

Interactions between Bacteria, Protozoa and Nematodes in Soil

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Abstract. Bacteria, protozoa and nematodes interact closely in soil ecosystems. Protozoa and nematodes eat bacteria (and occasionally each other), while bacteria defend themselves using chemical substances, resistant cell walls, irregular shapes and motility. Protozoa and nematodes are very different types of organisms, and hence apply very different feeding mechanisms; thus many protozoa can pick and choose individual bacterial cells, whereas nematodes ingest bacterial patches more uncritically. Protozoa and nematodes are both aquatic organisms whose activity depends on available soil water, but differences in size, motility, resting stages and reproductive strategies mean that the soil physico-chemical environment influences the activity of protozoa and nematodes differently. For example, the relative importance of protozoa compared to nematodes may shift towards protozoa in very clay-rich soils. The interactions between the three organism groups have major ecological consequences such as modification of the bacterial communities and increased nitrogen mineralisation, both of which affect plant growth. Increased nitrogen mineralisation will usually be beneficial for plant growth, whereas the grazing-induced changes in the bacterial communities can be both beneficial and detrimental to plants. Selective protozoan grazing can favour plant inhibiting bacteria. This may be a problem in clay rich soils where protozoa have better life conditions than nematodes.

Key words: Amoebae, bacteria, colonisation, competition, feeding types, flagellates, interactions, migration, nematodes, physico-chemical environment, plant growth, population growth, predation, protozoa, soil, soil texture, trophic interactions.

1. INTRODUCTION

The organisms belowground play equally significant roles in the terrestrial ecosystems as the organisms that live aboveground. First and foremost, these organisms degrade and mineralize dead organic matter, so plants can reclaim the nutrients. The soil food web includes two major degradation pathways (Moore *et al.* 2005); the fungal pathway and the bacterial pathway (Fig. 1). Here, we will only focus on the bacterial

pathway that is the bacteria, their grazers and the predators on the bacterial grazers (Fig. 1). These organisms shape the bacterial communities, and hence, stimulate, (and occasionally impede), bacterial processes. The most important bacterial grazers in soil are the protozoa and the nematodes (Fig. 2). These two groups differ in a number of ways; hence their ways of exploiting the bacterial food resource, and their impact on the bacterial communities, differ. Most of the differences between the two groups stem from the fact that protozoa and nematodes are very different organisms. Protozoa are unicellular protists (~ 2 µm to more than 50 µm), whereas the nematodes (~ 30 µm to mm size range) are multi-cellular metazoans.

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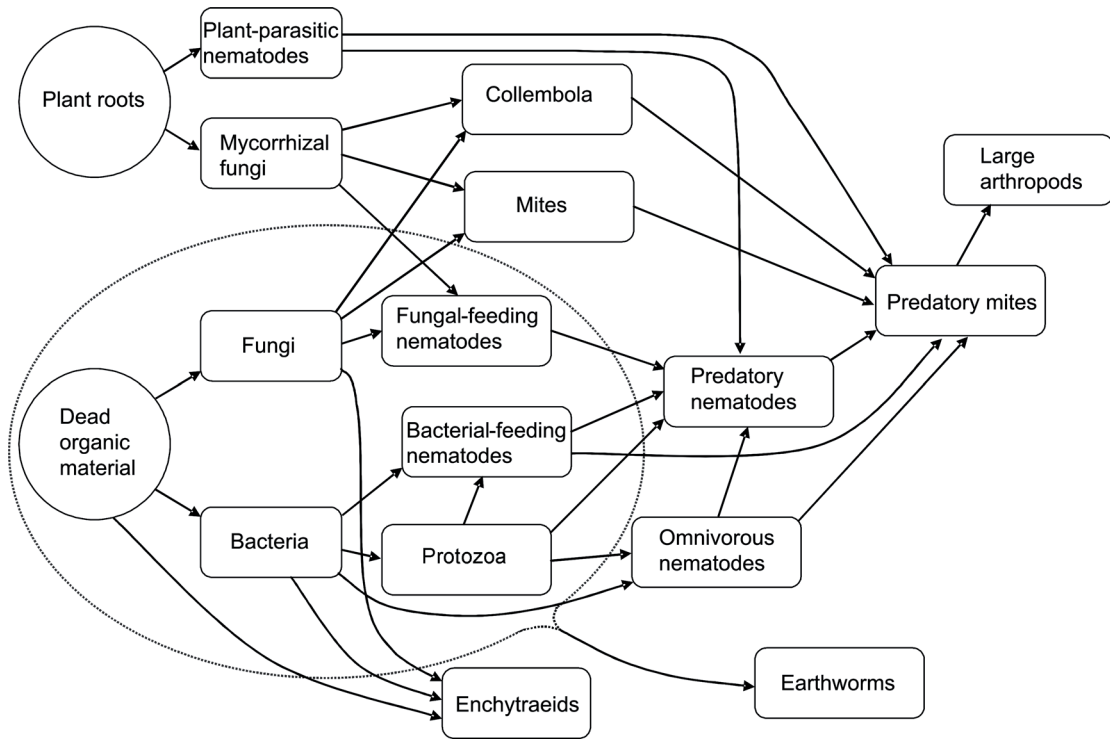


Fig. 1. Diagram of a simplified soil food web showing important trophic links. The diagram is combined and modified from several sources (see e.g. Holtkamp 2008, Hunt *et al.* 1987).

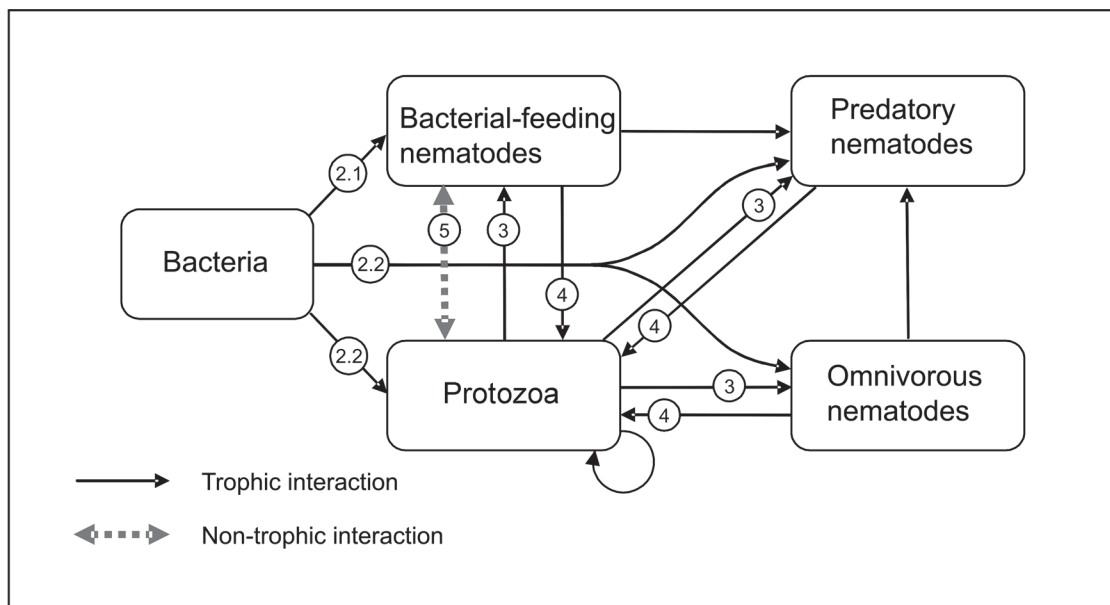


Fig. 2. A diagram illustrating the interactions between bacteria, protozoa and nematodes, which are treated in this paper. Numbers in circles refer to the section of the paper in which the particular interaction is discussed.

In a taxonomic sense, the nematodes make up a well-defined, monophyletic group of metazoans. Representatives of the group can be found in very different environments. Some are parasitic, and some are free-living freshwater, marine or soil organisms. Ecologists often use a functional approach to categorize soil living nematodes, i.e. fungal feeding, plant feeding, bacterivorous, predatory and omnivorous nematodes. Here, we only discuss the three latter groups as we only are concerned with the bacterial pathway. The term “protozoa” refers to a ragbag of unicellular organisms of non-monophyletic origin (Hausman *et al.* 2003). Here, we are only concerned with free-living (non-parasitic) phagotrophic forms. A few free-living forms feed on fungi (Ekelund 1998) and other substrates (Ekelund and Rønn 1994), but most free-living forms are bacterial feeders. Free-living protozoa in soil are often divided into four groups for practical purposes: naked and testate amoebae, flagellates and ciliates. Only the ciliates are monophyletic.

Nematodes are usually larger than protozoa (although there is some size overlap), and hence generally have longer generation-times. Nematodes have a much better ability to move around in the soil than protozoa, and are therefore more mobile than protozoa. The “protozoan individual” is much smaller than the nematodal individual, hence with the same biomass, protozoa are represented with many more individuals; for statistical reasons this makes a particular protozoan strain much less susceptible to become extinct.

2. FEEDING ON BACTERIA

Protozoa and nematodes use fundamentally different mechanisms for feeding. We discuss these mechanisms below in some detail, as they form a necessary background for understanding the function of the two organism groups in the soil ecosystem.

2.1. Protozoan ingestion of bacteria

Protozoa take up food particles by phagocytosis, which means that individual food particles are enclosed by an invagination of the cell membrane thereby forming a food vacuole (Hausmann *et al.* 2003). Inside the food vacuole enzymatic degradation facilitates the digestion. All protozoa ultimately take up food particles by phagocytosis but the mechanisms they employ for

apprehending and concentrating food particles from the environment before the food is enclosed in a food vacuole are very different. The different mechanisms for food uptake can be categorised in different ways and different authors use different terminology; therefore we attempt to clarify the subject.

Basically, protozoa can take up bacteria in free suspension or bacteria attached to surfaces. Although feeding on attached bacteria is very important in the environment, most studies on protozoan feeding ecology have focused on suspension feeders. This focus is probably somewhat misleading. Especially in soil, most bacteria are associated with surfaces and a major part of the protozoa either crawl, wobble, swim along, or are attached to surfaces. Parameters as e.g. growth rates have often been determined as a function of cell density; i.e. cells/ml (Ekelund 1996). However, the volume/surface-ratio of a container in which such experiments are performed will increase with the power of 3/2 when size increases; i.e. its volume will increase more than its surface. Because most bacteria and protozoa are surface-associated, the inner surfaces of relatively larger containers of the same geometry will have higher densities of bacteria and protozoa. Protozoan growth rates increase with bacterial density; therefore a relatively larger container with the same bacterial concentration will seemingly yield higher growth rates than smaller containers with the same geometry and the same bacterial concentration.

Fenchel (1987) divided protozoan feeding into three different types: **(1) feeding by direct interception**, **(2) filter-feeding** and **(3) diffusion feeding**. We recommend the scheme of Boenigk and Arndt (2002) using the three terms proposed by Fenchel only for suspension feeding protozoa and to use the term **(4) raptorial feeding** for mobile protozoa that actively search and engulf individual food particles and **(5) grasping** for the feeding process where protozoa ingest attached bacteria.

(1) Direct interception in Fenchels (1987) terminology means that the protozoa intercept and engulf individual food particles (bacterial cells). In line with Boenigk and Arndt (2000) we suggest a more narrow definition of interception feeding and restrict its use to describe the feeding process where protozoa, in particular flagellates, create a water current and directly intercept individual food particles in free suspension transported with this current, in soil *Spumella* and *Bodo saltans* use this method.

(2) **Filter-feeders** actively create a water current and food particles are gathered and concentrated by a filter and transported to the cell surface. Filter-feeding is particularly common among bacterial-feeding ciliates which retain bacterial prey on a ciliary filter (Fenchel 1980). In soil, water is mostly found as water-films and in small soil pores, and although filter-feeding protozoa do occur in soil, e.g. ciliates (Foissner 1987), choanoflagellates (Ekelund and Patterson 1997) and *Phalans-terium solitarium* (Ekelund 2002), their numbers are small (Ekelund *et al.* 2001) as is their importance.

(3) **Diffusion feeding:** The last mechanism for feeding on suspended bacteria, diffusion feeding, relies on the motility of the prey and not the predator. Prey items get entangled in pseudopodial networks or excreted mucus (Hausman *et al.* 2003). Diffusion feeding is mainly found among foraminiferans and “organisms with heliozoan morphology” and is rare in soil, though “organisms with heliozoan morphology” occasionally are seen in soil though in low numbers (Sandon 1927, Ekelund *et al.* 2001).

(4) **Raptorial feeding** refers to mobile protozoa that actively search for food particles often along surfaces (Boenigk and Arndt 2000). Various bodonids (e.g. *Rhyncomonas nasuta*, *Neobodo designis*) and *Heteromita globosa* would be classified as raptorial feeders.

(5) **Grasping** (feeding on attached bacteria, e.g. in biofilms). Most bacteria in soil are associated with surfaces of soil particles or decomposing organic material. Hence, feeding on attached bacteria is widespread among soil protozoa and is usually found among naked amoebae, testate amoebae and surface-gliding flagellates, in particular many cercozoans.

Quantitative accounts of protozoan diversity in soil are rare; hence the significance of the different feeding types is difficult to estimate. We used data from Ekelund *et al.* (2001) to calculate that three samples from a clay-loam soil (Griffiths *et al.* 2000) contained 0.3–12% interception feeders, 0.2–2% filter-feeders, 0.01–0.3% diffusion-feeders, 63–69% raptorial feeders, and 21–30% grasping feeders. These numbers (and many hours in front of the microscope) allow us to conclude that the most important protozoan feeding mechanisms in soil are raptorial feeding (i.e. direct active capture of suspended bacteria) and grasping. There is no sharp distinction between these two strategies as can be observed in cultures of surface-gliding flagellates which sometimes feed on surface-associated bacteria and sometimes feed on individual free bacteria just above the surface.

2.2. Nematodal ingestion of bacteria

With some exceptions, the food source of free-living nematodes can be determined based on the morphology of the stoma or mouth cavity. Plant- and fungal feeding nematodes are equipped with a needle-like structure used for puncturing and emptying plant cells or fungal hyphae. Most predatory nematodes have large barrel shaped mouth cavities that may carry teeth for retaining prey. The bacterial feeders have a mouth cavity lined by five sclerotized plates, which form either a narrow tubular mouth cavity or a wider and barrel shaped mouth cavity; they are never equipped with tooth structures.

In contrast to protozoa, bacterial-feeding nematodes do not ingest individual food items. Instead, they take up bacterial cells present in the environment through a pumping action of the muscular pharynx. The food particles are sucked in by the pumping action, the food particles are retained and excess liquid is expelled through the mouth opening. The pumping action generates a pressure that forces food into the intestine (Avery and Shtonda 2003, Woombs and Laybourn-Parry 1984). Hence, nematode feeding is essentially to be considered as filter-feeding (Avery and Shtonda 2003), and the width of the stoma determines the maximum size of particles ingested.

Many bacterial feeding nematodes have a terminal bulb, a muscular bundle at the posterior end of the pharynx, equipped with sclerotized cuticula, which is used for grinding food particles (Munn and Munn 2002). The grinding action of the terminal bulb means that nematodes have some possibility for physical disruption of bacterial aggregates and bacterial cell walls, whereas soil protozoa rely completely on enzymatic digestion. This has been suggested as an explanation that gram positive bacteria (with thick cell walls) appeared to be a better food source for the nematode *Caenorhabditis elegans* than they did for the flagellate *Cercomonas* (Bjørnlund *et al.* 2006).

2.3. Selective feeding on bacteria by protozoa and nematodes

Bacteria are not equally susceptible to predation by protozoa and nematodes; hence presence of protozoa (Kreuzer *et al.* 2006, Rosenberg *et al.* 2009, Rønn *et al.* 2002) and nematodes (Djigal *et al.* 2004, Griffiths *et al.* 1999) changes the composition of bacterial communities. Selective feeding on bacteria can result from simple physical constraints of the feeding apparatus. For example, size-selective feeding is typical for fil-

ter-feeding ciliates and filter- and interception-feeding flagellates, which feed most efficiently on intermediate-sized bacteria (Hahn and Höfle 2001, Jürgens and Güde 1994, Jürgens and Matz 2002), whereas many surface-associated amoebae and flagellates, which engulf their prey by simple phagocytosis, are less constrained by prey size and may be able to ingest very large prey (Ekelund and Rønn 1994).

Other factors such as high bacterial motility can also reduce protozoan feeding efficiency (Boenigk *et al.* 2001, Matz and Jürgens 2003, Matz and Kjelleberg 2005, Matz *et al.* 2002). The ability of bacteria to form dense micro-colonies through excretion of polysaccharides (Klinge 1958, Matz and Kjelleberg 2005) or form biofilms (Matz *et al.* 2004) also reduce their chance of being consumed by grazers. These selection mechanisms can be regarded as passive mechanisms since only simple physical interactions are needed to explain the selection process. However, it appears that protozoa are also able to select actively among equally-sized food particles in a mixture (Jürgens and DeMott 1995, Thurman *et al.* 2010). These active selection mechanisms are probably related to surface-properties of the bacterial cell (Wildschutte *et al.* 2004, Wootton *et al.* 2007) and presumably depend on the cell to cell contact that occurs before and during the phagocytotic feeding process. Boenigk *et al.* (2001) demonstrated that flagellates were able to ingest cyanobacterial cells within a few minutes after ingestion, whereas other bacteria were processed further in the food vacuole.

Some bacteria produce toxic compounds that inhibit or kill protozoan cells (Jousset *et al.* 2006, 2008; Matz *et al.* 2004; Pedersen *et al.* 2010, 2011) and hence it is highly advantageous for protozoa to be able to avoid feeding on these bacterial cells. Bacteria also vary in their nutritional value and protozoan growth rates depend strongly on the type of bacterial food they are offered (Bjørnlund *et al.* 2006, Rønn *et al.* 2001a, Weekers *et al.* 1993). However, it is less clear to what extent protozoa are able to select bacteria solely on the basis of their nutritional value (Lekfeldt and Rønn 2008).

Bacterivorous nematodes, on the other hand, are not in close contact with their prey prior to ingestion and hence do not have the same possibilities for active selection of specific prey bacteria as protozoa do. However, since they are functionally filter-feeders some of the passive selection mechanisms also apply to nematode feeding. Hence, feeding by *Caenorhabditis elegans* is size-selective with relatively small cells being

ingested more efficiently than larger ones (Avery and Shtonda 2003). Furthermore, nematodes do respond to chemical cues and laboratory studies have shown that they are attracted to patches with certain bacteria (Salinas *et al.* 2007). In addition to such chemotactic behaviour, Shtonda and Avery (2006) demonstrated that the food seeking behaviour of *C. elegans* depended on the food quality of the bacteria in its immediate surroundings so that the time spent feeding on high-quality food bacteria was optimised. Hence, if different patches in soil are colonised by different bacteria, it is possible that nematodes actively seek up and preferentially feed on certain patches and avoid others.

3. NEMATODES FEEDING ON PROTOZOA

Predatory and omnivorous nematodes may feed on protozoa (Small 1987, Yeates *et al.* 1993), but the quantitative importance of this trophic interaction is not well understood. It is generally difficult to quantify trophic transfers in soil food webs. This is partly due to the obvious difficulties of direct observing organism interactions in the soil environment, and partly because organisms of the soil decomposer food web are generally not specialists (Giller 1991). Even if predatory nematodes do not feed specifically on protozoa, they are likely to ingest protozoa as a by-catch when feeding on e.g. other nematodes. Similarly, predatory nematodes can not avoid taking up bacteria during feeding and part of their diet will consist of bacterial cells and other microorganisms. Hence, there is probably not any clear distinction between the predatory and bacterial-feeding mode of life for the nematodes.

Feeding strategy of predatory nematodes also depends on developmental stage. Initial stages of some nematodes from the families Diplogasteridae and Mononchidae are mainly bacterial feeders whereas later stages are predatory on nematodes and protozoa (Yeates 1987). The same is true for bacterial-feeding nematodes. Bacterial-feeding nematodes feed most voraciously in micro-sites with high bacterial density. These sites usually also harbour high protozoan densities and small flagellates and amoebae will be ingested along with the bacteria. Bjørnlund and Rønn (2008) reported that individuals of the bacterial-feeding nematode *Caenorhabditis elegans* that had reached the third larval stage were large enough to feed on a flagellate belonging to the genus *Cercomonas*.

Nematodes feeding on protozoa can have important implications for the trophic transfer through the decomposer food webs. Elliott *et al.* (1980) demonstrated that the nematode *Mesodiplogaster* grew better in the presence of amoebae than when only bacteria were present. The stimulatory effect of amoebae was largest in fine-textured soils indicating that the amoebae made food available to the nematodes by feeding in small pores inaccessible to the nematodes. Recently, Bjørnlund *et al.* (2009) reported a similar phenomenon from a study of interactions between the bacterial strain, *Pseudomonas* DSS73, a flagellate (*Cercomonas* sp.) and a nematode (*C. elegans*). This bacterium produces an exoproduct, amphisin, which provides the bacterium with antibiotic properties. The nematodes were not able to reproduce in cultures where this bacterial strain was the only available food source. The flagellates were less sensitive to the bacterial exoproducts and were able to multiply using this bacterium as food. In cultures where bacteria, flagellates and nematodes all were present, the nematodes were able to reproduce using the flagellate as food. This illustrates the important role the interaction between protozoa and nematodes may have for the transfer of bacterial biomass to higher trophic levels in the food web.

4. THE FOOD CHAIN REVERSED – PROTOZOAN FEEDING ON NEMATODES

Although the majority of soil protozoa feed mainly on bacteria, the protozoa as a group feed on a very wide spectrum of food items (Ekelund and Rønn 1994). Several large amoebae in Vampyrellidae (Hess *et al.* 2012) are able to feed on nematodes. Hence, the large amoeba *Theratromyxa weberi* engulfs nematodes and forms digestive cysts (Sayre 1973, Sayre and Wergin 1989, Weber *et al.* 1952, Winslow and Williams 1957) and other giant amoebae generally considered to be mycophagous have also been reported to feed on nematodes (Anderson and Patrick 1978; Old and Darbyshire 1978, 1980). These organisms were mainly studied in laboratory cultures due to their potential role as biological control agents of plant pathogenic fungi and/or nematodes but their ecological significance in the soil environment is virtually unknown. Some testate amoebae are also able to ingest and feed on nematodes (Yeates and Foissner 1995). The nematodes are mainly attacked from the tail end. Again, it is not known how important this tro-

phic interaction is in the environment, but the authors note that many extracted nematodes from the studied soil appeared to have tail damages resembling those inflicted by the testate amoebae. This suggests that this interaction could be of significance in sites with high abundance of testate amoebae (e.g. moist forest litter or bogs).

5. COMPETITION VS. DIRECT TROPHIC INTERACTION

Since bacterial-feeding nematodes and protozoa utilize the same food resource, they can affect each other negatively through resource competition. However, the interaction is more complicated since they also feed on each other as discussed above. Georgieva *et al.* (2005) found a negative correlation between abundance of flagellates and nematodes belonging to the family Neodiplogasteridae and suggested that this was due to intra-guild predation. The complexity of possible interactions between different protozoa and nematodes means that it can be difficult to predict the outcome of their mutual interaction. Interestingly, it has also been observed that bacterial-feeding nematodes can have a stimulatory effect on protozoan abundance – possibly due to a stimulation of bacterial production (Rønn *et al.* 2001b).

Protozoa and nematodes can also affect each other through non-trophic interaction and these interactions can significantly affect the relative success of each of the two groups. Recently, Bjørnlund and Rønn (2008) discovered that a small flagellate belonging to the genus *Cercomonas* is able to attack and kill larvae of *Caenorhabditis elegans*. Since amoebae can feed on nematodes (see above) and small marine planktonic flagellates can feed on much larger diatoms (Kühn *et al.* 2004, Schnepf and Kühn 2000), it would seem reasonable that the flagellates killed the nematodes to feed on them. However, so far no evidence has been found that the flagellates utilize the nematodes as a food resource (Bjørnlund and Rønn 2008, Bjørnlund *et al.* 2009). The mechanism behind this process is not clear but it is evident that the flagellate will benefit from this since *C. elegans* is both a competitor for bacterial food as well as a potential predator (see above). In line with this, Neidig *et al.* (2010) demonstrated that the amoeba *Acanthamoeba castellanii* excretes substances that are inhibitory and repellent towards *C. elegans* and that the nematode on the other hand excretes substances that in-

hibit the amoeba. The full implications of these intriguing interactions still need to be unravelled.

6. INTERACTIONS WITH THE PHYSICO-CHEMICAL ENVIRONMENT

The organisms that we deal with here are fundamentally aquatic creatures visiting a terrestrial world; hence the environment presents some serious challenges, which primarily relate to the complex soil matrix with its physical constraints and the inevitable risk of drought. Soil structure and water accessibility are closely interrelated quantities. The essential measure for soil-water accessibility is the water potential, which depends on both soil texture and structure and water content. The water potential must be determined empirically for the particular soil in question. When the water potential is known, the pore neck diameter of largest water-filled pores can be determined theoretically by the Kelvin equation, which in a simplified version states that: $D = 300/P$, where D is the pore neck diameter of largest water-filled pores (μm), and P is the water potential (kPa) (Carson *et al.* 2010).

Water drains more freely through large pores than through small pores and when soils dry, the largest pores empty first. The drier the soil, the smaller the largest water-filled pores will be and since small flagellates and naked amoebae have access to smaller pores than nematodes they should theoretically be able to maintain activity at lower water potential than nematodes. We notice, though, that our current understanding of the relationship between water potential, water-filled soil pores and activity of particular organisms is too simplistic. Several studies have documented nematode activity and/or population growth at water potentials that would theoretically only leave water in pores too small to accommodate nematodes (Freckman *et al.* 1987, Griffiths *et al.* 1995, Yeates *et al.* 2002).

6.1. Soil texture

Soils differ in a number of parameters, which creates different life-conditions for the organisms that inhabit them. One major parameter is the particle size distribution; the so-called soil texture. Soils with different textures have different distribution of soil pore sizes. Fine-textured soil with high content of small clay particles has a relatively higher proportion of small pores than soils with more coarse-grained sand particles.

Protozoa and nematodes are too small to physically move soil particles; hence they can only reach bacteria in soil pores with openings so large that they can enter them. Consequently, small flagellates and naked amoebae can access bacteria in water-filled pores with openings down to 2–3 μm (Kuikman *et al.* 1991, Rutherford and Juma 1992), whereas even the smallest nematodes are restricted from bacteria in soil pores smaller than approximately 30 μm (Jones and Thomasson 1976). Hence, protozoa have a competitive advantage over nematodes in fine-textured soils. In accordance with this, Rønn *et al.* (1995) found relatively higher nematode population growth in coarse-textured soils, whereas growth of naked amoebae was higher in fine-textured soils (Fig. 3). Likewise, in a subsequent experiment, with sterilized soil of varying clay content re-inoculated with non-sterilized soil, protozoa, but not nematodes responded positively to increasing clay content (Rønn *et al.* 2001b).

6.2. Soil moisture

Even severe drought will not eradicate protozoan populations, as they can survive prolonged drought as inactive cysts. Apparently, protozoa currently put some proportion of their production into a “cyst bank” to prepare for drought periods; this proportion increases with cell density. Hence, formation of cysts is not primarily induced by water stress, but rather by internal regulation within protozoan populations. This is a necessary adaptation, since drying of individual soil pores occur very fast without leaving time for encystment (Ekelund *et al.* 2002).

Drought sensitivity varies between nematode taxa; some are very sensitive to prolonged drought whereas others can survive desiccation. For example, the juveniles of the bacterial feeding family Rhabditidae form an inactive stage, termed “dauer larva” primarily as a response to nutritional stress. The cuticle of dauer larvae has reduced permeability and therefore also protects the inactive juvenile from water loss. Anhydrobiosis is an extreme mechanism of drought survival, where the nematode stops metabolizing, but regains activity remarkably fast after rehydration. For instance, *Filenchus polyhyppus* survived 39 years in an anhydrobiotic state in a dry herbarium (Steiner and Albin 1946). Further, desiccation tolerance varies between nematode taxa, and generally bacterial feeding Cephalobidae remain active at lower water availability than Rhabditidae (Bouwman and Zwart 1994, Griffiths *et al.* 1995, Yeates *et al.* 2002). Bouwman and Zwart (1994)

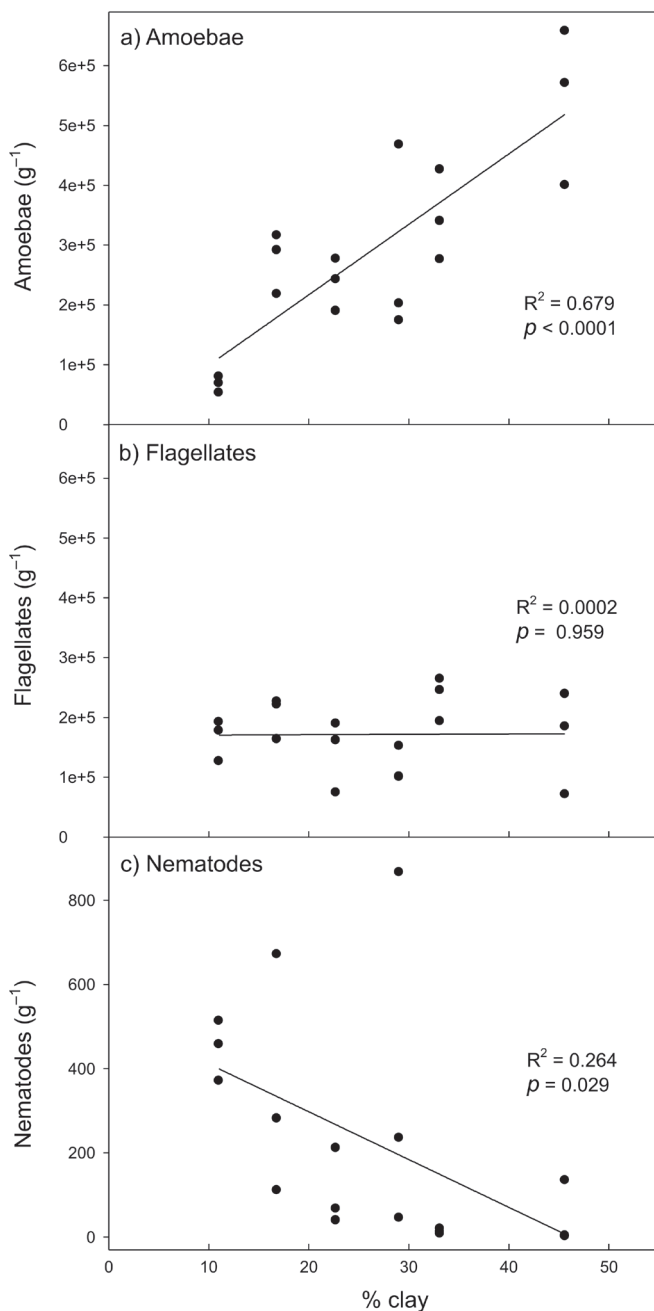


Fig. 3. Effect of soil texture on protozoa and nematodes. The graph shows the abundance of amoebae (a), flagellates (b), and nematodes (c) in soils with varying clay content. The soils were amended with glucose and incubated for two weeks before enumeration of organisms. Data from Rønn *et al.* (1995).

likewise observed different sensitivity to desiccation between protozoan taxa with higher drought tolerance for *Cercomonas* sp. than for *Spumella* sp.

6.3. Migration and colonisation

Protozoan cysts have a high dispersal potential, and can easily be spread even with the wind (Altenburger *et al.* 2010). Therefore, protozoa can colonize new substrates very fast by relatively rapid excystment and resumed binary fission, beginning from very few individuals, whereas nematodes are slower colonisers that have to migrate through the soil matrix to colonize new patches (Griffiths and Caul 1993).

Chemical gradients from decomposing organic matter, plant roots, bacteria etc. attract nematodes; in particular CO₂ gradients and gradients of other metabolic waste products (Anderson *et al.* 1997, Croll 1970, Grewal and Wright 1992, Griffiths and Caul 1993, Moens *et al.* 1999). The architecture of soil pore networks affects chemotactic attraction of nematodes; thus the diffusion pathway of volatile cues may be blocked by solid particles (Young *et al.* 1998). Protozoan migration in soil is much less significant (Griffiths and Caul 1993) amounting to maximum a few cm day⁻¹ (Adl 2007).

Soil moisture also affects nematode migration ability in the soil matrix. When the water potential decreases, water will only be present in gradually smaller pore volumes, and the hydraulic connectivity between water filled pores will decrease. This leaves nematode populations in individual moist pores isolated from each other. Young *et al.* (1998) demonstrated that the migration of nematodes in sand towards the food bacterium *Escherichia coli* decreased with decreasing water potential of the sand. Likewise, migration of bacterial-feeding nematodes into decaying barley leaves was higher at a water potential of -10 kPa (corresponding to water-filled pores with pore-necks smaller than 30 µm) than at -1, -500 and -1,000 kPa (Griffiths *et al.* 1995). Further, diffusion rates of gaseous attractants, e.g. CO₂, depend on soil water content, and nematode migration towards hotspots of high microbial activity may be restricted at high moisture levels, simply because the diffusion rates of gaseous attractants through water are orders of magnitude slower than through air.

The differences in migration potential of protozoa and nematodes have consequences for the colonization of freshly added organic substrates. Vestergaard *et al.* (2001) found that the relative importance of protozoa and nematodes depended on particle size of the decomposing plant material. Protozoa were relatively more important in soils with finely ground plant material whereas nematodes benefited when plant material was added as larger particles. When finely ground organic

resources are evenly distributed in the soil the small, numerous, fast-growing protozoa are able to resume activity right away. On the other hand, when the material is more heterogeneously distributed as large particles, the nematodes benefit from the larger food concentration and their ability to move toward the resource (Vestergaard *et al.* 2001).

7. ECOLOGICAL CONSEQUENCES OF INTERACTIONS

Grazing affects bacterial populations. There is plenty of evidence that protozoan grazing significantly reduces bacterial abundance in systems with high microbial activity (Ekelund *et al.* 2009, Rosenberg *et al.* 2009, Rønn *et al.* 2002). Thirup *et al.* (2000) even showed that regular fluctuations in bacterial numbers were followed by regular but delayed fluctuations in protozoan numbers. Similarly, several microcosm experiments have demonstrated reduced bacterial abundance or microbial biomass in the presence of bacterial-feeding nematodes (Bouwman *et al.* 1994, Djigal *et al.* 2004, Xiao *et al.* 2010). Grazing-induced reduction of bacterial abundance is often accompanied by increased bacterial activity (Ekelund and Rønn 1994); in particular nitrification is also often stimulated by grazing (Bouwman *et al.* 1994, Griffiths 1989, Verhagen *et al.* 1993, Xiao *et al.* 2010).

7.1. Why does grazing stimulate bacterial activity?

Protozoan and nematode carbon (C) to nitrogen (N) ratios are comparable to C to N ratios in the bacterial biomass (Zwart *et al.* 1994, Griffiths 1994). Hence, due to respiratory carbon loss the grazers ingest excess nitrogen when they ingest bacteria, which they excrete as ammonia (NH₃). Therefore, part of the grazing-induced stimulation of bacterial activity can be explained simply by N in excess from the grazers. Additionally, it has been suggested that bacterial populations that are grazed will be more metabolically active, because senescent cells are removed from the population and because fast-growing bacterial strains have a selective advantage over slower-growing strains. Finally, protozoan production of bacterial-stimulating metabolites may likewise stimulate bacterial activity (Ekelund and Rønn 1994).

7.2. Changes in the composition of bacterial communities

Grazing also alters the composition of bacterial communities as evidenced by DNA-based profiling of both ammonia-oxidizing bacterial communities (Xiao *et al.* 2010) and soil bacterial communities inoculated either with or without protozoan and/or nematode grazers (Djigal *et al.* 1994, De Mesel *et al.* 2004, Ekelund *et al.* 2009, Griffiths *et al.* 1999, Rosenberg *et al.* 2009, Rønn *et al.* 2002). Generally, Actinobacteria are favoured by protozoan grazing (Ekelund *et al.* 2009, Rønn *et al.* 2002). Grazing-induced shifts in bacterial community composition can be explained by direct effects of selective feeding on certain bacterial types (see above) or by changes in the competitive balance between different bacterial populations as a result of the general effect of grazing on the environmental conditions (e.g. increased availability of nitrogen, phosphorus etc.).

Bacterial feeding nematodes are unable to discriminate between individual bacterial cells as many protozoa can; hence, the two groups will affect bacterial populations differently. Pedersen *et al.* (2009) showed that in the presence of the flagellate *Cercomonas*, the bacterium *Pseudomonas fluorescens* CHA0 increased its abundance as compared to two other *Pseudomonas* strains, which were both eaten by the flagellate. In contrast, the number of *P. fluorescens* CHA0 declined relatively when the system was subjected to nematode predation pressure. Bjørnlund *et al.* (2012) further showed that *Arthrobacter* sp. (an Actinobacterium) thrived better when grazed by protozoa than when grazed by nematodes. As the bacterium is harmful to plants, this caused reduced plant performance in the experimental systems.

7.3. Nitrogen mineralisation and plant growth

Protozoan presence usually increases nitrogen mineralization and plant N uptake (Alphei *et al.* 1996; Bouwman *et al.* 1994; Clarholm 1985; Ekelund *et al.* 2009; Griffiths 1986; Ingham *et al.* 1985; Kuikman *et al.* 1990, 1991; Rønn *et al.* 2002). For instance, Velvet Grass (*Holcus lanatus*) grown with a mixture of bacterial and protozoan species attained almost twice the biomass as plants grown with bacteria only, most likely the result of the increased mineralization of soil nitrogen sources and subsequent increased plant nitrogen uptake (Ekelund *et al.* 2009). Similarly, Mosquito Grass (*Bouteloua gracilis*) grown with *Pseudomonas* strains and the bacterial feeding nematodes *Pelodera* sp. and

Acroboloides sp. reached more than twice the biomass of plants grown with the bacterial strains alone. In this case, nitrogen mineralization as well as plant N and P uptake were also stimulated by the presence of the bacterial grazer (Ingham *et al.* 1985).

7.4. Why does grazing stimulate plant growth?

Apart from the increased N-mineralisation, the altered bacterial community composition imposed by grazing has been suggested as an alternative explanation to the stimulation of plant growth. This concept is derived from experiments in which protozoan stimulation of plant growth was not easily explained by protozoan-induced increase in nutrient availability (Alphei *et al.* 1996, Jentschke *et al.* 1995). However, root branching of spruce seedlings was more pronounced in the presence of protozoa, which led Jentschke *et al.* (1995) to hypothesize that growth responses to protozoan presence could be hormonal. They hypothesized that selective grazing might favour survival of less desirable prey bacteria that produce the plant growth hormone indole-3-acetic acid (IAA).

This hypothesis was subsequently tested in experimental systems with mixed soil bacteria growing on agar plates. In these experiments, the amoeba *Acanthamoeba castellanii* increased the proportion of IAA producing bacteria and induced root branching in plant seedlings (Bonkowski and Brandt 2002). In a more recent study *Acanthamoeba castellanii* altered internal root auxin and cytokinin responses and root branching patterns (Krome *et al.* 2010). However, other studies found no differences between soil systems with and without protozoa with regard to abundance of IAA producing bacteria (Ekelund *et al.* 2009), nor had protozoan diversity (Vestergård *et al.* 2007) any effect on the relative abundance of IAA producers.

8. CONCLUSION

Bacteria, protozoa, and nematodes coexist closely in the soil ecosystem. Mutually, the three groups affect each other; both positively and negatively. Both nematodes and protozoa stimulate nitrogen mineralisation and plant growth through their effect on the soil bacteria, but because the two groups of organisms have different ways of food-uptake, movement and colonisation, they affect bacteria differently. This means that they shape bacterial communities differently, and that they also can affect plant growth differently.

Natural soils harbour many different bacteria, protozoa and nematodes, and interactions leading to both positive and negative effects on plant growth will occur. Still, different soil types offer different life conditions to protozoa and nematodes and, as described above, parameters such as e.g. soil texture (Rønn *et al.* 1995) and distribution of organic resources (Vestergaard *et al.* 2001) affect the relative importance of protozoa and nematodes. Consequently, we anticipate that e.g. the negative effects on plant growth of protozoan grazing mentioned above (sect. 7.2) can potentially occur under such environmental conditions that favour protozoan activity.

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REFERENCES

- Adl S. M. (2007) Motility and migration rate of protozoa in soil columns. *Soil Biol. Biochem.* **39**: 700–703
- Alphei J., Bonkowski M., Scheu S. (1996) Protozoa, Nematoda and Lumbricidae in the rhizosphere of *Hordelymus europaeus* (Poaceae): faunal interactions, response of microorganisms and effects on plant growth. *Oecologia* **106**: 111–126
- Altenburger A., Ekelund F., Jacobsen C. S. (2010) Protozoa and their bacterial prey colonize sterile soil fast. *Soil Biol. Biochem.* **42**: 1636–1639
- Anderson A. R. A., Young I. M., Sleeman B. D., Griffiths B. S., Robertson W. M. (1997) Nematode movement along a chemical gradient in a structurally heterogeneous environment. 1. Experiment. *Fund. Appl. Nematol.* **20**: 157–163
- Anderson T. R., Patrick Z. A. (1978) Mycophagous amoeboid organisms from soil that perforate spores of *Thielaviopsis basicola* and *Cochliobolus sativus*. *Phytopathology* **68**: 1618–1626
- Avery L., Shtonda B. B. (2003) Food transport in the *C. elegans* pharynx. *J. Exp. Biol.* **206**: 2441–2457
- Bjørnlund L., Rønn, R. (2008) ‘David and Goliath’ of the soil food web – flagellates that kill nematodes. *Soil Biol. Biochem.* **40**: 2032–2039
- Bjørnlund L., Liu M., Rønn R., Christensen S., Ekelund F. (2012) Nematodes and protozoa affect plants differently; depending on soil nutrient status. *Eur. J. Soil Biol.* **50**: 28–31
- Bjørnlund L., Mørk S., Vestergård M., Rønn R. (2006) Trophic interactions between rhizosphere bacteria and bacterial feeders influenced by phosphate and aphids in barley. *Biol. Fert. Soils* **43**: 1–11
- Bjørnlund L., Rønn R., Pechy-Tarr M., Maurhofer M., Keel C., Nybroe O. (2009) Functional GacS in *Pseudomonas* DSS73 prevents digestion by *Caenorhabditis elegans* and protects the nematode from killer flagellates. *ISME J.* **3**: 770–779
- Bonkowski M., Brandt F. (2002) Do soil protozoa enhance plant growth by hormonal effects? *Soil Biol. Biochem.* **34**: 1709–1715
- Boenigk J., Arndt H. (2000) Comparative studies on the feeding behavior of two heterotrophic nanoflagellates: the filterfeeding choanoflagellate *Monosiga ovata* and the raptorial-feeding ki-

- netoplastid *Rhynchomonas nasuta*. *Aquat. Microbial. Ecol.* **22**: 243–249
- Boenigk J., Arndt H. (2002) Bacterivory by heterotrophic flagellates: community structure and feeding strategies. *A. van Leeuw.* **81**: 465–480
- Boenigk J., Matz C., Jürgens K., Arndt H. (2001) The influence of preculture conditions and food quality on the ingestion and digestion process of three species of heterotrophic nanoflagellates. *Microb. Ecol.* **42**: 168–176
- Bouwman L. A., Zwart K. B. (1994) The ecology of bacterivorous protozoans and nematodes in arable soil. *Agr. Ecosyst. Environ.* **51**: 145–160
- Bouwman L. A., Bloem J., Van den Boogert P. H. J. F., Bremer F., Hoenderboom G. H. J., De Ruiter P. C. (1994) Short-term and long-term effects of bacterivorous nematodes and nematophagous fungi on carbon and nitrogen mineralization in microcosms. *Biol. Fert. Soils* **17**: 249–256
- Carson J. K., Gonzalez-Quinones V., Murphy D. V., Hinz C., Shaw J. A., Gleeson D. B. (2010) Low Pore Connectivity Increases Bacterial Diversity in Soil. *Appl. Environ. Microbiol.* **12**: 3936–3942
- Clarholm M. (1985) Interactions of bacteria, protozoa and plants leading to mineralization of soil nitrogen. *Soil Biol. Biochem.* **17**: 181–187
- Croll N. A. (1970) *The Behaviour of Nematodes – their activities, senses and responses*. Edward Arnold, London
- De Mesel I., Derycke S., Moens T., Van der Gucht K., Vincx M., Swings J. (2004) Top-down impact of bacterivorous nematodes on the bacterial community structure: a microcosm study. *Environ. Microbiol.* **6**: 733–744
- Djigal D., Brauman A., Diop T. A., Chotte J. L., Villenave C. (2004) Influence of bacterial-feeding nematodes (Cephalobidae) on soil microbial communities during maize growth. *Soil Biol. Biochem.* **36**: 323–331
- Ekelund F. (1996) Growth kinetics of five common heterotrophic soil flagellates. *Eur. J. Soil Biol.* **32**: 15–24.
- Ekelund F. (1998) Enumeration and abundance of mycophagous protozoa in soil, with special emphasis on heterotrophic flagellates. *Soil Biol. Biochem.* **30**: 1343–1347
- Ekelund F. (2002) a study of the soil flagellate *Phalansterium solitarium* Sandon 1924 with preliminary data on its ultrastructure. *Protistology* **2**: 152–158
- Ekelund F., Patterson D. J. (1997) Some heterotrophic flagellates from a cultivated soil in Australia. *Arch. Protistenkd.* **148**: 461–478
- Ekelund F., Rønn R. (1994) Notes on protozoa in agricultural soil with emphasis on heterotrophic flagellates and naked amoebae and their ecology. *FEMS Microbiol. Rev.* **15**: 321–353
- Ekelund F., Rønn R., Griffiths B. S. (2001) Quantitative estimation of flagellate community structure and diversity in soil samples. *Protist* **152**: 301–314
- Ekelund F., Frederiksen H. B., Rønn R. (2002) Population dynamics of active and total ciliate populations in arable soil amended with wheat. *Appl. Environ. Microbiol.* **68**: 1096–1101
- Ekelund F., Saj S., Vestergård M., Bertaux J., Mikola J. (2009) The “soil microbial loop” is not always needed to explain protozoan stimulation of plants. *Soil Biol. Biochem.* **41**: 2336–2342
- Elliott E. T., Anderson R. V., Coleman D. C., Cole C. V. (1980) Habitable pore space and microbial trophic interactions. *Oikos* **35**: 327–335
- Fenchel T. (1980) Relation between particle size selection and clearance in suspension-feeding ciliates. *Limnol. Oceanogr.* **25**: 733–738.
- Fenchel T. (1987). *Ecology of protozoa. The biology of free-living phagotrophic protists*. Springer-Verlag, Berlin
- Foissner W. (1987) Soil protozoa: Fundamental problems, ecological significance, adaptations in ciliates and testaceans, bioindicators and guide to the literature. *Progr. Protistol.* **2**: 69–212
- Freckman D. W., Whitford W. G., Steinberger Y. (1987) Effect of irrigation on nematode population dynamics and activity in desert soils. *Biol. Fert. Soils* **3**: 3–10
- Georgieva S., Christensen S., Petersen H., Gjelstrup P., Thorup-Kristensen K. (2005) Early decomposer assemblages of soil organisms in litterbags with vetch and rye roots. *Soil Biol. Biochem.* **37**: 1145–1155
- Giller P. S. (1991) The diversity of soil communities, the ‘poor man’s tropical rainforest’. *Biodivers. Conserv.* **5**: 135–168
- Grewal P. S., Wright D. J. (1992) Migration of *Caenorhabditis elegans* (Nematoda: Rhabditidae) larvae towards bacteria and the nature of the bacterial stimulus. *Fund. Appl. Nematol.* **15**: 159–166
- Griffiths B. S. (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 B. S. (1989) Enhanced nitrification in the presence of bacteriophagous protozoa. *Soil Biol. Biochem.* **21**: 1045–1051
- Griffiths B. S. (1994) Soil Nutrient Flow. In: *Soil Protozoa*, (Ed. J. F. Darbyshire). CAB International, Wallingford, 65–91
- Griffiths B. S., Caul S. (1993) Migration of bacterial-feeding nematodes, but not protozoa, to decomposing grass residues. *Biol. Fert. Soils* **15**: 201–207
- Griffiths B. S., Young I. M., Caul S. (1995) Nematode and protozoan population-dynamics on decomposing barley leaves incubated at different soil matric potentials. *Pedobiologia* **39**: 454–461
- Griffiths B. S., Bonkowski M., Dobson G., Caul S. (1999) Changes in soil microbial community structure in the presence of microbial-feeding nematodes and protozoa. *Pedobiologia* **43**: 297–304
- Griffiths B. S., Ritz K., Bardgett R. D., Cook R., Christensen S., Ekelund F., Sørensen S. J., Bååth E., Bloem J., de Ruiter P. C., Dolfing J., Nicolardot B. (2000) Ecosystem response of pasture soil communities to fumigation-induced microbial diversity reductions: an examination of the biodiversity-ecosystem function relationship. *Oikos* **90**: 279–294
- Hahn M. W., Höfle M. G. (2001) Grazing of protozoa and its effect on populations of aquatic bacteria. *FEMS Microbiol. Ecol.* **35**: 113–127
- Hausman K., Hülsman N., Radek R. (2003) *Protistology*. E. Schweizerbart’sche Verlagsbuchhandlung, Berlin, Stuttgart
- Hess S., Sausen N., Melkonian M. (2012) Shedding light on vampires: The phylogeny of vampyrellid amoebae revisited. *Plos One* **7**: e31165
- Holtkamp R., Kardol P., van der Wal A., Dekker S. C., van der Putten W., de Ruiter P. C. (2008) Soil food web structure during ecosystem development after land abandonment. *Appl. Soil Ecol.* **39**: 23–34
- Hunt H. W., Coleman D. C., Ingham E. R., Elliott E. T., Moore J. C., Rose S. L., Reid C. P. P., Morley C. R. (1987) The detrital food web in a shortgrass prairie. *Biol. Fert. Soils* **3**: 57–68
- Ingham R. E., Trofymow J. A., Ingham E. R., Coleman D. C. (1985) Interactions of bacteria, fungi, and their nematode grazers: effects on nutrient cycling and plant growth. *Ecol. Monogr.* **55**: 119–140
- Jentschke G., Bonkowski M., Godbold D. L., Scheu S. (1995) Soil protozoa and forest tree growth: non-nutritional effects and interactions with mycorrhizae. *Biol. Fert. Soils* **20**: 263–269

- Jones F. G. W., Thomasson A. J. (1976) Bulk density as an indicator of pore space in soils usable by nematodes. *Nematologica* **22**: 133–137
- Jousset A., Lara E., Wall L. G., Valverde C. (2006) Secondary metabolites help biocontrol strain *Pseudomonas fluorescens* CHA0 to escape protozoan grazing. *Appl. Environ. Microbiol.* **72**: 7083–7090
- Jousset A., Scheu S., Bonkowski M. (2008) Secondary metabolite production facilitates establishment of rhizobacteria by reducing both protozoan predation and the competitive effects of indigenous bacteria. *Funct. Ecol.* **22**: 714–719
- Jürgens K., DeMott W. R. (1995) Behavioral flexibility in prey selection by bacterivorous nanoflagellates. *Limnol. Oceanogr.* **40**: 1503–1507
- Jürgens K., Güde H. (1994) The potential importance of grazing-resistant bacteria in planktonic systems. *Mar. Ecol. Prog. Ser.* **112**: 169–188
- Jürgens K., Matz C. (2002) Predation as a shaping force for the phenotypic and genotypic composition of planktonic bacteria. *A. van Leeuw.* **81**: 413–434
- Klinge K. (1958) *Pseudomonas-fluorescens*-Polysaccharide als Schutz gegen Phagozytose durch Amöben. *Naturwissenschaften* **22**: 550–551
- Kreuzer K., Adanczyk J., Iijima M., Wagner M., Scheu S., Bonkowski M. (2006) Grazing of a common species of soil protozoa (*Acanthamoeba castellanii*) affects rhizosphere bacterial community composition and root architecture of rice (*Oryza sativa* L.). *Soil Biol. Biochem.* **38**: 1665–1672
- Krome K., Rosenberg K., Dickler C., Kreuzer K., Ludwig-Müller J., Ullrich-Eberius C., Scheu S., Bonkowski M. (2010) Soil bacteria and protozoa affect root branching via effects on the auxin and cytokinin balance in plants. *Plant Soil* **328**: 191–201
- Kühn S., Medlin L., Eller G. (2004) Phylogenetic position of the parasitoid nanoflagellate *Pirsonia* inferred from nuclear-encoded small subunit ribosomal DNA and a description of *Pseudopirsonia* n. gen. and *Pseudopirsonia mucosa* (Drebes) comb. nov. *Protist* **155**: 143–156
- Kuikman P. J., Van Elsas J. D., Jansen A. G., Burgers S. L. G. E., Van Veen J. A. (1990) Population dynamics and activity of bacteria and protozoa in relation to their spatial distribution in soil. *Soil Biol. Biochem.* **22**: 1063–1073
- Kuikman P. J., Jansen A. G., Van Veen J. A. (1991) ¹⁵N-Nitrogen mineralization from bacteria by protozoan grazing at different soil moisture regimes. *Soil Biol. Biochem.* **23**: 193–200
- Lekfeldt J. D. S., Rønn R. (2008) A common soil flagellate (*Cercomonas* sp.) grows slowly when feeding on the bacterium *Rhodococcus fascians* in isolation, but does not discriminate against it in a mixed culture with *Sphingopyxis wittflariensis*. *FEMS Microbiol. Ecol.* **65**: 113–124
- Matz C., Jürgens K. (2003) Interaction of nutrient limitation and protozoan grazing determines the phenotypic structure of a bacterial community. *Microb. Ecol.* **45**: 384–398
- Matz C., Kjelleberg S. (2005) Off the hook – how bacteria survive protozoan grazing. *Trends Microbiol.* **13**: 302–307
- Matz C., Boenigk J., Arndt H., Jürgens K. (2002) Role of bacterial phenotypic traits in selective feeding of the heterotrophic nanoflagellate *Spumella* sp. *Aquat. Microb. Ecol.* **27**: 137–148
- Matz C., Bergfeld T., Rice S. A., Kjelleberg S. (2004) Microcolonies, quorum sensing and cytotoxicity determine the survival of *Pseudomonas aeruginosa* biofilms exposed to protozoan grazing. *Environ. Microbiol.* **6**: 218–226.
- Moens T., Verbeeck L., De Maeyer A., Swings J., Vincx M. (1999) Selective attraction of marine bacterivorous nematodes to their bacterial food. *Mar. Ecol. Prog.-Ser.* **176**: 165–178.
- Moore J. C., McCann K., de Ruiter P. C. (2005) Modeling trophic pathways, nutrient cycling, and dynamic stability in soils. *Pedobiologia* **49**: 499–510
- Munn E. A., Munn P. D. (2002) Feeding and digestion. In: *The biology of nematodes*, (Ed. D. L. Lee). Taylor & Francis, Singapore, 211–232
- Neidig N., Jousset A., Nunes F., Bonkowski M., Paul R. J., Scheu S. (2010) Interference between bacterial feeding nematodes and amoebae relies on innate and inducible mutual toxicity. *Funct. Ecol.* **24**: 1133–1138
- Old K. M., Darbyshire J. F. (1978) Soil fungi as food for giant amoebae. *Soil Biol. Biochem.* **11**: 93–100
- Old K. M., Darbyshire J. F. (1980) *Arachnula impatiens* Cienk., a mycophagous giant amoeba from soil. *Protistologica* **16**: 277–287
- Pedersen A. L., Nybroe O., Winding A., Ekelund F., Bjørnlund L. (2009) Bacterial feeders, the nematode *Caenorhabditis elegans* and the flagellate *Cercomonas longicauda*, have different effects on outcome of competition among the *Pseudomonas* biocontrol strains CHA0 and DSS73. *Microb. Ecol.* **57**: 501–509
- Pedersen A. L., Ekelund F., Johansen A., Winding A. (2010) Interaction of bacterial feeding flagellates and *Pseudomonas* spp. *Biol. Fert. Soils* **46**: 151–158
- Pedersen A. L., Winding A., Altenburger A., Ekelund F. (2011) Protozoan growth rates on secondary-metabolite-producing *Pseudomonas* spp. correlate with high-level protozoan taxonomy. *FEMS Microbiol. Lett.* **316**: 16–22
- 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
- Rutherford P. M., Juma N. G. (1992) Influence of texture on habitable pore space and bacterial-protozoan populations in soil. *Biol. Fert. Soils* **12**: 221–227
- Rønn R., Thomsen I. K., Jensen B. (1995) Naked amoebae, flagellates and nematodes in soil of different texture. *Eur. J. Soil Biol.* **31**: 135–141
- Rønn R., Ekelund F., Grunert J. (2001a). Protozoan response to addition of the bacteria *Mycobacterium chlorophenolicus* and *Pseudomonas chlororaphis* to soil microcosms. *Biol. Fert. Soils.* **33**: 126–131
- Rønn R. M., Griffiths B. S., Young I. M. (2001b) Protozoa, nematodes and N-mineralization across a prescribed soil textural gradient. *Pedobiologia* **45**: 481–495
- Rønn R., McCaig A. E., Griffiths B. S., Prosser J. I. (2002) Impact of protozoan grazing on bacterial community structure in soil microcosms. *Appl. Environ. Microbiol.* **68**: 6094–6105
- Salinas K. A., Edenborn S. L., Sextstone A. J., Kotcon J. B. (2007) Bacterial preferences of the bacterivorous soil nematode *Cephalobus brevicauda* (Cephalobidae): Effect of bacterial type and size. *Pedobiologia* **51**: 55–64
- Sandon H. (1927) The composition and distribution of the protozoan fauna of the soil. Oliver and Boyd, Edinburgh and London
- Sayre R. M. (1973) *Theratomyxa weberi*, an amoeba predatory on plant-parasitic nematodes. *J. Nematol.* **5**: 258–264
- Sayre R. M., Wergin W. P. (1989) Morphology and fine structure of the trophozoites of *Theratomyxa weberi* (Protozoa: Vampyrellidae) predacious on soil nematodes. *Can. J. Microbiol.* **35**: 589–602

- Schnepf E., Kühn S. F. (2000) Food uptake and fine structure of *Cryothecomonas longipes* sp. nov., a marine nanoflagellate incertae sedis feeding phagotrophically on large diatoms. *Helgoland Marine Research* **54**: 18–32
- Shtonda B. B., Avery L. (2006) Dietary choice behavior in *Caenorhabditis elegans*. *J. Exp. Biol.* **209**: 89–102
- Small R. W. (1987) a review of the prey of predatory soil nematodes. *Pedobiologia* **30**: 179–206
- Steiner G., Albin F. M. (1946) Resuscitation of the nematode *Tylenchus polyhyppnus*, n. sp. after almost 39 years dormancy. *Journal of the Washington Academy of Science* **36**: 97–99
- Thirup L., Ekelund F., Johnsen K., Jacobsen C. S. (2000) Population dynamics of the fast-growing sub-populations of *Pseudomonas* and total bacteria, and their protozoan grazers, revealed by fenpropimorph treatment. *Soil Biol. Biochem.* **32**: 1615–1623
- Thurman J., Parry J. D., Hill P. J., Laybourn-Parry J. (2010). The filter-feeding ciliates *Colpidium striatum* and *Tetrahymena pyriformis* display selective feeding behaviours in the presence of mixed, equally-sized, bacterial prey. *Protist* **161**: 577–588
- Verhagen F. J. M., Duyts, H., Laanbroek H. J. (1993) Effects of grazing by flagellates on the competition for ammonium between nitrifying and heterotrophic bacteria in soil columns. *Appl. Environ. Microbiol.* **59**: 2099–2106
- Vestergaard P., Rønn R., Christensen S. (2001) Reduced particle size of plant material does not stimulate decomposition, but affects the microbivorous microfauna. *Soil Biol. Biochem.* **33**: 1805–1810
- Vestergård M., Bjørnlund L., Henry F., Rønn R. (2007) Decreasing prevalence of rhizosphere IAA producing and seedling root growth promoting bacteria with barley development irrespective of protozoan grazing regime. *Plant Soil* **295**: 115–125
- Weber A. P., Zwillenberg L. O., Van der Laan P. A. (1952) a predacious amoeboid organism destroying larvae of the potato root eelworm and other nematodes. *Nature* **169**: 834–835
- Weekers P. H. H., Bodelier P. L. E., Wijen J. P. H., Vogels G. D. (1993) Effects of grazing by the free-living soil amoebae *Acanthamoeba castellanii*, *Acanthamoeba polyphaga*, and *Hartmannella vermiformis* on various bacteria. *Appl. Environ. Microbiol.* **59**: 2317–2319
- Wildschutte H., Wolfe D. M., Tamewitz A., Lawrence J. G. (2004) Protozoan predation, diversifying selection, and the evolution of antigenic diversity in *Salmonella*. *Proc. Nat. Acad. Sci.* **101**: 10644–10649
- Winslow R. D., Williams T. D. (1957) Amoeboid organisms attacking larvae of the potato root eelworm (*Heterodera rostochiensis* Woll.) in England and the beet eelworm (*H. schachtii* Schm.) in Canada. *Eur. J. Plant Pathol.* **63**: 242–243
- Woomb's M., Laybourn-Parry J. (1984) Feeding biology of *Diplogasteritus nudicapitatus* and *Rhabditis curvicaudata* (Nematoda) related to food concentration and temperature, in sewage treatment plants. *Oecologia* **64**: 163–167
- Wootton E. C., Zubkov M. V., Jones D. H., Jones R. H., Martel C. M., Thornton C. A., Robert E. C. (2007) Biochemical prey recognition by planktonic protozoa. *Environ. Microbiol.* **9**: 216–222
- Xiao H., Griffiths B., Chen X., Liu M., Jiao J., Hu F., Li H. (2010) Influence of bacterial-feeding nematodes on nitrification and the ammonia-oxidizing bacteria (AOB) community composition. *Appl. Soil Ecol.* **45**: 131–137
- Yeates G. W. (1987) Nematode feeding and activity: the importance of development stages. *Biol. Fertil. Soils* **3**: 143–146
- Yeates G. W., Bongers T., De Goede R. G. M., Freckman D., Georgieva S. S. (1993) Feeding habits in soil nematode families and genera – an outline for soil ecologists. *J. Nematol.* **25**: 315–331
- Yeates G. W., Foissner W. (1995) Testate amoebae as predators of nematodes. *Biol. Fert. Soils* **20**: 1–7
- Yeates G. W., Dando J. L., Shepherd T. G. (2002) Pressure plate studies to determine how soil moisture affects access of bacterial-feeding nematodes to food in soil. *Eur. J. Soil Sci.* **53**: 355–365
- Young I. M., Griffiths B. S., Robertson W. M., McNicol J. W. (1998) Nematode (*Caenorhabditis elegans*) movement in sand as affected by particle size, moisture and the presence of bacteria (*Escherichia coli*). *Eur. J. Soil Sci.* **49**: 237–241
- Zwart K. B., Kuikman P. J., Van Veen J. A. (1994) Rhizosphere Protozoa: Their significance in nutrient dynamics. In: Soil Protozoa, (Ed. J. F. Darbyshire). CAB International, Wallingford, 93–121

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