998) traced the fate of ^{13}C-enriched acetate and methane incorporated into PLFAs to link specific environmental processes to the identity of the microbial groups involved. In the context of food-web studies, PLFA and NLFA extraction in combination with stable isotope probing can

give important information about the rate (F_j) and fate (w_{ij}) of feeding interactions, and is highly complementary to other culture-independent methods (Maxfield and Evershed, 2011). Moreover, as the ^{13}C value of a specific fatty acid is dependent on the carbon pool it is derived from, i.e., de novo synthesis or dietary routing, a ^{13}C label introduced into a food web can be used to examine the route of the ^{13}C-pulse through the web by FA–SIP (Ruess and Chamberlain, 2010).

The DNA-based stable isotope probing (DNA–SIP) technique separates stable isotope labeled "heavy" DNA from unlabeled "light" DNA by density gradient centrifugation, after which the DNA can be identified both on a high taxonomic resolution. The possibility to derive high-quality taxonomic information regarding the labeled community is the main advantage of DNA–SIP over FA–SIP. On the other hand, DNA–SIP is less sensitive because low DNA synthesis rates limit the enrichment. Therefore a higher level of stable isotope enrichment compared to FA–SIP is required in order to get sufficient labeling. The low sensitivity of DNA–SIP applies to a lesser extent for the method of RNA-based stable isotope probing (RNA–SIP). RNA–SIP was reported for the first time by Manefield *et al.* (2002) who studied the degradation of ^{13}C labeled phenol in an industrial bioreactor. RNA synthesis rates are higher compared to DNA, which strengthens the sensitivity of RNA–SIP. Another advantage of RNA is the large amount of information it can give on both the phylogenetic (rRNA) and the functional (mRNA) gene diversity of the labeled organism. Therefore a combination of RNA–SIP (i.e., identity and functional gene diversity) with FA–SIP (i.e., carbon flux) is ideal to link the identity and function of key biota in the food web.

Over the last decade, SIP was mainly used to trace defined microbial groups responsible for the primary degradation of specific substrates (Radajewski *et al.*, 2000). However, labeling occurs also through secondary assimilation of labeled substrates. This so-called "cross-feeding" already occurred during the DNA–SIP experiments of Radajewski *et al*. (2000). Cross-feeding was often seen as an issue, since organisms that are not involved in the primary assimilation of a substrate become labeled. However, the phenomenon of cross-feeding has great potential to increase our insight into trophic interactions among multiple trophic levels in the same habitat (Friedrich, 2011). Experiments designed in time series provide an excellent opportunity to capture the dynamic nature of carbon flow through soil food webs by studying cross-feeding patterns (Drigo *et al.*, 2010).

Since its first introduction, SIP has been combined with different molecular techniques, and new applications continue to be introduced (Abraham, 2014). Huang *et al.* (2007) studied the incorporation of ^{13}C in microbial cells by combining stable-isotope Raman microscopy with fluorescence *in situ* hybridization (FISH), called Raman–FISH. This method can gain increased insight into the incorporation of carbon in organisms on the cellular level, increasing the detailed understanding of energy flows in food webs. Also the combination of SIP with Raman spectroscopy has led to increased insight in the carbon flows of food webs on the individual microbial level, by achieving a better understanding of energy flows and metabolic pathways in the context of complex food webs (Li *et al.*, 2013). One of the upcoming applications of SIP is to determine both identity and function by targeting organisms with the use of so-called Chip–SIP. This

approach uses phylogenetic microarrays and secondary ion mass spectronomy (NanoSIMS) as a high-sensitivity and high-throughput method to test genomics-generated hypotheses about biogeochemical function in any natural environment (Mayali *et al.*, 2012). Although the use of Chip–SIP is still in its developing phase, it is definitely a promising tool to study the functioning of energy flows within food webs.

16.3.3.2 Quantitative Fatty Acid Signature Analysis

A prospect for the future in soil food-web analysis is the application of quantitative fatty acid signature analysis (QFASA), which makes it possible to assign feeding rates (F_j) to predator diets. QFASA was recently developed as a tool to estimate predator diets in marine mammals such as grey seals, polar bears, and seabirds (Iverson *et al.*, 2004; Thiemann *et al.*, 2008; Williams and Buck, 2010). At present, its wide-scale application is hampered by the lack in information on specific (lipid) pathways and current metabolism (life cycle, starvation) for most soil animal grazers and predators. Nevertheless, for Collembola, metabolism and pattern of lipids are well known in regard to food quality, environmental factors, and biotic constraints such as life cycle and starvation (Holmstrup *et al.*, 2002; Haubert *et al.*, 2004, 2008; van Dooremalen and Ellers, 2010), which offers a starting point for establishment of QFASA in soil food webs. Other promising areas for further investigations using fatty acids are comparison of resources and fecal profiles to assign both consumption on, as well as propagation of, dietary organisms (Buse *et al.*, 2014) or to disentangle trophic from mutualistic processes in plant–microbe–fauna interactions (Ngosong *et al.*, 2014).

16.3.3.3 Nematode Community Analysis

The empirical method of nematode faunal analysis uses nematode assemblages to assign conditions to the soil micro-food web such as major decomposition pathways, nutrient status, or disturbance (Ferris *et al.*, 2001). Exploring the soil nematode community structure provides an empirical method within the framework of feeding guilds and can be used to determine the interaction of the component species within a guild, but also to ask questions regarding the interactions between the various guilds that compose the larger community. Thus nematode communities can serve as a model for general processes in the soil food web and as a tool to link microbial and faunal food webs. The latter is particularly important as food-web models still lack sufficient quantitative empirical data on carbon and energy fluxes between microbes and fauna.

The soil micro-food web consists of basal organic resources derived from photoautotrophs (e.g., plant litter and root exudates), the microflora (bacteria and fungi), and the micro- and mesofauna that feed upon the microflora or on each other (Wardle *et al.*, 1998). Within this web, nematodes (Figure 16.3) are the most abundant and diverse multicellular organisms with millions of individuals and up to 200 species per square meter (Yeates, 2010). Moreover, nematodes have established functional groups at each trophic level and feed on bacteria, fungi, algae, or roots as well as on other microfauna (Yeates *et al.*, 1993). Due to these diverse biological interactions, nematodes hold a central position in both bottom–up and top–down controlled food webs (Ferris, 2010a; Yeates, 2010). In particular those nematodes that graze on bacteria and fungi

Table 16.3 Nematode faunal analysis and the respective community indices according to Bongers (1990), Freckman (1988), and Ferris *et al.* (2001) used to assign food-web conditions.

Food-web condition	Nematode faunal analysis
Bottom–up effect of resources	Density and biomass of trophic groups
Decomposition pathways and energy flux	Channel index, fungal-to-bacterial feeder ratio
Enrichment and structure	Enrichment index, structure index
Disturbance or maturity	Maturity index
Natural or managed conditions	Plant parasite index

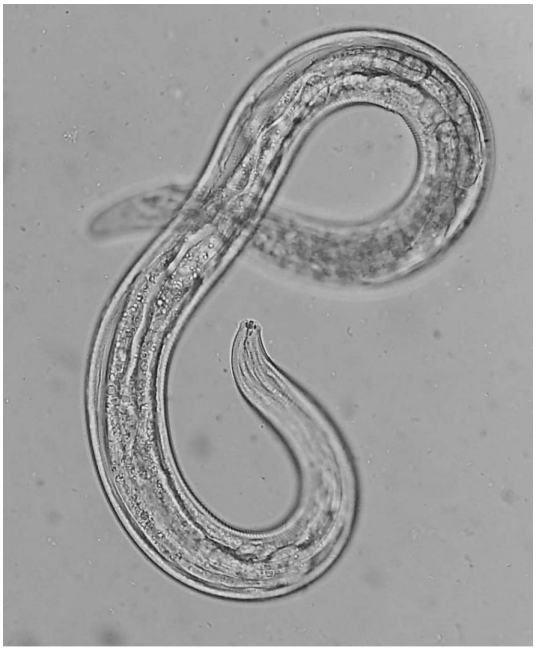

Figure 16.3 *Acrobeloides buetschlii*, an opportunistic nematode species and common bacterial feeder in the soil. (Picture by Veronika Bartel.)

play important roles in influencing soil microbial biomass, activity, and mineralization processes (Bardgett *et al.*, 1999; Griffiths *et al.*, 1999). Thus although nematodes represent only a small amount of biomass in the soil, their key position in the micro-food web impacts on ecosystem-level processes such as energy flow and nutrient cycling (e.g., Yeates *et al.*, 2009; Ferris, 2010a; Neher, 2010).

Nematode abundance (B_j), diversity, and effect on soil processes make nematode assemblages useful indicators of food-web conditions. By addressing the changes in horizontal as well as vertical diversity the nematode faunal analysis concept allows determining structure and function of the food web (Table 16.3). Nematode community indices based on life-history traits or trophic groups are applied to assign soil

decomposition pathways (channel index) as well as enrichment and structure (enrichment and structure index) of food webs in grassland, arable, and forest soil (Ferris *et al.*, 2001; Ruess, 2003; Ruess and Ferris, 2004). In particular, the channel index is a useful tool to determine the major fluxes of carbon and energy through the soil food web in terms of feeding rates (F_j). Plant effects, i.e., aboveground impact, are expressed in the plant parasite index (PPI; Bongers, 1990), whereas the maturity index (MI; Bongers, 1990) is a measure for disturbance and successional stage. Moreover, the establishment of functional groups in both primary production-based (herbivore) as well as decomposition-based (detrital) food chains can allow for a linkage between these two fundamental pathways (see Figure 16.2).

Only recently, metabolic footprints of nematodes were introduced as metrics for the magnitude of services provided by feeding guilds in the soil food web (Ferris, 2010b). This approach takes advantage of the standardized morphometric characteristics used in nematode taxonomic description. This comprehensive database facilitates assessment of body volume and weight, which can be converted to carbon metabolism by prescribed coefficients. Until now, soil food-web models generally apply abundance data as a proxy, partly combined with values on respiration or functional response. However, this does not take into account the relationship between prey and predator body sizes, which was reported to systematically differ across habitats and consumer types (Brose *et al.*, 2006). Body-size relationship is an important factor for interaction strength patterns in food webs and thus affects resilience and stability (Jonsson, 2014). Including metabolic footprints into soil food-web analysis, e.g., by making the energy conversion efficiency (e_j) body-size dependent, provides an opportunity for a more detailed interpretation of energy-flow webs and improves the accuracy of quantitative models. Overall, nematode faunal analysis provides a useful tool for assessing the importance of the different energy channels (i.e., bacterial, fungal, plant), as well as food chains (i.e., herbivore, detrital), in soil food webs and thus for determining food-web functioning and energy flux.

16.3.3.4 Controlled Laboratory Experiments

Since soil food webs are often difficult to tease apart because of the large number of intertwined and potentially confounding effects, controlled laboratory experiments offer an ideal complement to field experiments. Controlled small-scale experiments offer the possibility to follow in detail single interactions under defined conditions (von Berg *et al.*, 2012). The results can be then straightforwardly connected to genetic and physiological data on the studied organisms (Brose *et al.*, 2008; Brose, 2010; Neidig *et al.*, 2011). In connection with empirical and theoretical predictions (Baiser *et al.*, 2010), laboratory experiments can serve as a basis for parameterizing more complex food webs (Brose *et al.*, 2008; Brose, 2010). Novel approaches of molecular marking of food items also hold great promise for experiments in such controlled environments, including the quantification of food consumption in functional-response experiments. For example, Mora *et al.* (2014) showed that silica particles containing encapsualted DNA can be used to label food items with the label being detectable for several days post

consumption. Moreover, the label gets transferred across trophic levels and includes the possibility to quantify prey uptake using real-time PCR.

Laboratory feeding choice experiments are commonly used to study feeding preferences of soil organisms (w_{ij}). Even generalist predators show distinct feeding preferences. Selective feeding depends on several parameters, such as body mass ratio, and total and relative abundance (Kalinkat *et al.*, 2011). Further, prey properties, such as the presence of defensive structures, and active selection processes by the predators can influence which prey will be consumed first (Jousset, 2012). Prey selection has profound impacts on the stability and evolutionary dynamics of the whole community. In controlled systems, it is possible to mix preys at different relative and total abundances and thus accurately determine under which conditions predators will eat which prey. Microcosms can be set up to mimic several environment types such as plant-root systems (Jousset *et al.*, 2009), lakes (Jürgens and Simek, 2000), or litter (Vuvic-Pestic *et al.*, 2010). Prey abundance in the diet can be tracked with a vast array of available methods. For instance, prey staining and imaging allow us to count remaining, unconsumed prey (Jousset *et al.*, 2009) or even vacuole content for protozoa (Jezbera *et al.*, 2005). Experimental work can subsequently be combined with DNA-based methods and field data, for example for determination of active predation versus scavenging of dead prey (Heidemann *et al.*, 2011).

16.4 Discussion and Conclusions

Soil food-web ecologists have just started to exploit (molecular and biochemical) empirical tools to study subterranean feeding networks. Winemiller and Layman (2005) concluded that empirical food-web research is lagging behind theoretical research. The overview provided in this chapter illustrates many of the advancements that have been made since then in empirically testing soil food-web models. However, a big challenge remains in bringing theoretical and empirical food-web scientists together to take full advantage of the range of possibilities that empirical methods offer for food-web modeling. Table 16.4 provides an overview of the methods discussed and the type of information each can provide to empirically based soil food-web models.

The choice of specific empirical methods will largely depend on the type of questions asked in empirical studies, as well as the properties of food webs that one wishes to obtain. It is therefore especially important to note that the properties of food webs vary depending on the techniques used to reconstruct a food-web model (Wirta *et al.*, 2014). It is essential to have a good consideration of multiple empirical techniques, as displayed in the conceptual diagram of Figure 16.4.

Figure 16.4 provides an overview of the types of empirical methods that are suitable to study specific trophic levels, or the soil food web as a whole. Most methods presented allow for the detection of trophic connections in most of the trophic levels of the soil food web. Exceptions are PLFA–SIP analyses (focusing on the microbial part of the soil food web), but combined with different types of lipid analyses, lipids could be traced further into the soil food web. Nematode community analyses focus mainly on the

Table 16.4 Overview of the empirical methods discussed. For each method it is specified what type of necessary data the method can provide in terms of empirically based soil food-web modeling.

Methods	Type of data
DNA-based techniques on dietary samples	Presence/absence of specific organisms Frequency of interaction (f_j)
qPCR on fecal samples	Frequency of interaction, potentially diet composition (f_j)
Lipid analyses	Frequency of interaction (f_j)
Fatty acid analyses on fecal pellets	Frequency of interaction (f_j), assimilation efficiency
Quantitative fatty acid signatures (QFASA)	Feeding rates (F_j)
Stable isotope probing (SIP)	Feeding rates (F_j), prey preferences (w_{ij})
FA–SIP	*Carbon flux*
DNA–SIP	*Identity*
RNA–SIP	*Phylogenetic (rRNA) and functional (mRNA) gene diversity*
Nematode community analyses	Feeding rates (F_j) Population size (B_j)
Food-choice experiments	Prey (substrate) preferences (w_{ij})

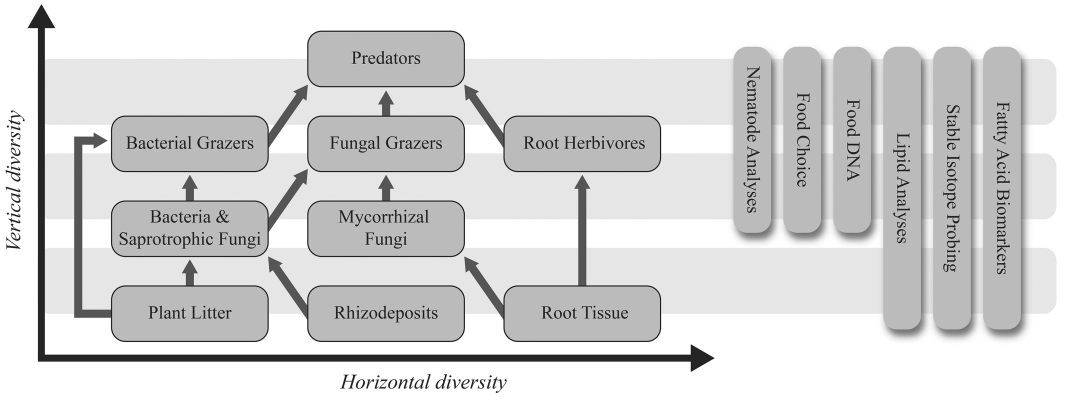

Figure 16.4 Conceptual diagram of a soil food web, showing the main feeding guilds and major pathways of carbon and energy. Horizontal diversity refers to diversity within trophic levels and vertical diversity refers to diversity between trophic levels. The right side of the diagram displays the proposed empirical methods over the range of trophic levels they can be applied to.

higher trophic levels of the soil food web, acting as "connectors" between the unexploited microbial part of the soil food web and the faunal food part of the food web that has been described in much more detail. Identifying food remains with the help of DNA-based techniques offers a high specificity and sensitivity, and opens up entire new possibilities to examine trophic interactions. Especially in combination with controlled feeding experiments, this method is of great value to determine exact feeding interactions, as well as feeding preferences; a combination of results that is of high value for establishing empirically based soil food-web models.

In the history of soil food-web modeling, there has been a strong divergence between soil food web models that were based upon primary producers (herbivory based) and models that relied on dead organic matter (detrital based) (see Figure 16.2). Those two types of food webs have been studied in separate areas of research due to large differences in empirical approaches. We expect that emerging methods, as described in this chapter, will yield large advances in bringing production-based and detrital-based food chains closer together and even link the two fields of research. Not only are the new arising empirical methods able to link different types of food webs, the snap-shot dietary information provided by, for example, DNA-based methods is also ideally suited for assessing the temporal dynamics in soil food webs, a topic that remains largely unexplored. Existing soil food-web models could also be further improved by including host–parasitoid relationships. The molecular techniques discussed also offer an effective way to study endoparasitism by detecting, for instance, the DNA of parasites and parasitoids within the host sample (Agustí *et al.*, 2005; Gariepy *et al.*, 2008; Traugott *et al.*, 2013; Hrček and Godfray, 2015). Pooling empirical techniques into combined detrital- and herbivory-based food webs with host–parasitoid food webs, has therefore a great potential to better understand the detailed interactions within soil food webs, as well as the functioning of soil food webs as a whole. Only recently, combining stable isotope analysis of bulk tissues as well as fatty acids gave new insight into the allocation and transfer of plant-derived carbon through a food web in an arable soil. The study of Pausch *et al*. (2015) revealed that saprotrophic fungi, not bacteria, are most active in these processes, challenging previous views on the dominance of bacteria in root carbon dynamics in arable soil.

Recent advances in empirical methods will open up new possibilities to study important areas of food-web model research, e.g., the link between microbial diversity and the functioning of soil food webs or the link between nematode diversity and their impact on soil food-web structure. Emerging empirical techniques, as described in this chapter, can bring a much higher resolution into food-web models that will certainly revolutionize our view of soil food webs. The high specificity at which trophic links can be identified raises the characterization of trophic niches for soil invertebrates to a completely new level, allowing a critical evaluation of the commonly used grouping of specific species into feeding guilds. Although empirically based soil food-web models that make use of feeding guilds have proven their value and utility (e.g., Hunt *et al.*, 1987; de Ruiter *et al.*, 1993; Berg *et al.*, 2001; Schröter *et al.*, 2003) an increased level of detail will be of great value for predictive models that focus on spatial and temporal patterns, as well as models that highlight the importance of specific parts of the soil food web, such as the soil microbial community, by bringing in greater phylogenetic resolution.

References

Abraham, W.-R. (2014). Applications and impacts of stable isotope probing for analysis of microbial interactions. *Journal of Appled Microbiology and Biotechnology*, **98**, 4817–4828.

Agustí, N., Bourguet, D., Spataro, T., *et al.* (2005). Detection, identification and geographical distribution of European corn borer larval parasitoids using molecular markers. *Molecular Ecology*, **14**, 3267–3274.

Alvarez, H. M. and Steinbüchel, A. (2002). Triacylglycerols in prokaryotic microorganisms. *Journal of Applied Microbiology and Biotechnology*, **60**, 367–376.

Andújar, C., Arribas, P., Ruzicka, F., *et al.* (2015). Phylogenetic community ecology of soil biodiversity using mitochondrial metagenomics. *Molecular Ecology*, **24**(14), 3603–3617. doi:10.1111/mec.13195.

Baiser, B., Russell, G. J., and Lockwood, J. L. (2010). Connectance determines invasion success via trophic interactions in model food webs. *Oikos*, **119**, 1970–1976.

Baker, R., Buckland, A., and Sheaves, M. (2014). Fish gut content analysis: robust measures of diet composition. *Fish and Fisheries*, **15**, 170–177.

Bardgett, R. D., Cook, R., Yeates, G. W., and Denton, C. S. (1999). The influence of nematodes on below-ground processes in grassland ecosystems. *Plant Soil*, **212**, 23–33.

Berg, M., de Ruiter, P. C., Didden, W. A. M., *et al.* (2001). Community food web, decomposition and nitrogen mineralisation in a stratified Scots pine forest soil. *Oikos* **94**, 130–142.

Berlow, E. L., Neutel, A.-M., Cohen, J. E., *et al.* (2004). Interaction strengths in food webs: issues and opportunities. *Journal of Animal Ecology*, **73**, 585–598.

Bongers, T. (1990). The maturity index: an ecological measure of environmental disturbance based on nematode species composition. *Oecologica*, **83**, 14–19.

Bonkowski, M. (2004). Protozoa and plant growth: the microbial loop in soil revisited. *New Phytologist*, **162**, 617–631.

Boschker, H. T. S., Nold, S. C., Wellsbury, P., *et al.* (1998). Direct linking of microbial populations to specific biogeochemical processes by 13C-labelling of biomarkers. *Nature*, **392**, 801–805.

Boyer, S., Yeates, G. W., Wratten, S. D., Holyoake, A., and Cruickshank, R. H. (2011). Molecular and morphological analyses of faeces to investigate the diet of earthworm predators: example of a carnivorous land snail endemic to New Zealand. *Pedobiologia (Jena)*, **54**, 153–158.

Brose, U. (2010). Body-mass constraints on foraging behaviour determine population and food-web dynamics. *Functional Ecology*, **24**, 28–34.

Brose, U., Jonsson, T., Berlow, E. L., *et al.* (2006). Consumer-resource body-size relationships in natural food webs. *Ecology*, **87**, 2411–2417.

Brose, U., Ehnes, R. B., Rall, B. C., *et al.* (2008). Foraging theory predicts predator–prey energy fluxes. *Journal of Animal Ecology*, **77**, 1072–1078.

Buse, T., Ruess, L., and Filser, J. (2013). New trophic biomarkers for Collembola reared on algal diets. *Pedobiologia (Jena)*, **56**, 153–159.

Buse, T., Ruess, L., and Filser, J. (2014). Collembola gut passage shapes microbial communities in faecal pellets but not viability of dietary algal cells. *Chemoecology*, **24**, 79–84.

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

Chen, J., Ferris, H., Scow, K. M., and Graham, K. J. (2001). Fatty acid composition and dynamics of selected fungal-feeding nematodes and fungi. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, **130**, 135–144.

Clare, E. L. (2014). Molecular detection of trophic interactions: emerging trends, distinct advantages, significant considerations and conservation applications. *Evolutionary Applications*, **7**(9), 1144–1157. doi:10.1111/eva.12225.

Deagle, B. E. and Tollit, D. J. (2007). Quantitative analysis of prey DNA in pinniped faeces: potential to estimate diet composition? *Conservation Genetics*, **8**, 743–747.

Deagle, B. E., Kirkwood, R., Jarman, S. N. (2009). Analysis of Australian fur seal diet by pyrosequencing prey DNA in faeces. *Molecular Ecology*, **18**, 2022–2038.

Deagle, B. E., Jarman, S. N., Coissac, E., Pompanon, F., and Taberlet, P. (2014). DNA metabarcoding and the cytochrome c oxidase subunit I marker. *Biology Letters*, **10**, dx.doi.org/10.1098/rsbl.2014.0562.

de Ruiter, P. C., Moore, J. C., Zwart, K. B., *et al.* (1993). Simulation of nitrogen mineralization in the below-ground food webs of two winter wheat fields. *Journal of Applied Ecology*, **30**, 95–106.

de Ruiter, P. C., Wolters, V., and Moore, J. C. (2005). *Dynamics Food Webs: Multispecies Assemblages, Ecosystem Development and Environmental Change*. San Diego, CA: Academic Press.

Drigo, B., Pijl, A. S., Duyts, H., *et al.* (2010). Shifting carbon flow from roots into associated microbial communities in response to elevated atmospheric CO_2. *Proceeding of the National Academy of Sciences of the USA*, **107**, 10938–10942.

Eggers, T. and Jones, T. H. (2000). You are what you eat… or are you? *Trends in Ecology and Evolution*, **15**, 265–266.

Ferlian, O., Scheu, S., and Pollierer, M. M. (2012). Trophic interactions in centipedes (Chilopoda, Myriapoda) as indicated by fatty acid patterns: variations with life stage, forest age and season. *Soil Biology and Biochemistry*, **52**, 33–42.

Ferris, H. (2010a). Contribution of nematodes to the structure and function of the soil food web. *Journal of Nematology*, **42**, 63–67.

Ferris, H. (2010b). Form and function: metabolic footprints of nematodes in the soil food web. *European Journal of Soil Biology*, **46**, 97–104.

Ferris, H., Bongers, T., and De Goede, R. G. M. (2001). A framework for soil food web diagnostics: extension of the nematode faunal analysis concept. *Applied Soil Ecology*, **18**, 13–29.

Freckman, D. W. (1988). Bacterivorous nematodes and organic-matter decomposition. *Agriculture Ecosystems and Environment*, **24**, 195–217.

Friedrich, M. W. (2011). Trophic interactions in microbial communities and food webs traced by stable isotope probing of nucleic acids. In *Stable Isotope Probing and Related Technologies*, ed. J. C. Murrell and A. S. Whiteley, American Society for Microbiology Press, pp. 203–232.

Gariepy, T., Kuhlmann, U., Gillott, C., and Erlandson, M. (2008). A large-scale comparison of conventional and molecular methods for the evaluation of host–parasitoid associations in non-target risk-assessment studies. *Journal of Applied Ecology*, **45**, 708–715.

Ghafouri, S. and McGhee, J. (2007). Bacterial residence time in the intestine of *Caenorhabditis elegans*. *Nematology*, **9**, 87–91.

Greenstone, M. H., Rowley, D. L., Weber, D. C., Payton, M. E., and Hawthorne, D. J. (2007). Feeding mode and prey detectability half-lives in molecular gut-content analysis: an example with two predators of the Colorado potato beetle. *Bulletin of Entomological Research*, **97**, 201–209.

Greenstone, M. H., Payton, M. E., Weber, D. C., and Simmons, A. M. (2014). The detectability half-life in arthropod predator–prey research: what it is, why we need it, how to measure it, and how to use it. *Molecular Ecology*, **23**, 3799–3813.

Griffiths, B. S., Ritz, K., Ebblewhite, N., and Dobson, G. (1999). Soil microbial community structure: effects of substrate loading rates. *Soil Biology and Biochemistry*, **31**, 145–153.

Harper, G. L., King, R. A., Dodd, C. S., *et al.* (2005). Rapid screening of invertebrate predators for multiple prey DNA targets. *Molecular Ecology*, **14**, 819–827.

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

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

Haubert, D., Häggblom, M. M., Scheu, S., and 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., *et al.* (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.

Heidemann, K., Scheu, S., Ruess, L., and Maraun, M. (2011). Molecular detection of nematode predation and scavenging in oribatid mites: laboratory and field experiments. *Soil Biology and Biochemistry*, **43**, 2229–2236.

Heidemann, K., Hennies, A., Schakowske, J., *et al.* (2014a). Free-living nematodes as prey for higher trophic levels of forest soil food webs. *Oikos*, **123**, 1199–1211.

Heidemann, K., Ruess, L., Scheu, S., and Maraun, M. (2014b). Nematode consumption by mite communities varies in different forest microhabitats as indicated by molecular gut content analysis. *Experimental and Applied Acarology*, **64**, 49–60.

Holmstrup, M., Hedlund, K., and 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**, 961–970.

Hrček. J. and Godfray. H. C. J. (2015). What do molecular methods bring to host–parasitoid food webs? *Trends in Parasitology*, **31**, 30–35.

Huang, W. E., Stoecker, K., Griffiths, R., *et al.* (2007). Raman–FISH: combining stable-isotope Raman spectroscopy and fluorescence in situ hybridization for the single cell analysis of identity and function. *Environmental Microbiology*, **9**, 1878–1889.

Hunt, H. W., Coleman, D. C., Ingham, E. R., *et al.* (1987). The detrital food web in a shortgrass prairie. *Biology and Fertility of Soils*, **3**, 57–68.

Hyslop, E. J. (1980). Stomach contents analysis: a review of methods and their application. *Journal of Fish Biology*, **17**, 411–429.

Iverson, S. J., Field, C., Bowen, W. D., and Blanchard, W. (2004). Quantitative fatty acid signature analysis: a new method of estimating predator diets. *Ecological Monographs*, **74**, 211–235.

Jezbera, J., Hornák, K., and Simek, K. (2005). Food selection by bacterivorous protists: insight from the analysis of the food vacuole content by means of fluorescence in situ hybridization. *FEMS Microbiol Ecology*, **52**, 351–363.

Jonsson, T. (2014). Trophic links and the relationship between predator and prey body sizes in food webs. *Community Ecology*, **15**, 54–64.

Jousset, A. (2012). Ecological and evolutive implications of bacterial defences against predators. *Environmental Microbiology*, **14**, 1830–1843.

Jousset. A., Rochat, L., Péchy-Tarr, M., *et al.* (2009). Predators promote defence of rhizosphere bacterial populations by selective feeding on non-toxic cheaters. *ISME Journal*, **3**, 666–674.

Juen, A. and Traugott, M. (2005). Detecting predation and scavenging by DNA gut-content analysis: a case study using a soil insect predator–prey system. *Oecologia*, **142**, 344–352.

Juen, A. and Traugott, M. (2007). Revealing species-specific trophic links in soil food webs: molecular identification of scarab predators. *Molecular Ecology*, **16**, 1545–1557.

Jürgens, K. and Simek, K. (2000). Functional response and particle size selection of *Halteria* cf. *grandinella*, a common freshwater oligotrichous ciliate. *Aquatic Microbial Ecology*, **22**, 57–68.

Kalinkat, G., Rall, B. C., Vucic-Pestic, O., and Brose, U. (2011). The allometry of prey preferences. *PLoS One*, **6**, e25937.

Kasper, M. L., Reeson, A. F., Cooper, S. J. B, Perry, K. D., and Austin, A. D. (2004). Assessment of prey overlap between a native (*Polistes humilis*) and an introduced (*Vespula germanica*) social wasp using morphology and phylogenetic analyses of 16S rDNA. *Molecular Ecology*, **13**, 2037–2048.

King, R. A., Read, D. S., Traugott, M., and Symondson, W. O. C. (2008). Molecular analysis of predation: a review of best practice for DNA-based approaches. *Molecular Ecology*, **17**, 947–963.

King, R. A., Vaughan, I. P., Bell, J. R., Bohan, D. A., and Symondson, W. O. C. (2010). Prey choice by carabid beetles feeding on an earthworm community analysed using species- and lineage-specific PCR primers. *Molecular Ecology*, **19**, 1721–1732.

Layman, C. A., Araujo, M. S., Boucek, R., *et al.* (2012). Applying stable isotopes to examine food-web structure: an overview of analytical tools. *Biological Reviews*, **87**, 545–562.

Li, M., Huang, W. E., Gibson, C. M., Fowler, P. W., and Jousset, A. (2013). Stable isotope probing and Raman spectroscopy for monitoring carbon flow in a food chain and revealing metabolic pathway. *Analytical Chemistry*, **85**, 1642–1649.

Lundgren, J. G. and Fergen, J. K. (2014). Predator community structure and trophic linkage strength to a focal prey. *Molecular Ecology*, **23**, 3790–3798.

MacArthur, R. H. (1972). Strong, or weak, interations? *Transactions of the Connecticut Academy of Arts and Sciences*, **44**, 177–188.

Manefield, M., Whiteley, A. S., Griffiths, R. I., and Bailey, M. J. (2002). RNA stable isotope probing, a novel means of linking microbial community function to phylogeny. *Rapid Communication in Mass Spectrometry*, **16**, 2179–2183.

Maxfield, P. J. and Evershed, R. P. (2011). Phospholipid fatty acid stable isotope probing techniques in microbial ecology. In *Stable Isotope Probing and Related Technologies*, ed. J. C. Murrell and A. S. Whiteley, American Society of Microbiology Press, pp. 37–71.

May, R. M. (1972). Will a large complex system be stable? *Nature*, **238**, 413–414.

May, R. M. (1974). *Stability and Complexity in Model Ecosystems*. Princeton, NJ: Princeton University Press.

Mayali, X., Weber, P. K., Brodie, E. L., *et al*. (2012). High-throughput isotopic analysis of RNA microarrays to quantify microbial resource use. *ISME Journal*, **6**, 1210–1221.

Memmott, J. (2009). Food webs: a ladder for picking strawberries or a practical tool for practical problems? *Philosophical Transactions of the Royal Society B: Biological Sciences*, **364**, 1693–1699.

Molkentin, J. and Giesemann, A. (2007). Differentiation of organically and conventionally produced milk by stable isotope and fatty acid analysis. *Analytical Bioanalytical Chemistry*, **388**, 297–305.

Moore, J. C. and de Ruiter, P. C. (2012). *Energetic Food Webs*. Oxford, UK: Oxford University Press.

Moore, J. C., de Ruiter, P. C., and Hunt, H. W. (1993). Soil invertebrate/micro-invertebrate interactions: disproportionate effects of species on food web structure and function. *Veterinary Parasitology*, **48**, 247–260.

Mora, C. A., Paunescu, D., Grass, R. N., and Stark, W. J. (2014). Silica particles with encapsulated DNA as trophic tracers. *Molecular Ecology Resources*, **15**(2), 231–241, doi:10.1111/1755–0998.12299.

Müller-Navarra, D. C., Brett, M. T., Liston, A. M., and Goldman, C. R. (2000). A highly unsaturated fatty acid predicts carbon transfer between primary producers and consumers. *Nature*, **403**, 74–77.

Neher, D. A. (2010). Ecology of plant and free-living nematodes in natural and agricultural soil. *Annual Review of Phytopathology*, **48**, 371–394.

Neidig, N., Paul, R. J., Scheu, S., and Jousset, A. (2011). Secondary metabolites of *Pseudomonas fluorescens* CHA0 drive complex non-trophic interactions with bacterivorous nematodes. *Microbial Ecology*, **61**, 853–859.

Neutel, A.-M., Heesterbeek, J. A. P., and de Ruiter, P. C. (2002). Stability in real food webs: weak links in long loops. *Science*, **296**, 1120–1123.

Ngosong, C., Gabriel, E., and Ruess, L. (2012). Use of the signature fatty acid 16:1ω5 as a tool to determine the distribution of arbuscular mycorrhizal fungi in soil. *Journal of Lipids*, **2012**. doi:10.1155/2012/236807.

Ngosong, C., Gabriel, E., and Ruess, L. (2014). Collembola grazing on arbuscular mycorrhiza fungi modulates nutrient allocation in plants. *Pedobiologia (Jena)*, **57**, 171–179.

O'Neill, R. V. (1969). Indirect estimation of energy fluxes in animal food webs. *Journal of Theoretical Biology*, **22**, 284–290.

Paine, R. T. (1980). Food webs: linkage, interation strength and community infrastructure. *Journal of Animal Ecology*, **49**, 667–685.

Paine, R. T. (1992). Food-web analysis through field measurement of per capita interaction strength. *Nature*, **355**, 73–75.

Pausch, J., Kramer, S., Scharroba, A., *et al*. (2015). Small but active: pool size does not matter for carbon incorporation in belowground food webs. *Functional Ecology*, **30** (3), 479–489.

Pimm, S. L. (1982). *Food Webs*. London, UK: Chapman and Hall.

Pollierer, M. M., Scheu, S., and Haubert, D. (2010). Taking it to the next level: trophic transfer of marker fatty acids from basal resource to predators. *Soil Biology and Biochemistry*, **42**, 919–925.

Pompanon, F., Deagle, B. E., Symondson, W. O. C., *et al.* (2012). Who is eating what: diet assessment using next generation sequencing. *Molecular Ecology*, **21**, 1931–1950.

Pond, D. W., Leakey, R. J. G., and 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.

Radajewski, S., Ineson, P., Parekh, N. R., and Murrell, J. C. (2000). Stable-isotope probing as a tool in microbial ecology. *Nature*, **403**, 646–649.

Ruess, L. (2003). Nematode soil faunal analysis of decomposition pathways in different ecosystems. *Nematology*, **5**, 179–181.

Ruess, L. and Chamberlain, P. M. (2010). The fat that matters: soil food web analysis using fatty acids and their carbon stable isotope signature. *Soil Biology and Biochemistry*, **42**, 1898–1910.

Ruess, L. and Ferris, H. (2004). Decomposition pathways and successional changes. *Nematology Monographs and Perspectives*, **2**, 547–556.

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

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

Ruess, L., Tiunov, A., Haubert, D., *et al*. (2005a). Carbon stable isotope fractionation and trophic transfer of fatty acids in fungal based soil food chains. *Soil Biology Biochemistry*, **37**, 945–953.

Ruess, L., Schütz, K., Haubert, D., *et al*. (2005b). Application of lipid analysis to understand trophic interactions in soil. *Ecology*, **86**, 2075–2082.

Ruess, L., Schütz, K., Migge-Kleian, S., *et al*. (2007). Lipid composition of Collembola and their food resources in deciduous forest stands: implications for feeding strategies. *Soil Biology and Biochemistry*, **39**, 1990–2000.

Sanders, D., Jones, C. G., Thébault, E., *et al*. (2014). Integrating ecosystem engineering and food webs. *Oikos*, **123**, 513–524.

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

Scheu, S., Ruess, L., and Bonkowski, M. (2005). Interactions between microorganisms and soil micro- and mesofauna. In *Microorganisms in Soil: Roles in Genesis and Functions*, ed. F. Buscot and A. Varma, Berlin: Springer-Verlag, pp. 253–277.

Schröter, D., Wolters, V., and de Ruiter, P. C. (2003). C and N mineralisation in the decomposer food webs of a European forest transect. *Oikos*, **102**, 294–308.

Seeber, J., Rief, A., Seeber, G. U. H., Meyer, E., and Traugott, M. (2010). Molecular identification of detritivorous soil invertebrates from their faecal pellets. *Soil Biology Biochemistry*, **42**, 1263–1267.

Selakovic, S., de Ruiter, P. C., and Heesterbeek, H. (2014). Infectious disease agents mediate interaction in food webs and ecosystems. *Proceedings of the Royal Society B: Biological Sciences*, **281**(1777), 20132709.

Sint, D., Raso, L., and Traugott, M. (2012). Advances in multiplex PCR: balancing primer efficiencies and improving detection success. *Methods in Ecology and Evolution*, **3**, 898–905.

Stübing, D., Hagen, W., and 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 and Oceanography*, **48**, 1685–1700.

Symondson, W. O. C. (2012). The molecular revolution: using polymerase chain reaction based methods to explore the role of predators in terrestrial food webs. In *Biodiversity and Insect Pests: Key Issues for Sustainable Management*, ed. G. M. Gurr, S. D. Wratten, W. E. Snyder, and D. M. Y. Read, New York: Wiley and Sons, pp. 166–184.

Thiemann, G. W., Iverson, S. J., and Stirling, I. (2008). Polar bear diets and arctic marine food webs: insights from fatty acid analysis. *Ecological Monographs*, **78**, 591–613.

Traugott, M., Kamenova, S., and Ruess, L. (2013). Empirically characterising trophic networks: what emerging DNA-based methods, stable isotope and fatty acid analyses can offer. *Advances in Ecological Research*, **49**, 177–224, doi:10.1016/B978-0–12-420002–9.00003–2.

van Dooremalen, C. and Ellers, J. (2010). A moderate change in temperature induces changes in fatty acid composition of storage and membrane lipids in a soil arthropod. *Journal Insect Physiology*, **56**, 178–184.

Vestheim, H. and Jarman, S. N. (2008). Blocking primers to enhance PCR amplification of rare sequences in mixed samples: a case study on prey DNA in Antarctic krill stomachs. *Frontiers in Zoology*, **5**, 12, DOI: 10.1186/1742–9994-5–12.

von Berg, K., Traugott, M., and Scheu, S. (2012). Scavenging and active predation in generalist predators: a mesocosm study employing DNA-based gut content analysis. *Pedobiologia (Jena)*, **55**, 1–5.

Vuvic-Pestic, O. K., Birkhofer, K., Rall, B. C., Scheu, S., and Brose, U. (2010). Habitat structure and prey aggregation determine the functional response in a soil predator–prey interaction. *Pedobiologica*, **53**, 307–312.

Waldner, T. and Traugott, M. (2012). DNA-based analysis of regurgitates: a noninvasive approach to examine the diet of invertebrate consumers. *Molecular Ecology Resoures*, **12**, 669–675.

Waldner, T., Sint, D., Juen, A., and Traugott, M. (2013). The effect of predator identity on post-feeding prey DNA detection success in soil-dwelling macro-invertebrates. *Soil Biology Biochemistry*, **63**, 116–123.

Wallinger, C., Staudacher, K., Schallhart, N., *et al.* (2013). The effect of plant identity and the level of plant decay on molecular gut content analysis in a herbivorous soil insect. *Molecular Ecology Resoures*, **13**, 75–83.

Wardle, D. A., Verhoef, H. A., and Clarholm, M. (1998). Trophic relationships in the soil microfood-web: predicting the responses to a changing global environment. *Global Change Biology*, **4**, 713–727.

White, D. C., Stair, J. O., and Ringelberg, D. B. (1996). Quantitative comparisons of in situ microbial biodiversity by signature biomarker analysis. *Journal of Industrial Microbiology*, **17**, 185–196.

Williams, C. T. and Buck, C. L. (2010). Using fatty acids as dietary tracers in seabird trophic ecology: theory, application and limitations. *Journal of Ornithology*, **151**, 531–543.

Winemiller, K. O. and Layman, C. A. (2005). Food web science: moving on the path from abstraction to prediction. In *Dynamic Food Webs*, ed. P. C. de Ruiter, J. C. Moore, and V. Wolters, San Diego: Academic Press, pp. 10–23.

Wirta, H. K., Hebert, P. D. N., Kaartinen, R., *et al.* (2014). Complementary molecular information changes our perception of food web structure. *Proceedings of the National Academy of Sciences of the United States of America*, **111**, 1885–1890.

Yeates, G. W. (2010). Nematodes in ecological webs. In *Encyclopedia of Life Sciences (ELS)*, New York: John Wiley and Sons. doi:10.1002/9780470015902.a0021913.

Yeates, G. W., Bongers, T., De Goede, R. G., Freckman, D. W., and Georgieva, S. S. (1993). Feeding habits in soil nematode families and genera: an outline for soil ecologists. *Journal of Nematology*, **25**, 315–331.

Yeates, G. W., Ferris, H., Moens, T., and van der Putten, W. H. (2009). The role of nematodes in ecosystems. In *Nematodes as Environmental Bioindicators*, ed. M. Wilson and T. Kakouli-Duarte, Wallingford, UK: CABI, pp. 1–44.

Zelles, L. (1999). Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: a review. *Biology and Fertility of Soils*, **29**, 111–129.

Zhang, G.-F., Lü, Z.-C., Wan, F.-H., and Lövei, G. L. (2007). Real-time PCR quantification of *Bemisia tabaci* (Homoptera: Aleyrodidae) B-biotype remains in predator guts. *Molecular Ecology Notes*, **7**, 947–954.