Microbial–Faunal Interactions in the Rhizosphere

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Abstract

Soils are home for a huge variety of organisms that are profoundly enriched in the rhizosphere. The most abundant ones, microbial bacteria (and to a lesser extent archaea) and fungi, directly compete for plant-derived nutrients that they use for reproduction. Predators of these minute microorganisms control their abundances, community structure and activity. Microbial protists, faunal nematodes and microarthropods are arguably the main bacterial and fungal predators, but also other groups including enchytraeids and even predatory bacteria, fungi and viruses contribute to microbial mortality. In this chapter, we introduce the major predators of microorganisms, their specific interactions with bacteria and fungi, and how predation on microorganisms affects nutrient cycling and eventually plant performance. We focus on protists and nematodes as the key microbial predators. We exemplify how this knowledge helps at better understanding microbial–faunal interactions, and how interactions among those

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microbial predators affect soil food webs. Overall, we show that the diversity of microbial predators is key to control rhizosphere microbiomes and, eventually, governs plant performance.

12.1 Introduction

Without soils there would not be human life. On soils we live and from soils we obtain most of our food. But soils are often still a mystery. We are well aware of the abiotic differences and, considering the importance of agricultural food production, we have gained profound knowledge on the importance of water and nutrients for the growth of plants. But our dependence on agriculturally produced food has also enhanced our knowledge on many other aspects in soil, including some biotic components. Plant pathogens are the most obvious example and a vast amount of knowledge has accumulated on pathogenic fungi, oomycetes, bacteria, nematodes and many more. This knowledge is needed as plant pathogens are causing profound economic losses, which has even resulted in mass starvation as illustrated by the Irish famine caused by the oomycete *Phytophthora infestans*. Yet, the rhizosphere contains overwhelming numbers of non-pathogenic (microbial) organisms with many of them directly mutually benefitting plant performance, such as mycorrhizal fungi and rhizobial bacteria. Most organisms, however, are thought to be neutral to plants, at least without directly impacting plant performance. This link is modified when higher trophic levels are included as they prey upon these 'plant-neutral' organisms. High trophic-level predators change prey communities, thereby releasing nutrients that benefit plants. These trophic interactions between microorganisms and their predators are the focus of this chapter and shown in Fig. 12.1.

12.2 Rhizosphere Organisms and Their Microbial Prey

12.2.1 Definitions and Background

Before going into more detail about *Microbial—faunal interactions*, we first define some terms used here. Obviously, the most prevalent term needing definition is *fauna* as it is already present in the title. The term fauna captures all animal life in contrast to flora (plants). *Microorganisms* include other life forms, especially pro-karyotic bacteria and archaea and eukaryotic fungi. *Protists* that include all other single-celled eukaryotes are also microorganisms including *protozoa* (heterotrophic single-celled organisms) and algae (autotrophic mostly single-celled organisms) (Geisen et al. 2018). We only recently obtained an understanding of the main taxa in soils and abiotic determinants, which has been identified to be soil moisture (Oliverio et al. 2020). Note that many organisms are mixotrophic and therefore *protists* can accommodate more precisely the entity of single-celled organisms than *protozoa* and *algae* (Geisen et al. 2018). Protozoa as the major soil-inhabiting
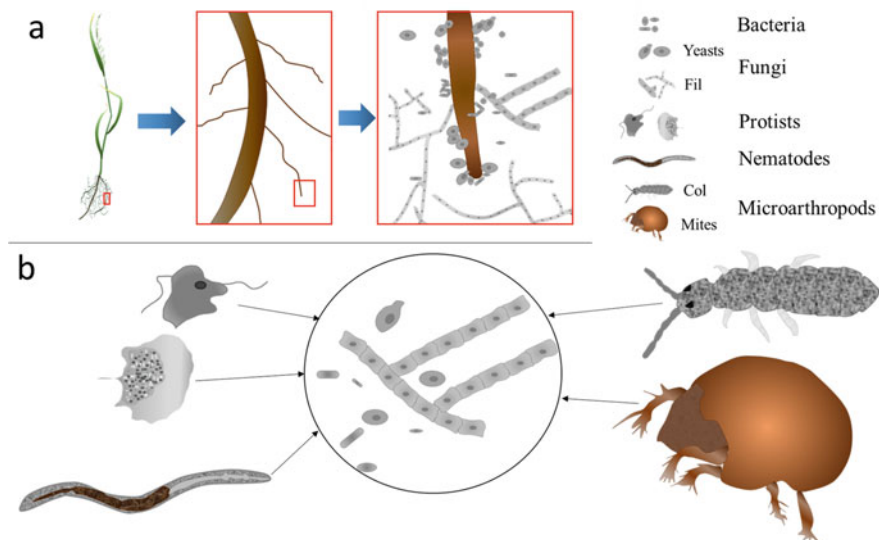
Fig. 12.1 (**a**) A representation of the rhizosphere and the associated microbiome; (**b**) Protists and microfauna as predators and thereby controllers of the rhizosphere microbiome

protists (but also algae seem to contribute to soil protist communities as recently shown (Seppey et al. 2017)) have often been divided into (naked and testate) amoebae, flagellates and ciliates. Protists are, unlike all other microbial groups, paraphyletic (having multiple phylogenetic origins) and are placed across the eukaryotic tree of life; fungi, animals and plants are single monophyletic (a single phylogenetic origin) clades within a phylogenetically diverse protist backbone. *Nematodes*, or roundworms, are the most abundant animals on Earth (van den Hoogen et al. 2019) and form a species-rich, trophically diverse phylum. Within the phylum Nematoda, lineages of predatory, omnivorous, fungivorous, bacterivorous and plant-parasitic nematodes arose independently multiple times (Quist et al. 2015; Schratzberger et al. 2019). Nematodes are abundant in nearly any soil, especially in colder, carbon-rich soils (van den Hoogen et al. 2019) and usually all trophic groups are represented in one soil sample (Fig. 12.2). In a hand full of soil, for example, you can expect from 500 to 20,000 nematodes (van den Hoogen et al. 2019). Because plant-roots leak water with nutrients into the soil, nematode densities in the close vicinity of plant roots are typically four to five times higher compared to the surrounding bulk soil. Yet, the diversification of many soil faunal groups goes even beyond the well-known species-rich arthropods. For instance, species numbers of nematode might be close to 1,000,000, of collembolans 10,000s and 100,000s—just in terrestrial soils (Geisen et al. 2019; Orgiazzi et al. 2016)!
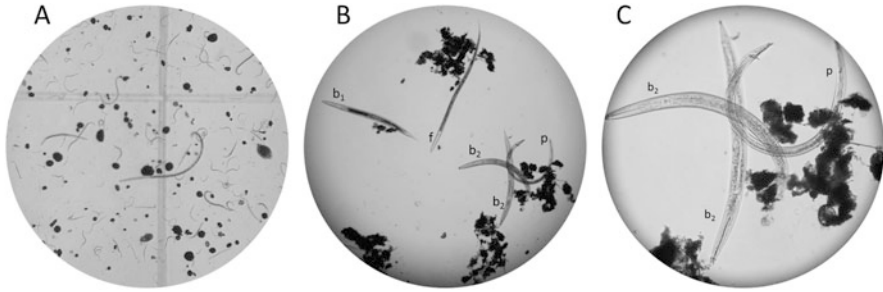
Fig. 12.2 Nematode suspension (**a**) containing diverse taxa at 40× magnification, (**b**) 100× magnification and (**c**) 200× magnification. For (**a**) and (**b**) trophic groups are shown Bacterivores (b_1: Rhabditidae and b_2: *Acrobeles sp.*, Cephalobidae), a fungivore (f) and a plant-parasite (p)

12.2.2 Why Are There Trophic-Level Interactions in Soils?

Mineral soils often lack (available) carbon, which is the key component for organic substances. One pathway carbon is entering soils is via plant-derived aboveground litter, which forms the base for the decomposer pathway. Another pathway is via relatively easily decomposable exudates and other root-derived components (Marschner 2012)—the focus of this chapter (note, these pathways are not necessarily disconnected and in many ways are linked). These compounds catalyse life in soils (Marschner 2012). Rhizospheres are highly enriched in soil biota, as microbial bacteria and fungi use root-derived carbon compounds for growth (Garbeva et al. 2008). These organisms compete for nutrients and are prone to predation by higher trophic levels and lysis from viral infections. Among organisms from higher trophic levels, protists are to be highlighted as the main predators of bacteria, while nematodes and collembolans are assumed to be the major fungal predators (de Ruiter et al. 1995). But there are plenty of other trophic interactions among microorganisms and fauna (Fig. 12.1b) that we will subsequently introduce. We will focus predominantly on protists and nematodes since these groups are assumed to consume most microbial biomass, but we will also include examples of enchytraeids and microarthropods, specifically mites and springtails. We acknowledge that other soil fauna, such as Tardigrada, Protura, Diplura, Isopoda and earthworms, consume microorganisms, at least in part of their diet. We recommend reading the Global Soil Biodiversity Atlas for additional information (Orgiazzi et al. 2016) and a recent comprehensive book (Nielsen 2019).

12.3 Consumers of Microorganisms

12.3.1 Bacterial Feeders

Protists and nematodes are suggested to be by far the major bacterial feeders in soils. Especially, amoebae are assumed to consume much of the bacterial biomass (Clarholm 1981; de Ruiter et al. 1995). Protists grow fast and therefore can respond quickly to increased bacterial abundances after changes in the environment, such as increased moisture and nutrient input. In a pioneering study, Clarholm (1981) found that protist abundances, especially of amoebae (Fig. 12.3), followed the patterns of bacteria after rainfall with abundances peaking within a few days. There have been cumulative reviews detailing the pivotal role protists play in controlling bacterial biomasses and we refer to those for further information (Bonkowski 2004; Clarholm 1985; Geisen et al. 2018).

Nematodes are the most abundant animals on Earth (van den Hoogen et al. 2019). They are key bacterivores and in some situations of higher importance as bacterivores than protists (Griffiths 1990). About half of all individuals within a nematode community are bacterial feeding (van den Hoogen et al. 2019; Quist et al. 2019). In the field of nematode ecology, many studies use the "functional group" or the "functional guild" approach. A "functional group", or trophic group, is a group
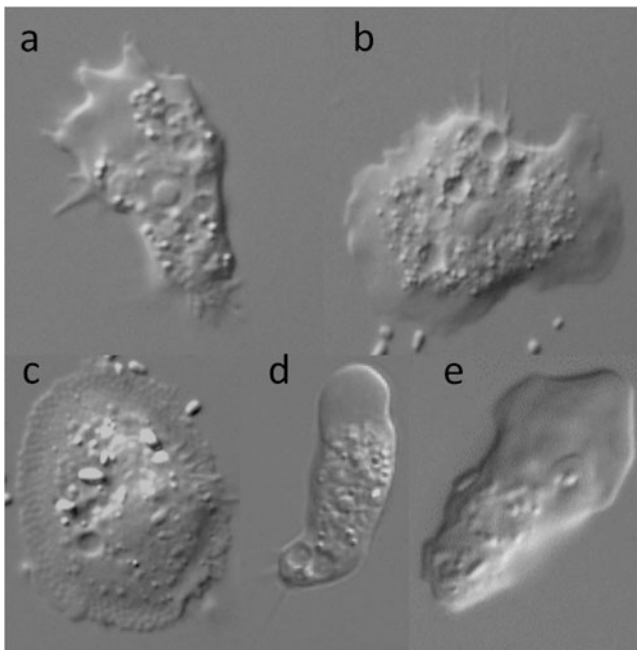
Fig. 12.3 Representation of common mostly bacterivorous amoeboid protists in soils. (**a**) *Acanthamoeba* sp.; (**b**) *Flamella* sp.; (**c**) *Cochliopodium* sp.; (**d**) *Allovahlkampfia* sp.; (**e**) *Stenamoeba* sp.

of organisms with the same feeding habits. For example, plant-parasitic nematodes in soil food webs are placed in the first trophic level, bacterivorous nematodes in the second and predatory nematodes in the third (Holtkamp et al. 2008). Based on their ecological characteristics, nematodes have been classified along a colonizer-persister (cp) scale (Bongers 1990). Nematodes in c-p category 1 and 2 can be seen as opportunistic, and have short generation times. On the other hand, taxa in c-p categories 4 and 5 reproduce relatively slow with single or a few eggs, have a long generation time and are highly sensitive to environmental stressors. Members of c-p categories 4 and 5 can be seen as K-strategists. A "functional guild" is a refinement of the functional group concept, and is defined as "taxa with the same feeding habits, and inferred function, in the food web" (Ferris et al. 2001). Examples of functional guilds are bacterivores (Ba_x), fungivores (Fu_x), carnivores (Ca_x) and omnivores (Om_x), whereby x can be 1–5 on the cp scale. One of the underlying assumptions is that members of such a guild show the same feeding habits. This might be roughly correct, as bacterivores feed (mainly) on bacteria, but for a better ecological understanding, it should be realized that (at least) individual taxa within this functional guild feed on distinct parts of the bacterial community. Most bacterivorous nematode taxa are r-strategist and placed in cp group 1 or 2. Feeding habits of nematodes are based on the nematodes' mouth morphologies (Yeates et al. 1993). The diversity of mouth morphologies of bacteria feeders points at different feeding strategies. The mouth of *Cephalobus, Anaplectus* and *Cruznema*, for example, are relatively simple cavities, whereas the heads of *Acrobeles* and *Wilsonema* have an elaborated labial region. Modified lips are called probolae, these fringe- or flap-like structures are probably used to capture their prey (Fig. 12.4a). Therefore, studies in rhizosphere ecology interested in taxon-specific interactions should analyse trophic interactions at lower taxonomic levels than functional groups or guilds.

Arguably to a lesser extent also other soil faunal groups feed on and control abundances of bacteria, including Collembola (Crotty et al. 2011; Rusek 1998), some mite species (Pollierer et al. 2012) and enchytraeids (O'Connor 1967; Waldrop et al. 2012).

New potentially important players that reduce bacterial growth have emerged in the past decade and it might be that intraguild microbial predation plays a key role in controlling bacterial abundances in soil. Predatory bacteria such as Myxobacteria (Petters et al. 2018) and viruses have been suggested to contribute substantially to bacterial reductions in the rhizosphere (Johnke et al. 2014).

12.3.2 Fungal Feeders

Until recently, protists were not considered as fungal feeding organisms in ecological studies (de Ruiter et al. 1995; Hunt et al. 1987). Yet, there is accumulating evidence that many phylogenetically diverse protist groups can feed on fungi (Geisen et al. 2016), some even being obligate fungal feeders (Petz et al. 1985, 1986). The biomass of fungal feeding protists in some systems might even be as large as the biomass of bacterial feeding protists (Ekelund 1998), underlying their
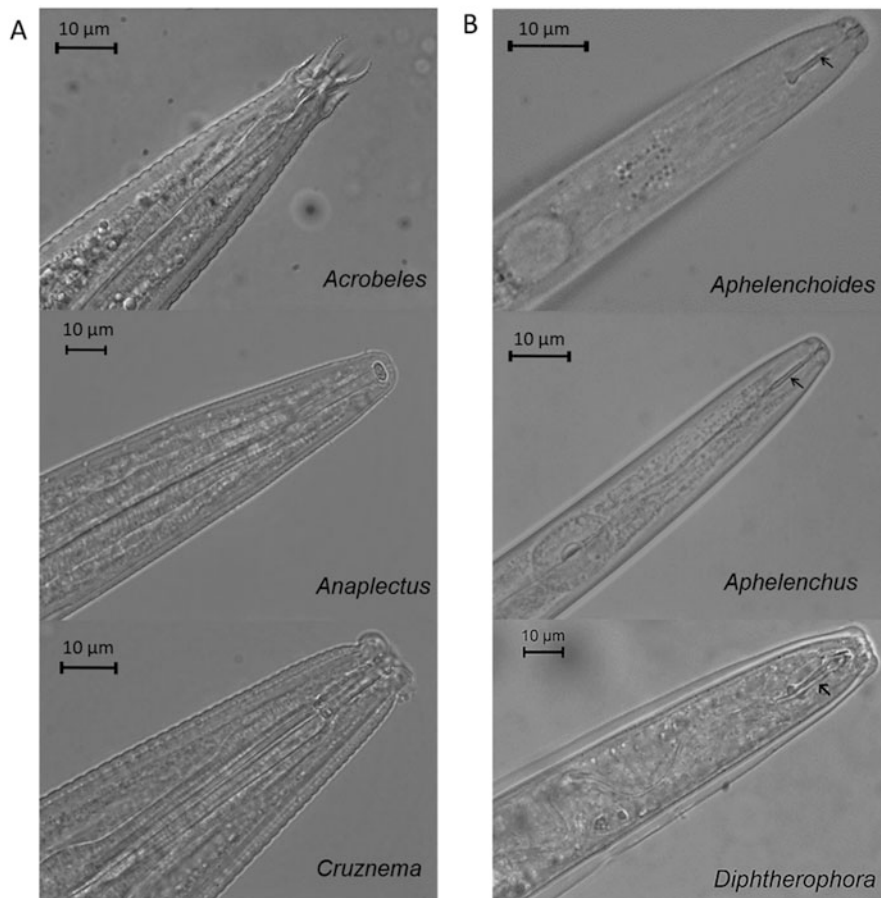
Fig. 12.4 Pictures of nematode head regions of (**a**) the bacterivorous genera *Acrobes*, *Anaplectus* and *Cruznema* and (**b**) the fungivorous genera *Aphelenchoides*, *Aphelenchus* and *Diphtherophora* (1000× magnification). Bacterivores usually have simple and small mouth cavities, but *Acrobeles* is characterized by three high labial probolae, which adopt a V-shape under the light microscope. Fungivores have a hardened piercing device called stylet that allows them to penetrate fungal tissue (indicated by arrows). Pictures were taken by Hanny van Megen, from the Laboratory of Nematology (WUR)

importance in soil food webs. It has yet to be determined if certain protist taxa can be used as biocontrol agents of pathogenic fungi (Chakraborty and Old 1982; Old and Oros 1980).

Again, feeding habits of nematodes are based on the nematode mouth morphologies (Yeates et al. 1993). All fungivorous nematodes are equipped with a piercing device that is used to puncture the fungal cell wall. The head regions and the so-called stylets to pierce fungal tissue of three fungivorous nematode genera are shown in Fig. 12.4b. Stylets of fungivores are very tiny compared to those of

plant-parasitic nematodes and look rather similar at first sight. Based on small subunit rDNA sequencing data, nematodes have been classified into 12 major clades, and fungivorous nematodes are found in four different clades (Quist et al. 2015). To nematode taxonomists, the morphologies of these devices are distinct: *Aphelenchus* (Clade 12) and *Aphelenchoides* (Clade 10) both have a stomatostyle, whereas *Diphtherophora* (Clade 1) has an onchiostyle. Distinct stylets could have led to different food preferences and feeding behaviour (see also Quist et al. 2014).

Collembola are often suggested to be specialist predators of fungi (de Ruiter et al. 1995) and several collembolan taxa are important fungal feeders (Rusek 1998). Mites, especially Oribatida, are also major consumers of fungi (Pollierer et al. 2012).

12.3.3 Protist Feeders

Much less knowledge exists on the predators of protists. Predatory nematodes have been assumed to be the main faunal group feeding on protists (de Ruiter et al. 1995). The mouth cavity of predatory nematodes from the Mononchida group allows them to ingest protist prey, predatory nematodes from the Dorylaimida group use a piercing device to suck body fluids from protists (Yeates et al. 1993). The assumption that nematodes are the major predators of protists was supported by a nicely conducted study that used stable isotope-labelled protists (Crotty et al. 2012). This study also found that Collembola, mites, earthworms and insect larvae were enriched in protist-derived isotopes suggesting a wide variety of animals to use protists as prey (Crotty et al. 2012). Collembola were already shown to feed on protists (Rusek 1998) suggesting that in systems with high collembolan numbers, this link might become of ecological importance.

Of arguably even higher importance of protist control is an intraguild control by protists themselves. Amoebae are assumed to be the main predators of flagellates (de Ruiter et al. 1995) and there is profound evidence that many larger protists depend on smaller protists as the main nutrient source (Geisen 2016). For instance, several larger amoebae, such as *Thecamoeba* spp., varioseans and vampyrellids can only be cultivated when supplemented with eukaryotic prey, especially smaller protists (Berney et al. 2015; Hess et al. 2012; Page 1977). However, we are missing a quantitative idea on the importance of protist intraguild predation and arguably, of the main nutrient flows from protists to other trophic levels.

12.3.4 Species-Specific Differences in Microbial Prey Preferences Among Soil Fauna

Most predatory groups can specifically select their prey items. Therefore, the classical food web representations give an overview of the suggested overall function of certain taxonomic groups, with details missing. Protists, for instance, specifically feed on their microbial prey with some groups of bacteria being preferentially fed upon while others are avoided. For instance, gram-positive bacteria and

secondary metabolite-producing pseudomonads are hardly being fed upon (Jousset 2012; Jousset et al. 2006), leading to changes in bacterial communities in the presence of protists (Bonkowski 2004; Jousset et al. 2009; Rosenberg et al. 2009). Protists also interact in a species-specific way with bacteria (Schulz-Bohm et al. 2017) suggesting that protist species have their own feeding niche.

Although nematodes' feeding preferences are usually considered at the trophic group level, different feeding preferences exist within trophic groups (Ettema 1998; Maharning et al. 2009; Neher et al. 2005; Porazinska et al. 1999; Quist et al. 2014). For example, the invasive plant *Solidago gigantea* was shown to give rise to a local increase in the fungal biomass in invaded habitats. Remarkably, only one fungivorous family (Aphelenchoididae) benefitted from the increase in fungal biomass, whereas the densities of two other fungivorous nematode families (Aphelenchidae and Diphtherophoridae) did not change at all (Quist et al. 2014). Nematode group-specific feeding on fungal groups that are promoted by *S. gigantea* is likely underlying this phenomenon.

Dietary choices of two bacteria feeding nematodes in vitro—*Mesorhabditis* sp. and *Acrobeloides* sp.—were recently shown to control their lifespan and reproduction rates. Preferences of both bacterivores were strongly related to water content, growth rate and metabolite concentration of the bacterial cells. Especially, *Mesorhabditis* had a clear preference for *Pseudomonas fluorescens* over three other bacterial taxa (Liu et al. 2017). The dietary choices of microbial feeding nematodes could control the structure and ecological functions of soil microbial communities.

Collembolan species differ in their preferred prey and actively select for specific microbial taxa (Jørgensen et al. 2003). For instance, Haubert et al. (2006) found that bacterial taxa differ in their nutritional value for collembolan predators. Even more, the diet of Collembola is species specific and expands widely beyond fungi and bacteria (Chahartaghi et al. 2005). Some Collembolans were found to feed on protists (Crotty et al. 2012). Also, mites do not equally feed on all prey taxa but select in a mite species-specific manner for their preferred fungal prey and can even incorporate litter and bacteria (Pollierer et al. 2012). Similarly, while most Collembola are suggested to feed predominantly on fungi, several species such as *Tomocerus baudoti* preferentially consume bacteria (Pollierer et al. 2012). Also, other animals such as mites feed on different fungi and it is assumed that dark pigmented fungi are generally a preferred food source than non-pigmented fungi (Maraun et al. 2003).

12.4 Microbial Predation and the Importance for Plant Performance

12.4.1 Faunal Interactions with Plant Pathogenic Microorganisms and Viruses

Fauna including microbial protists can directly protect plants by feeding on plant pathogenic microorganisms. Protists have recently been suggested to prey on plant pathogenic bacteria and thereby improve plant health (Xiong et al. 2020). Xiong et al. (2020) also suggested that the composition of protists early at plant growth can determine plant health later during growth, suggesting profound importance of protist predation on controlling pathogens. Some larger protist species can consume plant pathogenic fungi (Chakraborty and Old 1982; Geisen et al. 2016), yet their role as a biocontrol agent is debated (Chakraborty et al. 1983) and has yet to be confirmed.

Nematodes are mainly generalist feeders and microbial feeding nematodes feed also on plant pathogenic fungi and bacteria. As a biocontrol, the effect of nematodes feeding on obligate plant pathogens is probably negligible, since pathogens have parts that are inside the plant and out of reach of nematode feeding. On the other hand, plant-parasitic nematodes are important transmitters of soil-borne diseases (Holterman et al. 2017). For example, *Paratrichodorus* species have been found to transmit Tobacco rattle virus and Pea early browning virus (Taylor and Brown 1997) and in vineyards, the nepovirus Grapevine fanleaf virus, causal agent of grapevine fanleaf degeneration disease, spreads mainly via the nematode vector *Xiphinema index* (Hao et al. 2018). Soil bacteria and spores of fungi stick to the cuticula of nematodes, or stay alive in the nematode gut, increasing the transmission of bacteria. Entomopathogenic nematodes, *Steinernema* and *Heterorhabditis*, for example, keep bacteria in their gut, and release them when the nematode has entered an insect host, and thereby killing the insect (Bal et al. 2014). *Caenorhabditis elegans* was also found to have the ability to vector pathogenic bacteria (Anaid Diaz and Restif 2014). Yet, the role of this transmission of microorganisms on plant performance remains unknown. Plant pathogenic fungi are readily consumed by many fungivorous micro-arthropods which can result in profound increases in plant fitness (Sabatini and Innocenti 2001).

12.4.2 Faunal Interactions with Plant Mutualistic Microorganisms

Protists are so far not reported to prey on plant beneficial organisms. Protists are suggested to have positive effects on plant growth-promoting secondary metabolite-producing bacteria as they preferentially prey upon their bacterial competitors (Bonkowski 2004; Jousset et al. 2009).

Among the most well-known examples of plant mutualists are mycorrhizal fungi, particularly arbuscular mycorrhizal fungi (AMF). These fungi are obligate root symbionts and infect about 80% of all plant species. Mycorrhiza is the food source

of nearly all fungivores in the rhizosphere, an interaction that can potentially influence plant performance. Some nematodes feed on AMF. However, there seem to be species-specific differences in predation success of fungivorous nematodes on AMF. In vitro, for example, *Aphelenchoides saprophilus* multiplied on various AMF taxa, while *Tylolaimophorus* did not survive on any of these fungi (Ruess and Dighton 1996). While there are no studies reporting the active predation of protists on AMF, protists can indirectly affect the interaction between AMF and their plant host. In particular, protists have been shown to stimulate AMF, which resulted in positive effects on plants (Koller et al. 2013a, b).

Other common plant mutualistic microbial taxa are rhizobia, which were found to be reduced by protist predation (Heynen et al. 1988). However, as rhizobial bacteria are only affected in extremely high abundances and never consumed entirely (Danso and Alexander 1975), the effect on plant performance might be insignificant. However, as lower rhizobial colonization of plant roots was reported in the presence of high protist numbers in simplified systems (Ramirez and Alexander 1980), these interactions need further studies. While evidence of nematode predation on rhizobia is limited, nematodes might positively influence nodulation as they might serve as transport vehicles for rhizobia to better reach their hosts (Horiuchi et al. 2005).

Similar to nematodes, also microarthropods such as Collembola prey on AMF. However, it seems that Collembola preferentially prey on non-AMF fungal taxa and therefore might promote plant performance (Gange 2000).

12.4.3 Faunal Interactions with Plant Neutral Microorganisms

Plants need nutrients. And plants often compete with bacteria and fungi for the limited pool of especially nitrogen and phosphorus (Hodge et al. 2000). These nutrients would remain bound in microbial biomass especially as microbes are faster in exploiting available nutrients. However, predators release nutrients and over time plants take up an increasing fraction of available nutrients (Hodge et al. 2000). This predator-induced release of nutrients has been termed *microbial loop* with especially protists and in some conditions bacterivorous nematodes catalysing bacterial nutrient release (Bonkowski and Clarholm 2012; Rønn et al. 2012). However, the microbial loop should not be limited to these interactions and should encompass other microbial and faunal predators and prey.

12.5 Rhizosphere Food Webs Are Not Universally Identical

We often assume that food webs are universally identical and contain the same members with same links. However, this is unlikely to be the case and the single soil food web that is repetitively used in many systems is actually a specific food web for a shortgrass prairie in the United States (Hunt et al. 1987). Food webs are likely to differ as even microbial communities and their predators differ in their community structure depending on abiotic conditions (Garbeva et al. 2008), soil management

(Xiong et al. 2018), plant species and community identity (Garbeva et al. 2008; Ramirez et al. 2018), plant growth stage (Chap. 4) and many others. Especially, plants as sessile organisms are highly active in their selection of microbial community members and can recruit beneficial organisms such as upon pathogen attack (Berendsen et al. 2012). Even interactions of microbial predators can depend on soil type. For instance, protists might be more important bacterial predators than nematodes in clay-rich soils, while the opposite seems to be the case for very sandy soils (Rønn et al. 2012). However, differences in food-webs as well as microbial–faunal interactions among systems are rarely studied but should be prioritized in near-future efforts.

12.6 Non-Trophic Microbial–Faunal Interactions and Microbes That Prey on Fauna

There is increasing evidence that trophic interactions between microorganisms and their prey are just one piece of the puzzle that explains soil biodiversity. Evidently, there is competition in case microorganisms and fauna use the same prey items, such as protists and nematodes, that feed on bacteria. It has been proposed that protists might outcompete nematodes when feeding on bacteria as experimentally shown in a study investigating interactions between the ciliated protist *Colpoda steinii* and the nematode *Rhabditis* sp. (Griffiths 1986). Increased competitive ability of protists compared with nematodes is also supported by higher biomass gains when bacterial prey is increased (Griffiths and Caul 1993). Protist numbers usually increase faster than those of nematodes showing their higher turnover and potential importance in the initial control of bacterial abundances and bacterial community compositions (Christensen et al. 1992). However, nematodes become more dominant over time when bacterial abundances decrease suggesting that nematodes are more efficient bacterivores at lower resource availability, as proposed elsewhere (Griffiths 1990). In addition, nematodes might avoid competition by faster and more targeted migration to bacterial hotspots (Griffiths and Caul 1993). Even a direct non-trophic interaction between protists and nematodes were reported with both mutually inhibiting another (Neidig et al. 2010). These negative interactions were attributed to secondary metabolites produced by both protists and nematodes, suggesting a chemical warfare in order to increase competitiveness to feed on common bacterial prey (Neidig et al. 2010).

Beyond competition, there are direct trophic interactions between protists and nematodes. As detailed above, predatory nematodes are assumed to feed on protists (de Ruiter et al. 1995). On the other hand, some protist taxa can turn the tide and prey on nematodes. In initial studies, the giant amoeba *Theratromyxa weberi* was found to prey on plant pathogenic nematodes (Sayre 1973). Yet, the biocontrol efficiency was reported to be low and as these giant amoebae are hard to cultivate, research interest in these applied aspects faded. Later on, Yeates and Foissner (1995) discovered that two large testate amoebae, *Apodera vas* and *Difflugia lanceolata*, preyed upon a wide range of nematodes. More recently, Geisen et al. (2015b) found largely

by chance—we performed an experiment to test if small testate amoeba of the species *Cryptodifflugia operculata* are resistant to nematode predation due to their shell and can therefore outperform bacterivorous nematodes that compete for the same prey—that *C. operculata* actively hunts, kills and profits from ingesting nematode prey. Additional interactions between nematodes, protists and bacteria are nicely reviewed in Rønn et al. (2012).

There are several other examples of microorganisms that prey on larger fauna. Among the most well-known are nematophagous fungi that feed on nematodes (Gray 1987), while amoebophagous fungi feed on amoeboid protists (Michel et al. 2014). Entomopathogenic fungi can infect a wide range of fauna including insect larvae (Shah and Pell 2003) and a vast diversity of these organisms inhabit soils (Zimmermann 1986). Yet, their importance in food webs remains poorly understood. The same holds for other parasites, particularly protists that are highly diverse. In fact, the diversity of protists in the Neotropics was found to be dominated by parasitic lineages with most of them entirely being unknown to science (Mahé et al. 2017). This likely important function has only been found with emerging sequencing technologies (Bates et al. 2013; Geisen et al. 2015c; Grossmann et al. 2016) and these parasites were shown to inhabit different soil animal groups, including earthworms, mites and Collembola (Geisen et al. 2015a; Rusek 1998). This led Mahé et al. (2017) to propose that protists might be key drivers of animal diversity. Parasitic bacteria and fungi are also prevalent and can control soil fauna, such as bacteria reduce (plant-parasitic) nematode numbers (Siddiqui and Mahmood 1999). The functional importance of parasites in food webs, however, still needs to be quantified.

12.7 Open Gaps

While a lot of information on soil biodiversity has been accumulated, major gaps in our knowledge on soil biodiversity still exist (Cameron et al. 2018). New technologies help us fill gaps in our understanding particularly of the taxonomic diversity of bacteria and fungi, while research on the predators of these microbial groups is hardly increasing (Geisen et al. 2017). Especially, functional knowledge remains scarce as these studies need time-consuming and therefore expensive efforts. These include gut content analyses to determine species-specific feeding and stable isotope analyses to determine feeding and feeding importance.

Emerging technologies are in place to fill these gaps but it will still take several years (hopefully not decades) until we have filled our knowledge on the relative importance of predators of microorganisms, their role in structuring microbial communities and the importance in agriculture and natural ecosystems. Yet, this venue is promising and has to be pursued as it is of direct relevance for humanity. We might directly benefit from the possibility for directed applications of microbial predators to increase plant performance such as by increasing nutrient turnover and therefore increased plant uptake possibilities and by controlling microbe-induced plant diseases—leading to higher biomasses and lower disease incidence of plants.

References

Anaid Diaz S, Restif O (2014) Spread and transmission of bacterial pathogens in experimental populations of the nematode *Caenorhabditis elegans*. Appl Environ Microbiol 80:5411–5418

Bal HK, Taylor RAJ, Grewal PS (2014) Ambush foraging entomopathogenic nematodes employ 'sprinters' for long-distance dispersal in the absence of hosts. J Parasitol 100:422–432

Bates ST, Clemente JC, Flores GE, Walters WA, Parfrey LW, Knight R, Fierer N (2013) Global biogeography of highly diverse protistan communities in soil. ISME J 7:652–659

Berendsen RL, Pieterse CMJ, Bakker PAHM (2012) The rhizosphere microbiome and plant health. Trends Plant Sci 17:478–486

Berney C, Geisen S, Van Wichelen J, Nitsche F, Vanormelingen P, Bonkowski M, Bass D (2015) Expansion of the 'Reticulosphere': diversity of novel branching and network-forming amoebae helps to define Variosea (Amoebozoa). Protist 166:271–295

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

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

Bonkowski M, Clarholm M (2012) Stimulation of plant growth through interactions of bacteria and protozoa: testing the auxiliary microbial loop hypothesis. Acta Protozool 51:237–247

Cameron EK et al (2018) Global gaps in soil biodiversity data. Nat Ecol Evol 2(7):1042–1043

Chahartaghi M, Langel R, Scheu S, Ruess L (2005) Feeding guilds in Collembola based on nitrogen stable isotope ratios. Soil Biol Biochem 37:1718–1725

Chakraborty S, Old K (1982) Mycophagous soil amoeba: interactions with three plant pathogenic fungi. Soil Biol Biochem 14:247–255

Chakraborty S, Old KM, Warcup JH (1983) Amoebae from a take-all suppressive soil which feed on *Gaeumannomyces graminis tritici* and other soil fungi. Soil Biol Biochem 15:17–24

Christensen S, Griffiths BS, Ekelund F, Rønn R (1992) Huge increase in bacterivores on freshly killed barley roots. FEMS Microbiol Lett 86:303–309

Clarholm M (1981) Protozoan grazing of bacteria in soil—impact and importance. Microb Ecol 7:343–350

Clarholm M (1985) Interactions of bacteria, protozoa and plants leading to mineralization of soil nitrogen. Soil Biol Biochem 17:181–187

Crotty F, Blackshaw R, Murray P (2011) Tracking the flow of bacterially derived 13C and 15N through soil faunal feeding channels. Rapid Commun Mass Spectrom 25:1503–1513

Crotty F, Adl S, Blackshaw R, Murray P (2012) Protozoan pulses unveil their pivotal position within the soil food web. Microb Ecol 63:905–918

Danso SKA, Alexander M (1975) Regulation of predation by prey density: the protozoan *Rhizobium* relationship. Appl Environ Microbiol 29:515–521

de Ruiter PC, Neutel AM, Moore JC (1995) Energetics, patterns of interaction strengths, and stability in real ecosystems. Science 269:1257–1260

Ekelund F (1998) Enumeration and abundance of mycophagous protozoa in soil, with special emphasis on heterotrophic flagellates. Soil Biol Biochem 30:1343–1347

Ettema CH (1998) Soil nematode diversity: species coexistence and ecosystem function. J Nematol 30:159–169

Ferris H, Bongers T, De Goede RGM (2001) A framework for soil food web diagnostics: extension of the nematode faunal analysis concept. Appl Soil Ecol 18:13–29

Gange A (2000) Arbuscular mycorrhizal fungi, Collembola and plant growth. Trends Ecol Evol 15:369–372

Garbeva P, Elsas JD, Veen JA (2008) Rhizosphere microbial community and its response to plant species and soil history. Plant Soil 302:19–32

Geisen S (2016) The bacterial-fungal energy channel concept challenged by enormous functional versatility of soil protists. Soil Biol Biochem 102:22–25

Geisen S, Laros I, Vizcaíno A, Bonkowski M, de Groot GA (2015a) Not all are free-living: high-throughput DNA metabarcoding reveals a diverse community of protists parasitizing soil metazoa. Mol Ecol 24:4556–4569

Geisen S, Rosengarten J, Koller R, Mulder C, Urich T, Bonkowski M (2015b) Pack hunting by a common soil amoeba on nematodes. Environ Microbiol 17:4538–4546. https://doi.org/10.1111/1462-2920.12949

Geisen S, Tveit AT, Clark IM, Richter A, Svenning MM, Bonkowski M, Urich T (2015c) Metatranscriptomic census of active protists in soils. ISME J 9:2178–2190

Geisen S, Koller R, Hünninghaus M, Dumack K, Urich T, Bonkowski M (2016) The soil food web revisited: diverse and widespread mycophagous soil protists. Soil Biol Biochem 94:10–18

Geisen S et al (2017) Soil protistology rebooted: 30 fundamental questions to start with. Soil Biol Biochem 111:94–103

Geisen S et al (2018) Soil protists: a fertile frontier in soil biology research. FEMS Microbiol Rev 42:293–323

Geisen S et al (2019) A methodological framework to embrace soil biodiversity. Soil Biol Biochem 136:107536

Gray NF (1987) Nematophagous fungi with particular reference to their ecology. Biol Rev 62:245–304

Griffiths B (1986) Mineralization of nitrogen and phosphorus by mixed cultures of the ciliate protozoan *Colpoda steinii*, the nematode *Rhabditis* sp. and the bacterium *Pseudomonas fluorescens*. Soil Biol Biochem 18:637–641

Griffiths BS (1990) A comparison of microbial-feeding nematodes and protozoa in the rhizosphere of different plants. Biol Fertil Soils 9:83–88

Griffiths B, Caul S (1993) Migration of bacterial-feeding nematodes, but not protozoa, to decomposing grass residues. Biol Fertil Soils 15:201–207

Grossmann L et al (2016) Protistan community analysis: key findings of a large-scale molecular sampling. ISME J 10:2269–2279

Hao Z et al (2018) Arbuscular mycorrhiza affects grapevine fanleaf virus transmission by the nematode vector *Xiphinema index*. Appl Soil Ecol 129:107–111. https://doi.org/10.1016/j.apsoil.2018.05.007

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

Hess S, Sausen N, Melkonian M (2012) Shedding light on vampires: the phylogeny of vampyrellid amoebae revisited. PLoS One 7:e31165. https://doi.org/10.1371/journal.pone.0031165

Heynen CE, Van Elsas JD, Kuikman PJ, van Veen JA (1988) Dynamics of *Rhizobium leguminosarum biovar trifolii* introduced into soil; the effect of bentonite clay on predation by protozoa. Soil Biol Biochem 20:483–488

Hodge A, Robinson D, Fitter A (2000) Are microorganisms more effective than plants at competing for nitrogen? Trends Plant Sci 5:304–308

Holterman M et al (2017) Disparate gain and loss of parasitic abilities among nematode lineages. PLoS One 12:e0185445

Holtkamp R, Kardol P, van der Wal A, Dekker SC, van der Putten WH, de Ruiter PC (2008) Soil food web structure during ecosystem development after land abandonment. Appl Soil Ecol 39:23–34

Horiuchi J-I, Prithiviraj B, Bais HP, Kimball BA, Vivanco JM (2005) Soil nematodes mediate positive interactions between legume plants and rhizobium bacteria. Planta 222:848–857

Hunt HW et al (1987) The detrital food web in a shortgrass prairie. Biol Fertil Soils 3:57–68

Johnke J, Cohen Y, de Leeuw M, Kushmaro A, Jurkevitch E, Chatzinotas A (2014) Multiple micro-predators controlling bacterial communities in the environment. Curr Opin Biotechnol 27:185–190

Jørgensen HB, Elmholt S, Petersen H (2003) Collembolan dietary specialisation on soil grown fungi. Biol Fertil Soils 39:9–15. https://doi.org/10.1007/s00374-003-0674-6

Jousset A (2012) Ecological and evolutive implications of bacterial defences against predators. Environ Microbiol 14:1830–1843

Jousset A, Lara E, Wall LG, Valverde C (2006) Secondary metabolites help biocontrol strain *Pseudomonas fluorescens* CHA0 to escape protozoan grazing. Appl Environ Microbiol 72:7083–7090

Jousset A, Rochat L, Pechy-Tarr M, Keel C, Scheu S, Bonkowski M (2009) Predators promote defence of rhizosphere bacterial populations by selective feeding on non-toxic cheaters. ISME J 3:666–674

Koller R, Rodriguez A, Robin C, Scheu S, Bonkowski M (2013a) Protozoa enhance foraging efficiency of arbuscular mycorrhizal fungi for mineral nitrogen from organic matter in soil to the benefit of host plants. New Phytol 199:203–211

Koller R, Scheu S, Bonkowski M, Robin C (2013b) Protozoa stimulate N uptake and growth of arbuscular mycorrhizal plants. Soil Biol Biochem 65:204–210

Liu T, Yu L, Xu J, Yan X, Li H, Whalen JK, Hu F (2017) Bacterial traits and quality contribute to the diet choice and survival of bacterial-feeding nematodes. Soil Biol Biochem 115:467–474

Maharning AR, Mills AAS, Adl SM (2009) Soil community changes during secondary succession to naturalized grasslands. Appl Soil Ecol 41:137–147

Mahé F et al (2017) Parasites dominate hyperdiverse soil protist communities in Neotropical rainforests. Nat Ecol Evol 1:0091. https://doi.org/10.1038/s41559-017-0091

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. Eur J Soil Biol 39:85–95

Marschner P (2012) Rhizosphere biology. In: Marschner P (ed) Marschner's mineral nutrition of higher plants, 3rd edn. Academic Press, San Diego, pp 369–388. https://doi.org/10.1016/B978-0-12-384905-2.00015-7

Michel R, Walochnik J, Scheid P (2014) Article for the "free-living amoebae special issue": isolation and characterisation of various amoebophagous fungi and evaluation of their prey spectrum. Exp Parasitol 145:S131–S136

Neher DA, Wu J, Barbercheck ME, Anas O (2005) Ecosystem type affects interpretation of soil nematode community measures. Appl Soil Ecol 30:47–64

Neidig N, Jousset A, Nunes F, Bonkowski M, Paul RJ, Scheu S (2010) Interference between bacterial feeding nematodes and amoebae relies on innate and inducible mutual toxicity. Funct Ecol 24:1133–1138

Nielsen UN (2019) Soil fauna assemblages. Cambridge University Press, Cambridge

O'Connor F (1967) The enchytraeidae. In: Soil biology. Academic Press, New York, pp 213–257

Old KM, Oros JM (1980) Mycophagous amoebae in Australian forest soils. Soil Biol Biochem 12:169–175

Oliverio AM, Geisen S, Delgado-Baquerizo M, Maestre FT, Turner BL, Fierer N (2020) The global-scale distributions of soil protists and their contributions to belowground systems. Sci Adv 6:eaax8787

Orgiazzi A, Bardgett RD, Barrios E (2016) Global soil biodiversity atlas. European Commission, Brussels

Page FC (1977) The genus *Thecamoeba* (Protozoa, Gymnamoebia) species distinctions, locomotive morphology, and protozoan prey. J Nat Hist 11:25–63

Petters S, Soellinger A, Bengtsson MM, Urich T (2018) The soil microbial food web revisited with metatranscriptomics - predatory Myxobacteria as keystone taxon? bioRxiv. https://doi.org/10.1101/373365

Petz W, Foissner W, Adam H (1985) Culture, food selection and growth rate in the mycophagous ciliate *Grossglockneria acuta* Foissner, 1980: first evidence of autochthonous soil ciliates. Soil Biol Biochem 17:871–875

Petz W, Foissner W, Wirnsberger E, Krautgartner WD, Adam H (1986) Mycophagy, a new feeding strategy in autochthonous soil ciliates. Naturwissenschaften 73:560–562

Pollierer MM, Dyckmans J, Scheu S, Haubert D (2012) Carbon flux through fungi and bacteria into the forest soil animal food web as indicated by compound-specific 13C fatty acid analysis. Funct Ecol 26:978–990

Porazinska DL, Duncan LW, McSorley R, Graham JH (1999) Nematode communities as indicators of status and processes of a soil ecosystem influenced by agricultural management practices. Appl Soil Ecol 13:69–86

Quist CW, Vervoort MTW, Van Megen H, Gort G, Bakker J, Van der Putten WH, Helder J (2014) Selective alteration of soil food web components by invasive giant goldenrod Solidago gigantea in two distinct habitat types. Oikos 123:837–845

Quist CW, Smant G, Helder J (2015) Evolution of plant parasitism in the phylum Nematoda. Annu Rev Phytopathol 53:289–310

Quist CW, Gort G, Mooijman P, Brus DJ, Van den Elsen S, Kostenko O, Vervoort M, Bakker J, Van der Putten WH, Helder J (2019) Spatial distribution of soil nematodes relates to soil organic matter and life strategy. Soil Biol Biochem 136:107542

Ramirez C, Alexander M (1980) Evidence suggesting protozoan predation on *Rhizobium* associated with germinating seeds and in the rhizosphere of beans (*Phaseolus vulgaris* L.). Appl Environ Microbiol 40:492–499

Ramirez KS, Geisen S, Morriën E, Snoek BL, van der Putten WH (2018) Network analyses can advance above-belowground ecology. Trends Plant Sci 23:759–768

Rønn R, Vestergård M, Ekelund F (2012) Interactions between bacteria, protozoa and nematodes in soil. Acta Protozool 51:223–235

Rosenberg K, Bertaux J, Krome K, Hartmann A, Scheu S, Bonkowski M (2009) Soil amoebae rapidly change bacterial community composition in the rhizosphere of *Arabidopsis thaliana*. ISME J 3:675–684

Ruess L, Dighton J (1996) Cultural studies on soil nematodes and their fungal hosts. Nematologica 42:330–346

Rusek J (1998) Biodiversity of Collembola and their functional role in the ecosystem. Biodivers Conserv 7:1207–1219

Sabatini MA, Innocenti G (2001) Effects of Collembola on plant-pathogenic fungus interactions in simple experimental systems. Biol Fertil Soils 33:62–66

Sayre RM (1973) *Theratromyxa weberi*, an amoeba predatory on plant-parasitic nematodes. J Nematol 5:258–264

Schratzberger M, Holterman M, van Oevelen D, Helder J (2019) A worm's world: ecological flexibility pays off for free-living nematodes in sediments and soils. Bioscience 69:867–876

Schulz-Bohm K, Geisen S, Wubs ERJ, Song C, de Boer W, Garbeva P (2017) The prey's scent - volatile organic compound mediated interactions between soil bacteria and their protist predators. ISME J 11:817–820

Seppey CVW, Singer D, Dumack K, Fournier B, Belbahri L, Mitchell EAD, Lara E (2017) Distribution patterns of soil microbial eukaryotes suggests widespread algivory by phagotrophic protists as an alternative pathway for nutrient cycling. Soil Biol Biochem 112:68–76

Shah P, Pell J (2003) Entomopathogenic fungi as biological control agents. Appl Microbiol Biotechnol 61:413–423

Siddiqui Z, Mahmood I (1999) Role of bacteria in the management of plant parasitic nematodes: a review. Bioresour Technol 69:167–179

Taylor CE, Brown DJF (1997) Nematode vectors of plant viruses. CAB International, Wallingford

van den Hoogen J et al (2019) Soil nematode abundance and functional group composition at a global scale. Nature 572:194–198

Waldrop MP et al (2012) Bacterial and enchytraeid abundance accelerate soil carbon turnover along a lowland vegetation gradient in interior Alaska. Soil Biol Biochem 50:188–198

Xiong W et al (2018) Soil protist communities form a dynamic hub in the soil microbiome. ISME J 12:634–638

Xiong W et al (2020) Rhizosphere protists are key determinants of plant health. Microbiome 8:27

Yeates GW, Foissner W (1995) Testate amoebae as predators of nematodes. Biol Fertil Soils 20:1–7

Yeates GW, Bongers T, De Goede RGM, Freckman DW, Georgieva SS (1993) Feeding-habits in soil nematode families and genera - an outline for soil ecologists. J Nematol 25:315–331

Zimmermann G (1986) The 'Galleria bait method' for detection of entomopathogenic fungi in soil. J Appl Entomol 102:213–215