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The nematode–based food–chain of a temperate deciduous forest

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Contents

Summary

Soils are complex and heterogeneous habitats for soil animals. Trophic interactions between soil animals are depicted in soil food webs which form an essential component of terrestrial ecosystems. Soil food webs are based on predator-prey interactions and reflect the flux of matter and energy through ecological systems. The soil food web is compartmentalized in distinct energy channels that process energy in different ways. The main energy channels in forest systems are the bacterial, fungal and plant litter energy channel with the bacterial channel probably being the fastest. However, the importance of predator-prey interactions in these channels remains little understood. Especially nematodes are an understudied group in soil food webs since they are small, difficult to determine and also difficult to detect as prey organism.

I investigated the role of nematodes as prey for microarthropods using molecular gut content analysis. Therefore, specific primers for certain prey taxa were used to screen potential predators for presence of the respective prey. With this approach the consumption and distribution of certain prey among many potential predators can be investigated (bottom-up view). By screening many individuals the importance of predator species as antagonists of nematode prey species can be evaluated (top-down view). Thereby, molecular gut content analysis provides the opportunity to investigate predator-prey interactions allowing to trace trophic links between certain prey taxa and higher consumers of the soil food web.

In the first study (**Chapter 2**) I investigated if nematodes serve as prey for microarthropods. Therefore, the entomopathogenic nematodes *Phasmarhabditis hermaphrodita* and *Steinernema feltiae* were used as model organisms to investigate if soil mites, especially species that have been assumed to live as decomposers, include nematodes in their diet. Established molecular markers for the two nematode species were used in these studies. To confirm detection of predation events, I investigated how long nematode DNA can be traced in the gut of the oribatid mite species *Steganacarus magnus*. In the field I investigated if soil mites preferentially consume dead or living nematode prey and if active predation for nematode prey occurred. The results indicate that nematode DNA can be traced for up to 128 h in the gut of *S. magnus* confirming good detection of nematode prey during the experiments. However, the detection time of prey DNA varied between nematode species and depended on the exposure time of the nematodes to the mites. Soil mite species consumed the two model nematode species in the laboratory and in the field suggesting that nematodes form part of their regular diet. In the field experiment many 'classical decomposer' soil mite species fed on the nematodes *P. hermaphrodita* and *S. feltiae*. Living and dead nematodes were consumed indicating that both feeding modes, predation and scavenging, occur. The mites differentially

consumed the two nematode species depending on whether they were dead or alive pointing to nematode defence mechanisms that influence this predator-prey interaction.

It is very likely that microarthropods also feed on indigenous nematode species and may significantly impact nematode communities; however, no molecular markers exist to test this assumption. Therefore, I designed specific primers for four free-living bacterial feeding soil nematode species (*Acrobeloides buetschlii*, *Panagrellus redivivus*, *Plectus minimus* and *Plectus velox*) and established them for molecular gut content analyses of potential microarthropod predators (**Chapter 3**). The specificity of the molecular markers was confirmed by a non-target test to check for cross-reactions, and the sensitivity was confirmed by a two-fold serial dilution of prey DNA. The newly designed molecular markers amplify sensitively taxon-specific 18S rDNA up to 128 h after ingestion in the gut of a microarthropod predator. The detection time for the respective nematode species varied between nematode species, mite species and time of exposure indicating that these prey detection times differ for every predator-prey interaction. Abundant soil mite and collembolan species were shown to feed on these nematode species in the laboratory and on *A. buetschlii* and *Plectus* spp. in the field indicating that indigenous nematodes indeed form part of the diet of soil microarthropods including those previously assumed to live as detritivores. Nematode-predator interactions presumably contribute significantly to the flux of energy from root exudates via bacteria to higher trophic levels.

Forest soils are patchy habitats comprising different microhabitats, such as litter, moss and grass. These microhabitats significantly affect the density and distribution of nematode and microarthropod species, but also contribute to variations in interactions between soil animal species. I investigated if *A. buetschlii* and *Plectus* spp. were differentially consumed by fourteen abundant soil mite species including Mesostigmata and Oribatida from litter, moss and grass using molecular gut content analysis (**Chapter 4**). The mites differentially consumed the two nematode taxa related to their density and the consumption of nematodes differed between the habitats. Our results indicate shifts in trophic niches with changing habitat characteristics which likely contribute to the high diversity of microarthropods in deciduous forests.

By designing and establishing specific nematode markers for molecular gut content analysis to investigate the role of indigenous bacterial feeding nematodes as prey for microarthropods this thesis provides promising tools to investigate how carbon is channelled from roots over bacteria to higher trophic levels. Although the few analyzed nematode taxa only represent a small fraction of the nematode community of temperate forest soils, they were frequently detected as prey of microarthropods - including those previously thought to live as

decomposers - suggesting that the impact of microarthropods on nematodes is high. Overall, the results represent a major step forward for the understanding of soil animal food webs and highlight that the decomposer food web is more complex and trophically diverse than previously assumed.

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General Introduction

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1. Soil food webs and their main energy channels

Searching for food is essential for most terrestrial animals. It is one of the driving forces for niche differentiation, i.e. species numbers, in above- and below-ground systems. Predator-prey interactions are the base for channelling energy from lower to higher trophic levels and are depicted as links in food webs (Pimm 1991; Terborgh & Estes 2010). A major component of terrestrial ecosystems is the soil food web (Scheu & Setälä 2002; Bardgett & Wardle 2010). The density and distribution of species is affected by the patchiness of the soil which comprises different microhabitats such as litter, fine and coarse woody debris and patches of mosses and grass (Sulkava & Huhta 1998; Hohberg & Traunspurger 2005). The patchiness of soil habitats also contributes to variations in predator-prey interactions and allows coexistence of the high number of soil animal species (Anderson 1975; Mikola & Sulkava 2001; Proctor *et al.* 2002; Scheu & Setälä 2002; Maraun *et al.* 2003). Since soil systems are species-rich, the organisms typically are small and difficult to observe; due to these difficulties soil food webs are often viewed as 'black box' and trophic relationships are little understood (Wardle & Yeates 1993; Symondson 2002; Scheu *et al.* 2005).

The soil food web is compartmentalized in the bacterial, fungal and plant litter energy channel, and carbon and nutrients are transferred to higher trophic levels via these pathways (Moore & Hunt 1988; Scheu *et al.* 2005). The relative contribution of the different energy channels to carbon and nutrient cycling varies depending on forest type and microhabitat (Ruess 2003). Recent studies indicate that a wide range of predators rely on carbon from the bacterial channel (Pollierer *et al.* 2012) which is assumed to be the fastest as it has a high turnover rate and needs only ~72 hours to transfer carbon from microbial biomass to higher trophic levels, i.e. to predatory microarthropods (van Hees *et al.* 2005; Strickland *et al.* 2012). One of the most important links in the bacterial energy channel presumably are bacterial-feeding nematodes transferring carbon from bacteria to higher trophic levels (Freckman 1988; Crotty *et al.* 2011; Ferlian *et al.* 2012) since bacteria have a higher nutrient content (Griffiths & Caul 1993) than fungi and less defence mechanisms against grazing by nematodes (Wardle & Yeates 1993). However, this predator-prey interaction is still little understood.

2. Nematodes

Nematodes are the most diverse and abundant soil Metazoa on earth (Baxter 2003). They live freely in aquatic and terrestrial systems but also parasitic in animals and plants. Free-living nematodes are key players in soil systems (Moore *et al.* 2003; Ferris 2010; Yeates 2010) due to their nutrition, diversity, density and role as prey, thereby having an important function for

transferring carbon to higher trophic levels. Nematodes feed on bacteria, fungi, algae, plants, dead organic material, invertebrates or are omnivorous (Yeates 1993). They can be identified and determined to family level by their mouthparts which are often typical for their nutrition. Free-living soil nematodes usually reach a size of 0.3-3 mm, and forest soils can inhabit up to 100 species and up to several million individuals per square meter (Yeates *et al.* 2000). Especially bacterial feeding nematodes form one of the most important bacterial grazers in soil since they account for >50% and at hotspots of microbial activity sum up to about 90% of total free-living nematodes (Griffiths 1990; Zunke & Perry 1997; Scheu *et al.* 2005; Mulder & Vonk 2011). Therefore, they are an important food source for other soil animals including tardigrades (Hohberg & Traunspurger 2005), predatory nematodes (Small & Grootaert 1983), testate amoebae (Yeates & Foissner 1995), mites and collembolans (Rockett & Woodring 1966; Muraoka & Ishibashi 1976; Walter 1988a,b; Read *et al.* 2006).

Although some taxa of nematode predators are known, the involved species and their impact on the nematode community are still unclear. Therefore, it is still unknown if the nematode density is controlled by their resource ("bottom-up") or by predators ("top-down"; Ferris *et al.* 2001; Read *et al.* 2006).

Terrestrial nematodes are worldwide distributed, even occurring in Antarctica and colonize nearly every habitat of the world (Powers *et al.* 1998; Wu *et al.* 2011). In forest ecosystems free-living nematodes form part of the main energy channels and due the high densities and diversity of grazers especially of the fungal and bacterial energy channel. In agrosystems plant parasitic and entomopathogenic nematodes play a major role. Plant parasitic nematodes are host-specific and cause extensive crop losses as pests in monocultures, e.g. Oliveira *et al.* (2007) stated global crop loss in the order of US$ 78 billion per year relatable to the nematodes *Meloidogyne* spp., *Heterodera* spp., *Globodera* spp. and *Pratylenchus* spp., therefore, appropriate agents for biological control of these nematodes are in demand. Entomopathogenic nematodes are host specific and are used for biological control of larger pests, e.g. *Heterorhabditis bacteriophora* is specific to insect larvae and has been used against the western corn root worm (the beetle lavae *Diabrotica virgifera virgifera*; Toepfer *et al.* 2005). However, since they are spread out with water to the soil surface numerous nematode individuals are lost either by stress or by predation of soil animals, most importantly microarthropods (Dunphy & Webster 1986; Hyvönen & Persson 1996; Read *et al.* 2006).

Plant resources form the basis of soil food webs thereby governing plant-, bacterial- and fungal-feeding nematodes in soil. Structure, texture, soil mineralogy, and nutrient and water availability influence capturing these resources (Eisenhauer *et al.* 2011). In addition to resources nematode communities depend on habitat characteristics. Temperature, soil type

and moisture are the most important factors affecting nematode distribution (Yeates 1979; Verschoor *et al.* 2001). For plant-parasitic nematodes the presence of the host-plant is also important (Yeates & Boag 2004). If the temperature is low nematodes become inactive and are unable to develop (Yeates & Boag 2004), if the temperature is too high, nematodes also reduce their activity (Boag 1980; Burman & Pye 1980), paralyse or die (Wallace 1963). Nematodes need to pass through soil pores that have a diameter equal or greater than their body width to migrate through soils (Yeates & Boag 2004), whereas too small or too large soil pores avoid nematode concealment or allow predators access of their hiding place (Hohberg & Traunspurger 2005). Soil moisture is also an important factor since nematodes move in waterfilms and therefore water is essential for nematode migration (Wallace 1959). All these factors likely also influence the accessibility of nematodes as prey, since paralyzed, slow or immobile nematodes are easier to subdue for predators.

3. Microarthropods

Microarthropods (collembolans and mites) are abundant and species rich in soil systems. Stable isotope analyses indicate that collembolans and mites are very diverse in their nutrition and that their trophic level spans from primary and secondary decomposers (feeding predominantly on litter or fungi) to predators (feeding predominantly on nematodes and microarthropods; Schneider *et al.* 2004a Chahartaghi *et al.* 2005; Klarner *et al.* 2013). The degree of food specialization in microarthropods has been discussed intensively (Giller 1996; Schneider *et al.* 2004b) as it forms a major determinant of the diversity of microarthropods in the terrestrial systems (described as 'enigma of soil animal species diversity'; Anderson 1975; Scheu & Setälä 2002; Maraun *et al.* 2003a). Trophic niches are key to explain species richness since even small differences in food preferences may reduce competition (Maraun *et al.* 1998). Unfortunately, food resources and trophic niches of many soil microarthropod species still are little known (Scheu & Setälä 2002; Maraun *et al.* 2003a).

Collembolans are worldwide distributed and reach high densities of up to million individuals per square metre in forest soils (Petersen & Luxton, 1982) and first occurred in the early Devonian 400 million years ago *(*Grimaldi 2010). They are a species-rich group of small wingless hexapods and about 7000 species are described (Rusek 1998). Collembolans typically are equipped with a furca which increases mobility and allows escape from predators. Collembolans without furca avoid predation by excretion of a glue from pseudocells on the body surface. Collembolans are assumed to be mainly fungivorous (Chen *et al.* 1996; Hopkin 1997) but their nutrition is as diverse as that of mites (Rusek 1998; Chahartaghi *et al.* 2005) also including predators of nematodes (Read *et al.* 2006).

Soil mites (Acari) are spider-like arthropods possessing a sclerotized chitinous exoskeleton and four pairs of legs. Mites have a worldwide distribution and can be found in virtually all habitats (Walter & Proctor 1999; Köhler 1997). The Acari contain mainly predatory groups such as gamasid and uropodid mites (both Parasitiformes: Mesostigmata) and predominantly primary and secondary decomposers such as oribatid mites (Acariformes: Oribatida).

Most gamasid mites are exclusively predatory and only *Macrocheles vagabundus* and few genera from the superfamily Phytoseioidea are known to additionally include plant diets (Karg 1993). Gamasid mites contain large, aggressive and very mobile predators such as species of the genera *Pergamasus* and *Veigaia* that even subdue larger collembolans, whereas smaller species predominantly include small collembolans without furca and nematodes in their diet (Karg 1993; Koehler 1997, 1999; Klarner *et al.* 2013). Gamasid mites have an extra-intestinal digestion, an overall sensitivity to light but no optical orientation and detect prey by chemical or tactile stimuli *(*Karg 1993; Köhler 1997). Their prey choice seems to be limited by size, e.g. too large prey gets attacked but not killed whereas too small prey is ignored (Karg 1993).

Many uropodid mite species are known to feed on nematodes and therefore prefer organic matter with many nematodes as habitat which influences its distribution (Karg 1989; Köhler 1997). Rich uropodine communities in the range of 10,000 individuals per square meter can be found in organic materials such as deciduous forest litter and reflect the increase of pore volume and soil organic matter (Karg 1986; Karg 1989). Overall, gamasid and uropodid mites are top-predators in the mesofaunal food web and often form part of the highest trophic level in soil systems.

Oribatid mites are species-rich with about 10,000 described species worldwide (Schatz 2002) and reach high densities of up to 400,000 individuals per square meter in forest ecosystems (Maraun & Scheu 2000). Recently, the origin of oribatid mites has been dated back to the Precambrian some 570 million years ago by molecular clock analyses (Schaefer *et al.* 2010) and the oldest fossils are from Devonian sediments 380 million years ago (Shear *et al.* 1984; Norton *et al.* 1988). Oribatid mites convergently evolved defence mechanisms against predators such as ptychoid body form, hologastry and cuticular mineralization (Pachl *et al.* 2012). Today, adult oribatid mites are assumed to live in enemy-free space and avoid predation by other mesofauna taxa due to strongly sclerotized cuticle and chemical defence by secretions of opisthonotal glands (Peschel *et al.* 2006; Heethoff et al. 2011). Occasionally, however, they are consumed by large animals such as salamanders (Norton & McNamara 1978), frogs (Saporito *et al.* 2007), centipedes (Lebrun 1970), scydmaenid beetles (Mollemann & Walter 2001) and ants (Masuko 1994; Wilson 2005). Oribatid mites have been assumed to feed mainly on litter and fungi (Maraun & Scheu 2000), and hold a key position in

decomposition and mineralization processes of soils (Seastedt 1984). Therefore, they are classified as 'classical' decomposers and are often pooled as one functional group in food web analyses. However, recent studies indicate that the nutrition of oribatid mites is more diverse than presumably assumed and that they span over four trophic levels from primary decomposers up to predators that most likely feed on nematodes (Schneider *et al.* 2004a; Rockett & Woodring 1966; Muraoka & Ishibashi 1976; Oliveira *et al.* 2007; Norton & Behan-Pelletier 2009).

4. Studying trophic interactions in soil

Trophic interactions in soil are often difficult to observe and to evaluate which is due to a number of factors. First, soil animals are small; second, they live in opaque and inaccessible habitats; and third, direct observations are difficult without disturbing the system (Symondson 2002; Scheu *et al.* 2005). Therefore, analysis of predator's gut content (Symondson 2002; King *et al.* 2008), faeces (Seeber *et al.* 2010) or regurgitates (Waldner & Traugott 2012) are necessary to determine the ingested prey. Microscopic analyses allow to identify and quantify prey remnants (Symondson 2002), however, the technique relies on hard prey fragments and is unsuitable for soft bodied prey, such as earthworms but also nematodes which only provide sclerotized mouthparts for detection (Walter 1988a; Sunderland 1988). Moreover, numerous predatory soil organisms including microarthropods either digest their prey extra-intestinally or are liquid feeders consuming only fluid prey components. Therefore, analyses based on indigestible remains provide biased results, missing many predator-prey interactions (Dennison & Hodkinson 1983; Walter 1988a; Sunderland 1988; Symondson 2002; Sheppard & Harwood 2005).

Several indirect methods for studying predator-prey interactions in soil have been established in the last years, e.g. fatty acid analysis (Ruess *et al.* 2004) and the analysis of stable isotope ratios (^{15}N/^{14}N, ^{13}C/^{12}C; Maraun *et al.* 2011). These techniques allow insight into the trophic position of soil animal species and ascribing species to energy channels such as the bacterial and fungal decomposition pathway (Pollierer *et al.* 2012). However, determination of predator-prey interactions at the level of species or individuals are not possible. Other indirect methods have been used recently, e.g. gas chromatography–mass spectrometry analysis of prey alkaloid markers (Sloggett *et al.* 2009), protein electrophoresis (Traugott 2003), immunoassays using polyclonal or monoclonal antibodies (Sunderland 1988; Harwood *et al.* 2004), but the use of molecular markers for a certain prey was the most promising and best evaluated approach to detect neglected trophic links of the soil food web (Symondson 2002).

5. Molecular gut content analysis

Molecular gut content analysis is a novel and promising tool to detect predator-prey interactions in soil food webs. Molecular markers, i.e. primers for a certain prey taxon, are used to detect its DNA in the gut of predators to determine consumption and can also be used to identify parasite-host interactions (Traugott *et al.* 2008).

General or specific primers can be used as molecular markers. General primers amplify prey groups or families pointing to the prey spectrum of a predator but are prone to PCR bias (Vestheim & Jarman 2008). Specific primers amplify prey DNA on genus or species level and point to the distribution of a certain prey taxon within the food web, thereby identifying many potential predators in short time.

Primers for molecular gut content analyses need to be sensitive in detecting small amounts of prey DNA after ingestion, since the prey individual typically is smaller than the predator and only consumed partially (Eitzinger & Traugott 2011; King *et al.* 2008). They need to amplify their target specifically and not the DNA of the predator or alternative food. Therefore, they have to be tested against a wide range of non-target species for possible cross-reactions to exclude false positive results (Harwood *et al.* 2007; King *et al.* 2008). Prey DNA detection decreases with time since prey in the gut is digested by the predator (Sheppard *et al.* 2005; Read et al. 2006; von Berg *et al.* 2008; Gagnon *et al.* 2011). Therefore, the primers need to detect prey DNA long enough to allow identifying feeding events that occurred before sampling of soil animals in the field.

Mitochondrial or ribosomal multi-copy genes are preferred to single-copy genes for designing primers as this increases the probability of detecting prey DNA (King *et al.* 2008). Moreover, primers need to amplify short fragments (typically ~300 bp; Zaidi *et al.* 1999; Sint *et al.* 2011) to allow detection of degraded prey DNA by gut enzymes and to prolong detection time. However, since DNA can be detected between some hours and several days after feeding in the gut of a predator the method provides more than a short term 'snapshot' of feeding events (Pollierer *et al.* 2012).

Molecular gut content analysis also has limitations. The amplification of prey bands and its visualization shows only the presence or absence of prey DNA but neither the amount of prey consumed nor if the prey was dead or alive, i.e. does not allow to differentiate between predation and scavenging (Juen & Traugott 2005; Foltan *et al.* 2005; von Berg *et al.* 2012) nor to identify secondary predation (hyperpredation; Sheppard *et al.* 2005). Further, detection of cannibalism is impossible. A quantification of the prey/predator DNA ratio is possible by quantitative real-time PCR (qPCR; Weber & Lundgren 2009) but gives only hints which of the

predator individuals has consumed more than the other. Future work is necessary for allowing to relate the amount of prey DNA detected to the number (biomass) of prey individuals consumed. However, by screening a large number of predator individuals the method allows to determine trophic links and their strength in the food web if specific primers exist. Since the soil system is exceptionally rich in species the method needs primarily to be applied to abundant prey species that hold key positions in soil food webs such as nematodes.

Molecular markers have been designed and evaluated for three entomopathogenic nematodes *Phasmarhabditis hermaphrodita*, *Steinernema feltiae* and *Heterorhabditis megidis* and were used to investigate the gut content of collembolans (Read *et al.* 2006). The results suggest that these microarthropods indeed feed on nematodes in the laboratory and the field when available. If other soil microarthropods, such as mesostigmatid and oribatid mites, also include nematodes in their diet remains a goal for future work.

6. Objectives and chapter outline

This thesis focuses on the role of nematodes as prey for microarthropods. Therefore, molecular markers for entomopathogenic model nematodes (*Phasmarhabditis hermaphrodita* and *Steinernema feltiae*) were used to identify potential predators and to distinguish in a field experiment the importance of scavenging or predation as feeding mode (Chapter 2). Moreover, new molecular markers for free-living bacterial feeding nematodes (*Acrobeloides buetschlii*, *Plectus minimus*, *Plectus velox* and *Panagrellus redivivus*) were designed and optimized for molecular gut content analyses to investigate if microarthropods consume these nematode species in the field (Chapter 3). Further, using these primers we investigated if nematode consumption differs between microhabitats (litter, grass and moss) indicating trophic niche differentiation and contributing to the coexistence of the many soil animal species (Chapter 4).

We examined the following main hypothesis:

(1) Nematodes form important prey of many microarthropod taxa including those that have been assumed to live as decomposers. Therefore, established and new designed molecular markers were used (Chapter 2 and 3).
(2) Microarthropods prefer dead over living prey (scavenging vs. predation; Chapter 2).
(3) Nematode species are differentially consumed by mites pointing to differences in predator-prey interactions on species level (Chapter 2, 3, 4).

    (4) Consumption of nematodes by microarthropods differs between microhabitats, i.e. their trophic niches vary in space (Chapter 4).

In the following, the content of the chapters is summarized:

In **Chapter 2** we used two entomopathogenic nematode species (*Phasmarhabditis hermaphrodita* and *Steinernema feltiae*) as model organisms to investigate if abundant soil mites feed on nematodes, how long nematode DNA is detectable in predator guts, and if they prefer dead or living nematode prey. Established molecular markers were used in these analyses (Read *et al.* 2006). In the laboratory potential mite predators of nematode species were identified including those previously assumed to live as decomposers. Prey DNA was detectable in predators for up to 128 h but detection depended on the time the nematodes were exposed to the predator. Since these two nematode species are large predators needed long (up to 48 h) to subdue and consume them. Dead and living *P. hermaphrodita* and *S. feltiae* were added to the forest floor food web for 48 h. Then, soil mites were extracted and screened for nematode prey. Soil mites including species that were previously assumed to live as decomposers frequently consumed the nematodes. Soil mites consumed dead and living nematodes indicating that they function as both scavengers and predators. The two nematode species were differentially consumed depending on whether they were dead or alive, indicating that defence mechanisms alter predator-prey interactions. The results suggests that the mite species studied also feed on free living nematode species in the field thereby controlling nematode populations.

In **Chapter 3** we investigated the role of field-living nematodes as prey for soil microarthropods. Therefore, we designed new primers for free-living bacterial feeding nematode species including *Acrobeloides buetschlii*, *Plectus minimus*, *Plectus velox* and *Panagrellus redivivus* and used them for molecular gut content analyses of soil mite and collembolan species in the laboratory and the field. The molecular markers amplify short parts of the respective nematode 18S rDNA and allowed detecting even small amounts of DNA in the gut of predators. DNA of these four nematode species was detectable for up to 128 h in the gut of mite predators. Detection time varied between nematode species, mite species and time of exposure indicating that detection times are specific for every predator-prey interaction. Abundant mite and collembolan species proved to feed on the studied nematode species in the laboratory and on *A. buetschlii* and *Plectus* spp. in the field. We could not detect *P. redivivus* in mites from the study site since it does not occur there, however, *P. redivivus* is easy to culture in large numbers and can be used as model organism for experimental studies. The results suggest that in the field nematodes form an important component of the diet of soil

microarthropods and that this presumably contributes to the flux of energy from root exudates via bacteria to higher trophic levels.

In **Chapter 4** we used the newly designed molecular markers for *A. buetschlii* and *Plectus* spp. to investigate if the consumption of these nematode species differs between habitats. Therefore, we investigated the consumption of nematode prey in fourteen abundant soil mite species from three different habitats (litter, grass and moss). The mite species differentially consumed the two nematode taxa and consumption differed between the three habitats. The results suggest that feeding of mites on nematodes is more common than previously assumed and that their diet varies between microhabitats. Trophic niche differentiation and spatial variation of trophic niches is likely to contribute to the coexistence of the many mite species in the seemingly homogeneous soil habitat.

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Molecular detection of nematode predation and scavenging in oribatid mites: Laboratory and field experiments

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Abstract

Recent stable isotope analyses indicate that a number of putative detritivorous soil microarthropods is not typical detritivores but rather live as predators or scavengers. Using molecular gut content analyses the present study investigates if nematodes indeed form part of the diet of oribatid mites. First, in a no-choice laboratory feeding experiment two nematode species (*Phasmarhabditis hermaphrodita* and *Steinernema feltiae*) were offered to eight species of oribatid mites and one gamasid mite. Second, after feeding for 4 and 48 h on each nematode species the detection time of prey DNA in the oribatid mite species *Steganacarus magnus* was investigated. Third, in a field experiment nematode prey (*P. hermaphrodita* and *S. feltiae*) in the diet of microarthropods was investigated distinguishing between scavenging and predation. In the no-choice laboratory experiment not only the gamasid mite but also several of the studied oribatid mite species consumed nematodes. After feeding on nematodes for 4 h prey DNA was detectable in *S. magnus* for only 4 h, but after feeding for 48 h prey DNA was detectable for 128 h, indicating that the duration of feeding on prey is an important determinant for prey DNA detection. The field experiment confirmed that oribatid mite species including *Liacarus subterraneus*, *Platynothrus peltifer* and *S. magnus* intensively prey on nematodes. Interestingly, DNA of dead *P. hermaphrodita* was detectable to a similar degree as that of living individuals indicating that scavenging is of significant importance in decomposer food webs. Results of our study indicate that predation and scavenging on nematodes by "detritivorous" microarthropods in soil food webs need to be reconsidered.

1. Introduction

Predator-prey interactions are an important component of terrestrial food webs influencing population densities and energy fluxes through the system (Terborgh & Estes 2010). Knowledge on predator-prey interactions is of prime importance for understanding aboveground as well as belowground food webs and for evaluating the relative importance of top-down forces in decomposer systems (Milton & Kaspari 2007; Tylianakis *et al.* 2008). In contrast to aboveground systems, predator-prey interactions in belowground food webs are difficult to study since (1) trophic links are difficult to observe without disturbing the system, (2) most soil animals are small (<1 mm) and therefore direct observation is difficult, and (3) liquid-feeding hampers tracing the food in the gut (Symondson 2002; Read *et al.* 2006; Juen & Traugott 2007).

Molecular methods may help to overcome these limitations (King *et al.* 2008). First, extraorally digested prey can be detected in the predator gut (Kuusk *et al.* 2008). Second, the investigation of the gut content of soil-living predators using specific primers allows distinguishing the prey at the level of species (Read *et al.* 2006; Juen & Traugott 2007). Specific primers amplifying short DNA fragments allow detection of prey DNA in the gut of predators even days after prey consumption (Agusti *et al.* 1999; Zaidi *et al.* 1999; Hoogendorn & Heimpel 2001). Using specific primers allows identifying the spectrum of predators of single species and thereby investigating the level of specialism vs. generalism in soil food webs. Molecular gut content analyses, however, also have limitations. One particular weakness is that predation, i.e. feeding on living prey, cannot be separated from scavenging (Juen & Traugott 2005) and from secondary predation, i.e. the feeding of a predator by another predator (Sheppard *et al.* 2005). One of the first important studies using molecular markers for the detection of nematode DNA in predator guts was carried out by Read *et al.* (2006) who developed specific primers for three nematode species (*Phasmarhabditis hermaphrodita*, *Steinernema feltiae*, *Heterorhabditis megidis*).

Nematodes hold a key position in soil food webs (Yeates *et al.* 1993; Bongers & Ferris 1999; Moore *et al.* 2003), little is known on their role as prey for other soil animals and to what extent their density is controlled by predators. They are among the most diverse and abundant animals on earth (Baxter 2003). Nematodes live not only as parasites in animals and plants, but also freely in aquatic and soil systems, where they can reach densities of up to several million individuals and up to 100 species per square meter (Yeates *et al.* 2000). In soil they feed on bacteria, fungi, algae, invertebrates, plants or are omnivorous, and, due to their generally high density, they form an important food source for other soil animals (Yeates *et al.* 1993; Read *et al.* 2006).

Mites (Acari) also reach high diversity and density in soil systems (Maraun & Scheu 2000) comprising not only "classical detritivores", such as Oribatida, but also predators, such as Gamasina and Uropodina. Laboratory studies (Muraoka & Ishibashi 1976) as well as stable isotope analyses (Schneider *et al.* 2004) indicate that several species of oribatid mites can live also predatory, most likely on nematodes. Gamasid mites are free-living, motile, liquid-feeding predatory mites (Koehler 1997). They are assumed to be generalist predators that feed on a variety of prey including nematodes. Uropodid mites live in soil where they mainly consume not only nematodes, but also slugs, insect larvae and dead animals (Karg 1989; Raut & Panigrahi 1991; Scheu & Falca 2000).

The aims of this study were to investigate (1) if soil mite species (mainly from the taxa Oribatida, but also Gamasina and Uropodina) feed on nematodes, (2) if the nematode species are consumed to a different degree, (3) the detection time of prey DNA after different time periods of feeding by *Steganacarus magnus*, (4) the relevance of laboratory feeding experiments for understanding trophic interrelationships in the field, and (5) the relevance of predation and scavenging in soil food webs, i.e. if microarthropod predators discriminate between dead and living prey. Therefore, laboratory no-choice feeding experiments were established using the two model nematode species *S. feltiae* and *P. hermaphrodita*. In the laboratory, nematodes were offered separately to investigate if the studied mite species differentially feed on nematode species. To determine the detection time of prey DNA *S. magnus* was fed with each nematode species for 4 h and 48 h. To prove the validity of the results of the laboratory studies in the field the two nematode species were added to the soil of a beech forest. Each species was added dead and alive to separate the role of scavenging and predation for soil mite nutrition. Low natural abundance of the added model nematodes in field soil allowed separating them from the resident nematode community. We hypothesized that a large number of mite species, including putatively detritivorous taxa such as oribatid mites feed on nematodes in the field.

2. Material and methods

2.1 The organisms

Infective juveniles (dauerlarvae) of two pathogenic nematode species were used for this study. The infective non-feeding third stage of the rhabditid *P. hermaphrodita* (Schneider, 1859) has a length of ~1mm. *P. hermaphrodita* is a parasitic bacterivore that infects the mantle of slugs where it reproduces and kills the slug before the new larvae are spread to the soil. The entomopathogenic *S. feltiae* (Filipjev, 1934) dauerlarvae has a length of w0.8mm and

parasitizes the hemocoel of sciarid and drosophilid larvae. Juveniles of both species search in soil for new hosts. *P. hermaphrodita* and *S. feltiae* are cosmopolitan species; however, their density in the field is low. At the studied beech forest they could not be detected by standard soil sampling and nematode extraction making them ideal model prey species as there are no background signals in nematode consumers (K. Heidemann, unpubl. data). Both nematode species were supplied by 'prime factory' (www.schneckenprofi.de, Hennstedt, Germany). They are able to survive in soil for several weeks which is more than sufficient for the purpose of our experiment (Kaya & Gaugler 1993).

Three mite taxa, i.e. Oribatida, Gamasina and Uropodina, were studied as potential predators for the two nematode species. In the laboratory experiment the gamasid mite *Hypoaspis aculeifer* (Canestrini, 1883) and eight oribatid mite species were included: *Achipteria coleoptrata* (Linné, 1758), *Atropacarus striculus* (Koch, 1835), *Carabodes coriaceus* Koch, 1835, *Damaeus riparius* Nicolet, 1855, *Eupelops plicatus* (Koch, 1835), *Hypochthonius rufulus* Koch, 1835, *Steganacarus magnus* (Nicolet, 1855) and *Archegozetes longisetosus* Aoki, 1965. In the field experiment the gamasid mite *Pergamasus septentrionalis* (Oudemans, 1902), the uropodid mite *Uropoda cassidea* (Hermann, 1804) and seven species of oribatid mites were investigated: *Chamobates voigtsi* (Oudemans, 1902), *Liacarus subterraneus* (Koch, 1844), *Platynothrus peltifer* (Koch, 1839), *Nothrus silvestris* Nicolet, 1855 and *S. magnus*. All species are inhabitants of European forest soils except *A. longisetosus* which is tropical and was included as it is the most frequently cultured oribatid mite species.

2.2 The study site

The Kranichstein forest is an oak-beech forest near Darmstadt, Hesse, Germany. The annual temperature is 9.5 °C and the annual precipitation is about 700 mm. Soil pH is 3.6-4.3 and the humus form is moder. The tree layer is dominated by beech (*Fagus sylvatica*, L.); parent rock is Cisuralian (Rotliegendes, Early Permian; Schneider *et al.* 2007).

2.3 Test of primer specificity

Both nematode primer pairs used were tested against 16 non-target species by Read *et al.* (2006). Additionally, we tested them against further 74 non-target species (Table S1, Supporting information), including the 15 mite species used in this study. The animals were starved for seven days and stored separately at -80°C in 180 mL ATL-Buffer of the extraction

kit DNeasy® Blood & Tissue Kit (Qiagen GmbH, Hilden, Germany). After DNA extraction the mesofauna taxa were tested with the D3 primers, and the macrofauna taxa with general COI primers (Folmer *et al.* 1994), to check success of the DNA extraction. The specific primers for *P. hermaphrodita* and *S. feltiae* were used in a PCR with the non-target taxa and with the respective nematode species as positive control to check for cross-reactions.

2.4 Detection time of prey in consumers

Detection time of prey DNA in consumers was investigated using the oribatid mite *S. magnus* which were extracted from soil of the Göttinger forest and the Hainich forest. All individuals were starved for seven days. The two nematode species (*S. feltiae*, *P. hermaphrodita*) were offered separately to 20 individuals of *S. magnus* for 4 and 48 h. Afterward mites were checked for attached nematodes and starved for 0, 2, 4, 8, 16, 32, 64 and 128 h. After these time periods we checked if mites were alive to avoid false negatives, and stored individual mites separately at -80 °C in 180 mL ATL-Buffer of the extraction kit DNeasy® Blood & Tissue Kit (Qiagen GmbH, Hilden, Germany). During the experiment mites were kept in darkness at 13°C.

2.5 Laboratory experiment

Seven oribatid mite species were extracted from soil of the Kranichstein forest whereas *A. longisetosus* (Oribatida) and *H. aculeifer* (Gamasid mites) were taken from laboratory cultures. All species were starved for four days. The nematode species were offered separately to eight oribatid mites and one gamasid mite. The latter was included to compare the feeding of a well-known predator with putative detritivorous species. After four days of feeding, mites were checked for attached nematodes; no nematodes were found. Subsequently, the mites were placed separately in 96% ethanol and stored at -20 °C. Five replicates were run for each predator species on each nematode species, except *A. longisetosus* and *D. riparius* (only four replicates for *P. hermaphrodita*) and *H. rufulus* (only three replicates for *P. hermaphrodita*). As controls, five individuals of each species were starved and tested using the specific primers to prove that there was no contamination in the handling of the samples.

2.6   Field experiments

To separate between predation and scavenging two field experiments were established. In the first experiment each of two replicates of 1 m^2 received 4 million living *P. hermaphrodita* and four million dead *S. feltiae* in November 2007 (average air temperature of 7-8°C); in the second experiment each of two replicates received 4 million dead *P. hermaphrodita* and 4 million living *S. feltiae*. The nematodes were applied to the natural soil animal community in a water solution (1 l for each species) using a watering can. Nematodes were killed by freezing at -80°C for two days prior to application and were used as "dead nematodes" in the experiment.

After 48 h the litter of each plot was collected and soil mites were extracted by heat for ~6 h (Kempson *et al.* 1963). The mites were checked for attached nematodes which were never found. In the following 3 h the most abundant large mite species were collected, washed inwater for 1min and stored separately at -80°C in 180 mL ATL-Buffer of the extraction kit DNeasy® Blood & Tissue Kit (Qiagen GmbH, Hilden, Germany). Overall, the time between the sampling of the species in the field and the freezing was about 10 h.

2.7   Bipartite network analysis

For a visualization of the predator-prey interactions between the mites and the nematodes from the field experiment, the links were graphically presented in a network using the package 'bipartite' (Dormann *et al.* 2008) in the program R (R Development Core Team 2009). Negative observations, i.e. no detection of feeding on any of the two nematode species (irrespective if they were dead or alive), were pooled for this figure.

2.8   DNA extraction, PCR and sequencing

DNA from whole individuals of soil microarthropods was extracted following the protocol of the DNeasy® Blood & Tissue Kit (Qiagen). To verify the success of the DNA extractions and to confirm predator species identity a PCR with D3 primers amplifying a 320 bp fragment of the 28S rDNA was performed with every sample (Maraun *et al.* 2003). Negative samples were excluded from the study.

Detection of nematode DNA followed the modified protocol established by Read *et al.* (2006). In short, a PCR with the specific nematode primers developed by Read *et al.* (2006)

[Sf-F-1896 (forward primer) 5'-TTT AGG CCA TCC TGG AAA GAG G-3'; Sf-R-2080 (reverse primer) 5'-GCT AAA ACC GGT AAA GAA AG-3'] amplified a 203 bp fragment of the cytochrome oxidase I (COI) region. The PCR for the detection of *P. hermaphrodita* [Ph-F-1754 (forward primer) 5'-TGG GCG CCC CTG ATA TAA GAT-3'; Ph-R-1887 (reverse primer) 5'-CGG ATG ACC AAG GGT ACT TAA T-3'] amplified a 203 bp and a 154 bp fragment (primer sequences are given in detail since the orientation of the two reverse primers is wrong in Read *et al.* 2006).

PCRs with D3 primers and specific nematode primers were carried out as follows: PCR contained 1 mL (concentration 100 pmol mL^{-1}) of each primer (Operon/Eurofins, Ebersberg, Germany), 1 mL of BSA (3%), 1 mL of 25mMMgCl2 (Fermentas, St. Leon-Rot, Germany), 12.5 mL of HotStarTaq$^{®}$ Master Mix Kit (Qiagen) including polymerase, 2.5 mL template DNA and were filled up to 25 mL with RNase free water. Thermal cycle parameters included an initial denaturation step of 10 min at 95°C followed by 35 cycles of 95°C for 30 s, the respective annealing temperature (56°C for *S. feltiae* and 58°C for *P. hermaphrodita*) for 45 s and 72°C for 30 s, and a final extension stage of 72°C for 10 min. The PCR products were visualized on a 1% agarose gel. Positive samples were sequenced by Macrogen Inc. (Seoul, South Korea).

2.9   Statistical analysis

For the laboratory experiment a logistic regression (Hosmer & Lemeshow 1989; Sokal & Rohlf 1995) was used to test (i) if the mite species differently consumed the nematode prey offered, and (ii) if one of the two nematode species offered was generally preferred over the other. In the field experiment a logistic regression was used to analyze (i) if the mites selectively consumed one of the two nematode species, (ii) if dead nematodes were preferred over living ones (or vice versa), and (iii) if the mite species differentially fed on the two nematode species. The analyses were carried out for each treatment separately using SAS 9.13 (SAS Institute Inc., Cary, USA). Subsequently, mean and standard derivations of nematode detection rates were calculated by using the package 'simpleboot' in R (Simple Bootstrap Routines. R-package version 1.1e3; Roger D. Peng, 2008; R Development Core Team 2009). Additionally, 95% confidence limits were calculated by 9999 bootstrap resamples for each predator feeding on a certain prey.

3. Results

3.1 Test of primer specificity

The non-target test of 74 species with the primers for *P. hermaphrodita* showed no cross-reactions (Table 1; Supporting material) indicating that this primer pair is very specific. In contrast to Read *et al.* (2006), the non-target test with the primers for *S. feltiae* showed cross-reactions for the following 12 species: *Uroseius cylindricus* (Berlese, 1916), *Lithobius aulacopus* Latzel, 1880, *Lithobius lapidicola* Meinert, 1872, *Lithobius muticus* (Koch, 1847), *Lithobius crassipes* Koch, 1862, *Neanura muscorum* (Templeton, 1835), *Philonthus carbonarius* (Gravenhorst, 1802), *Polydesmus complanatus* (Linnaeus, 1761), *Glomeris* sp., *Porcellio* sp., *Trichoniscus pusillus* Brandt, 1833 and *Aporrectodea caliginosa* (Savigny, 1826). However, the primers showed no cross-reaction with the mite predators used in our study. It can therefore be assumed that the positive cross-reactions are not relevant for our study. We sequenced about 50% of all positive prey bands and never found any of the above species in the gut of the mite species.

3.2 Detection time of prey DNA in consumers

Immediately after the end of the 48 h feeding trial 100% of the individuals of *S. magnus* that were offered *P. hermaphrodita* were tested positive for the nematode DNA. This high percentage declined during the post-feeding phase to about 20% after 128 h (Fig. 1a). In contrast, immediately after the end of the 4 h feeding trial only 55% of the individuals of *S. magnus* that were offered *P. hermaphrodita* were tested positive for nematode DNA. This number declined during the post-feeding phase to 5% after 4 h; later no prey DNA was detectable anymore (Fig. 1b).

Prey DNA detection of *S. feltiae* was generally less frequent than that of *P. hermaphrodita*. Immediately after the end of the 48 h feeding trial 100% of the individuals of *S. magnus* that were offered *S. feltiae* were tested positive for the nematode DNA. This number declined quickly during the early post-feeding phase to about 20% after 4 h, however, prey DNA could still be detected 128 h post feeding (Fig.1c). Immediately after the end of the 4 h feeding trial no individuals of *S. magnus* were tested positive for nematode DNA (Fig. 1d).
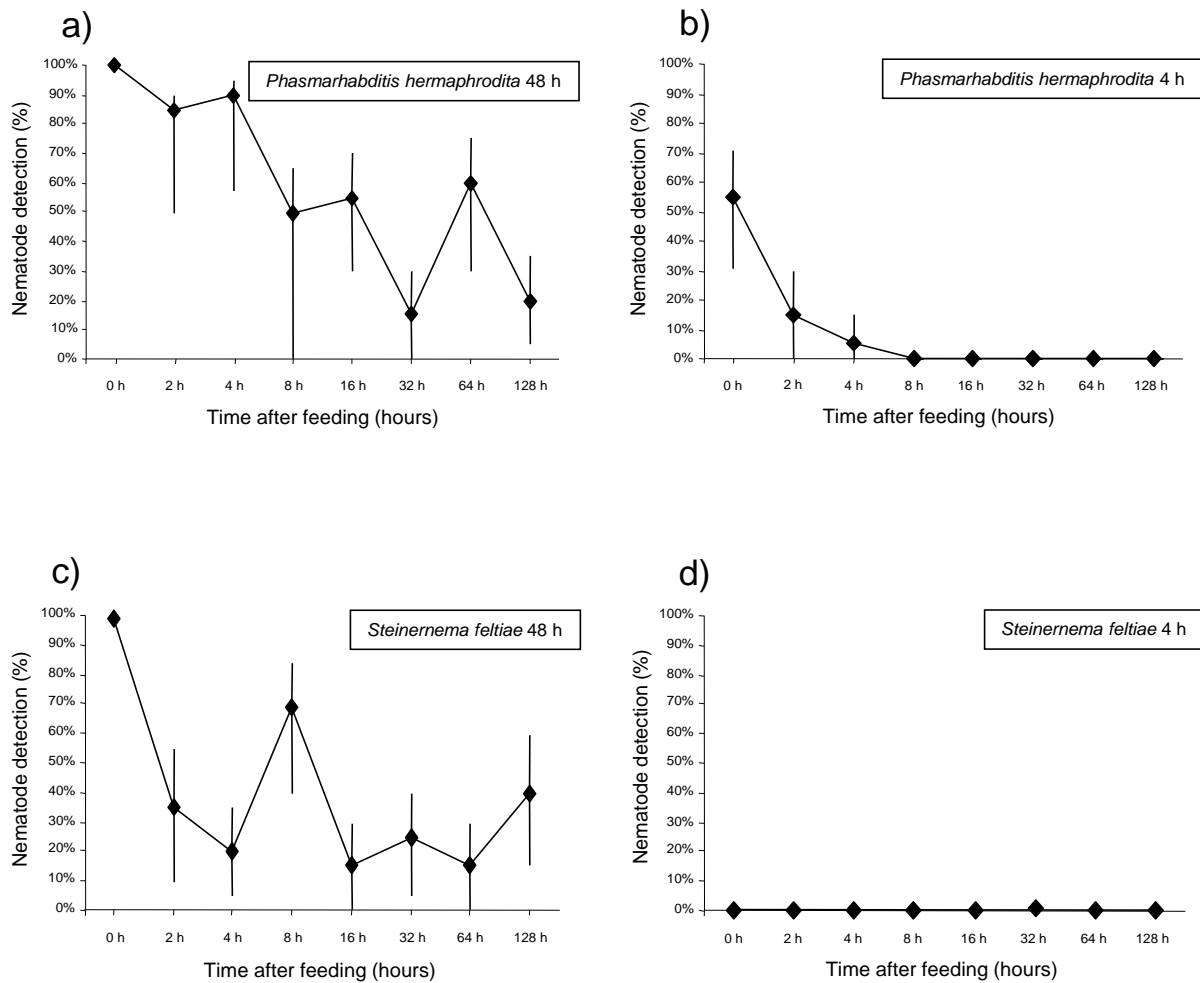
Figure 1: Detectability of *Steinernema feltiae* and *Phasmarhabditis hermaphrodita* DNA (part of the COI gene) in the gut of *Steganacarus magnus* after 0, 2, 4, 8, 16, 32, 64 and 128 h after feeding. The mites were fed for 48 h (a, c) and for 4 h (b, d) with the two nematode species. 20 individuals of *S. magnus* were tested per time-point. Data represent percentages of mite individuals in which nematode DNA were detected. The upper and lower confidence limits are indicated as error bars; for statistical analysis see text.

3.3   Laboratory experiment

Positive detection of nematode DNA in predator gut (in the following termed 'feeding' or 'consumption') significantly differed between mite species ($\chi^2 = 31.8$, df = 8, P< 0.0001; Fig. 2). Feeding on the two nematode species generally did not differ significantly ($\chi^2 = 0.56$, df = 1, P >0.05). All individuals of the oribatid mite species *D. riparius* and *S. magnus* consumed the nematode *P. hermaphrodita*; the other nematode species, *S. feltiae*, was only consumed occasionally by these two mite species. *A. longisetosus* preyed occasionally on *P.*

hermaphrodita, but consumed *S. feltiae* in all cases studied. *H. aculeifer* preyed occasionally on both nematode species (40% on *P. hermaphrodita* and 20% on *S. feltiae*). Only 20% of the investigated *A. striculus* consumed *P. hermaphrodita* but never *S. feltiae*, and *H. rufulus* occasionally consumed *S. feltiae* (40% of the tested individuals) but never *P. hermaphrodita*. *A. coleoptrata*, *C. coriaceus* and *E. plicatus* were not tested positive for any of the two offered nematode species.
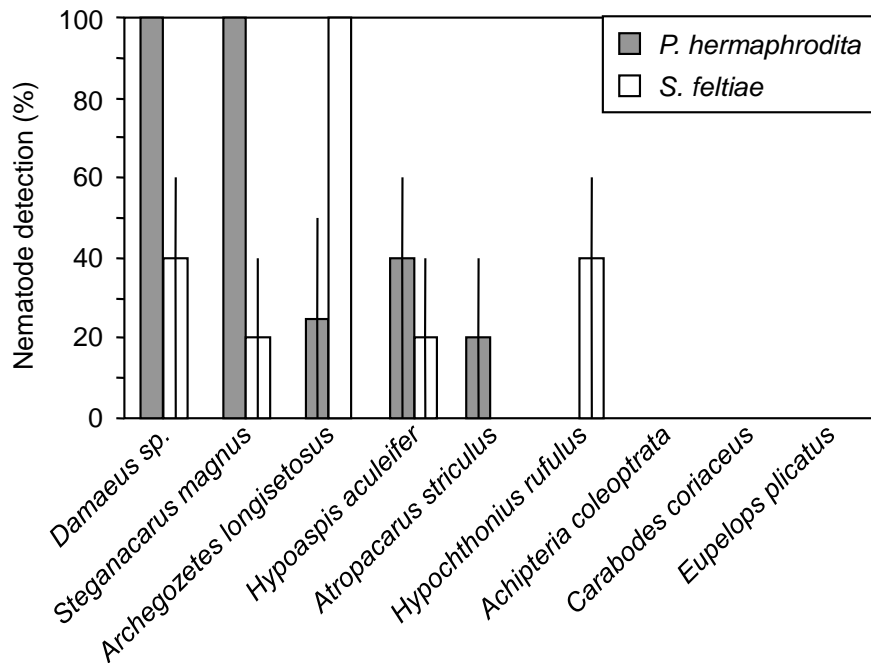
Figure 2: Detection of *Steinernema feltiae* and *Phasmarhabditis hermaphrodita* in the gut of nine mite species in no-choice laboratory experiments using part of the COI gene as molecular marker. Data represent percentages of mite individuals in which nematodes had been detected in the gut. The upper and lower confidence limits are indicated as error bars; for statistical analysis see text.

3.4 Field experiments

3.4.1 Experiment with living P. hermaphrodita and dead S. feltiae

DNA of the living *P. hermaphrodita* was more frequently detected in all studied mite species than that of the dead *S. feltiae* (χ^2 = 92.98, df = 1, P< 0.0001; Fig. 3a). The analyzed mite species fed differently on the two nematode species (χ^2 =107.74, df = 6, P<0.0001), and consumption tended to vary between the two nematode species (χ^2 = 18.36, df = 6, P< 0.054).Of the tested individuals of the gamasid mite *P. septentrionalis* 66% fed on the living

(*P. hermaphrodita*) and 32% on the dead nematode species (*S. feltiae*).*U. cassidea* fed frequently on *P. hermaphrodita* (85%) and somewhat less on *S. feltiae* (55%). Of the investigated individuals of *L. subterraneus* 85% fed on *P. hermaphrodita* and 92% on *S. feltiae*. A total of 40% of *P. peltifer* and 61% of *N. silvestris* preyed on the living nematode species and only 3% of *P. peltifer* on *S. feltiae*. No consumption of the dead nematode species was detected in *N. silvestris* and *S. magnus*. *S. magnus* only fed on *P. hermaphrodita* (39% of the tested individuals). Of the tested individuals of *C. voigtsi* 43% preyed on *P. hermaphrodita* and only 10% on *S. feltiae*.

3.4.2   Experiment with dead P. hermaphrodita and living S. feltiae

The dead nematode species was more frequently consumed by all mite species than the living one (χ^2 = 92.85, df = 1, P < 0.0001; Fig. 3b). The analyzed mite species fed differently on the two nematode species (χ^2 = 57.66; df = 6; P< 0.0001), and consumption varied between the two nematode species (χ^2 = 12.80, df = 6, P< 0.0463). The gamasid mite *P. septentrionalis* fed frequently (67% of the tested individuals) on the dead (*P. hermaphrodita*) and less (24%) on the living nematode species (*S. feltiae*). The uropodid mite (*U. cassidea*) only consumed *P. hermaphrodita* (about 25 % of the individuals) and never the living *S. feltiae*. The oribatid mites *L. subterraneus*, *S. magnus* and *C. voigtsi* fed frequently on *P. hermaphrodita* (100, 67 and 18%, respectively), but never the living nematode species. A total of 30% of the tested individuals of *P. peltifer* and 30% of *N. silvestris* consumed the dead nematode species and less the living one (7% and 6%, respectively).

a)

b)
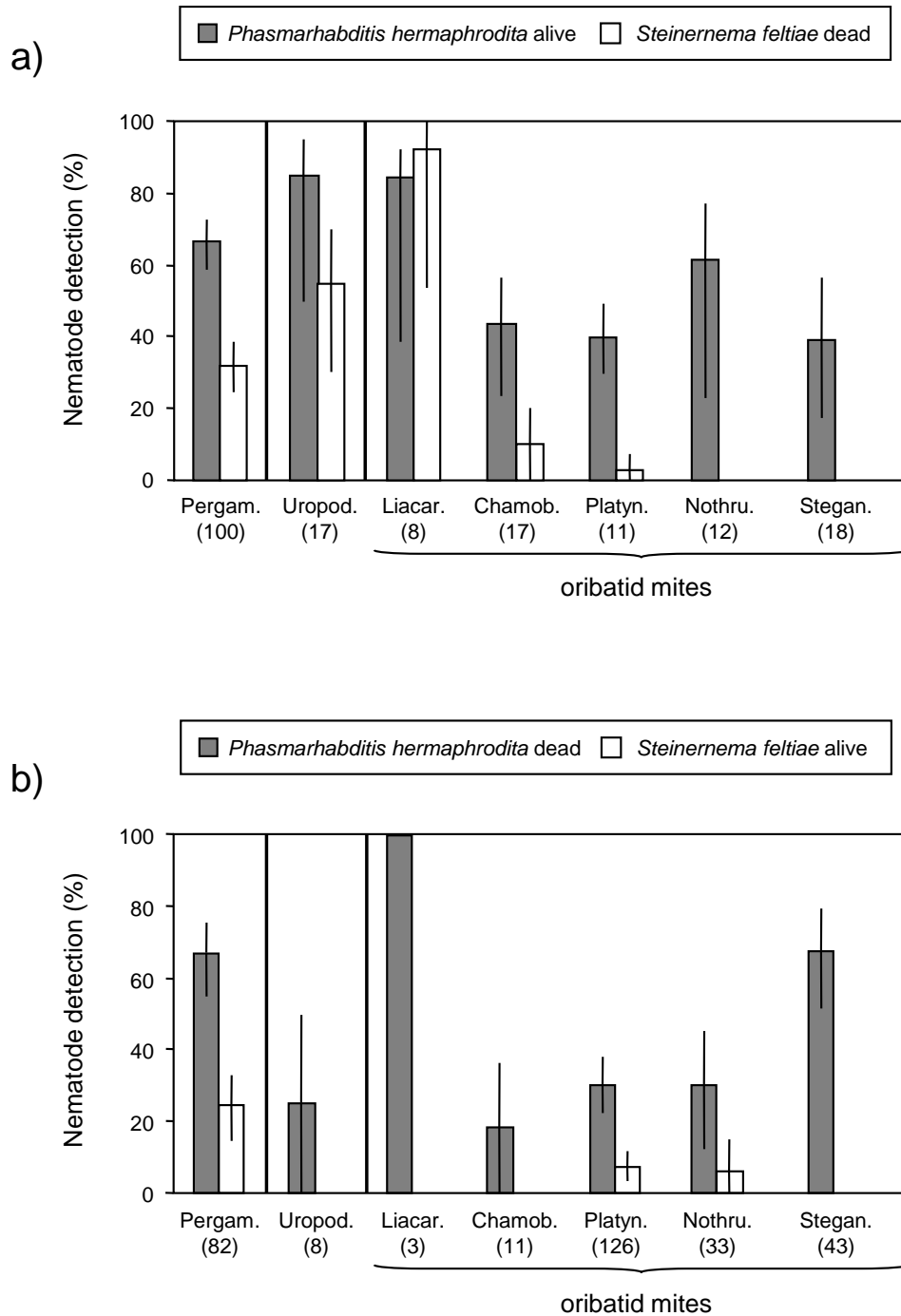
Figure 3: Detection of (a) living *Phasmarhabditis hermaphrodita* and dead *Steinernema feltiae* and (b) living *Steinernema feltiae* and dead *Phasmarhabditis hermaphrodita* in the gut of seven mite species (Pergam. = *Pergamasus septentrionalis*, Uropod. = *Uropoda cassidea*, Liacar. = *Liacarus subterraneus*, Platyn. = *Platynothrus peltifer*, Nothru. = *Nothrus silvestris*, Stegan. = *Steganacarus magnus*, Chamob. = *Chamobates voigtsi*) in the field using part of the COI gene as molecular marker. Data represent percentages of mite individuals in which nematodes were detected in the gut (the number of tested individuals is given in brackets). The upper and lower confidence limits are indicated as error bars; for statistical analysis see text.

3.5 Bipartite network

The bipartite network shows that living *P. hermaphrodita* were more frequently consumed than dead *P. hermaphrodita* and dead *S. feltiae*; living *S. feltiae* were hardly consumed by any mite species (Fig. 4). Feeding on nematodes was most pronounced in the gamasid mite *P. septentrionalis* and less pronounced in uropodid and oribatid mite species. In a number of cases the mite species did not feed on the nematode species offered.
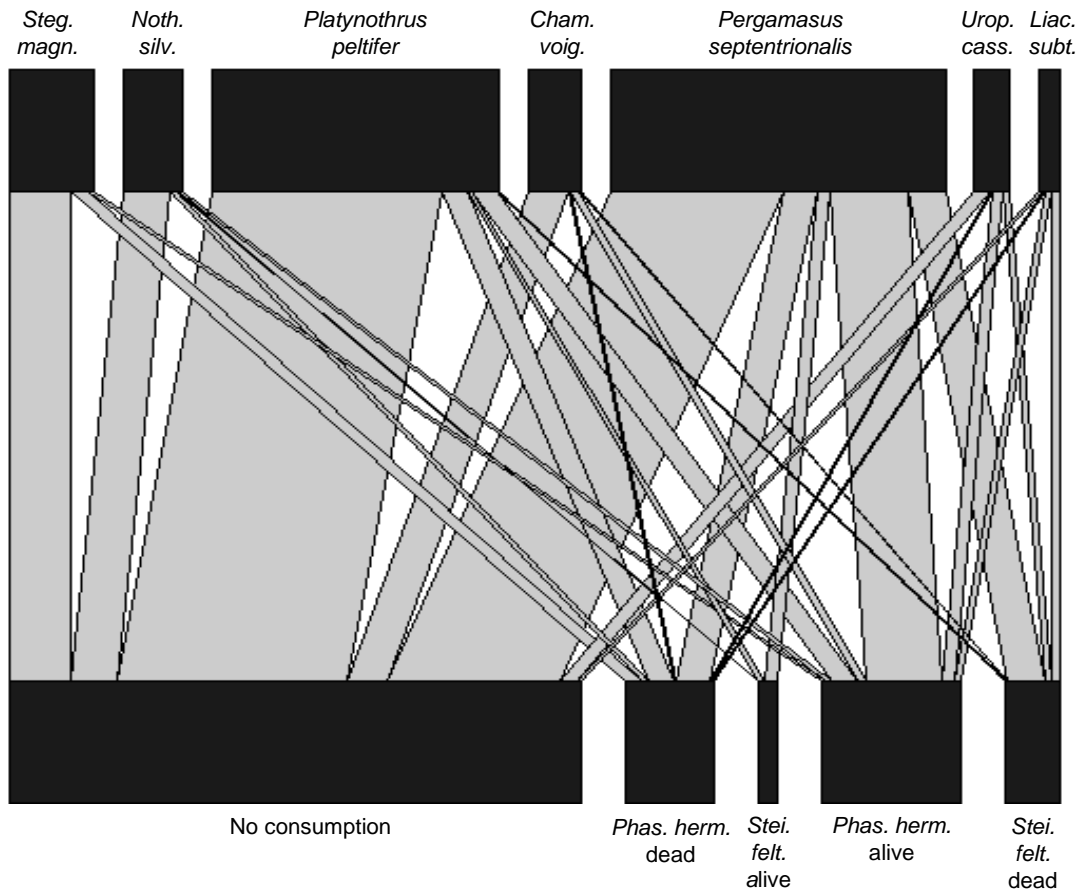
Figure 4: Visualization of predator-prey interactions as observed in the field experiment between seven mite species (Steg. magn. = *Steganacarus magnus*, Noth. silv. = *Nothrus silvestris*, Cham. voig. = *Chamobates voigtsi*, Urop. cass. = *Uropoda cassidea*, Liac. subt. = *Liacarus subterraneus*) and two nematode species (Phas. herm. = *Phasmarhabditis hermaphrodita*, Stei. felt. = *Steinernema feltiae*) using the R-package 'bipartite'. Upper bars represent the relative proportion of the respective taxa of the total number of investigated mite individuals. Lower bars represent the relative proportion of the consumed (or non-consumed) nematodes of all gut content measurements tested by PCR.

4. Discussion

4.1 Nematode predation in the field

Our findings indicate that typical detritivore microarthropods such as certain oribatid mite species indeed consume nematodes and thereby function as predators. Based on direct observations (Riha 1951) and stable isotope ratios (Pollierer *et al.* 2009) this has been hypothesized before but our data for the first time proof that it is indeed true in the field. These findings have major implications for the structure and functioning of detritivore food webs, e.g. they suggest that grouping of oribatid mites as single trophic/functional group is inadequate and that species identity matters for understanding soil food web dynamics. Using molecular markers Read *et al.* (2006) proved another abundant soil mesofauna group, Collembola (*Isotoma viridis*, *Isotomurus palustris*), to also feed on nematodes in the field suggesting that many putative detritivores in fact function as predators or scavengers.

Several of the oribatid mite species studied frequently consumed nematodes including *L. subterraneus*, *P. peltifer* and *S. magnus* even though they could have selected different food in the field. Other species of the genus *Liacarus* have been observed to feed on nematodes (Muraoka & Ishibashi 1976) but stable isotope analysis suggests that they predominantly live as detritivores (Schneider *et al.* 2004). The same applies to *P. peltifer* and *S. magnus*. Presumably, these species are opportunistic feeders that also feed on nematodes. *N. silvestris* and *C. voigtsi* only occasionally fed on nematodes suggesting that these species predominantly feed on fungi and dead organic matter as assumed previously (Pande & Berthet 1973; Muraoka & Ishibashi 1976; Schneider *et al.* 2004).

As expected the predatory gamasid and uropodid mites, *P. septentrionalis* and *U. cassidea*, consumed nematodes whether dead or alive, supporting the hypothesis that they function as predators and scavengers in belowground systems (Peschel *et al.* 2006; Walter & Proctor 1998).

Overall our results suggest that *P. hermaphrodita* was more frequently consumed than *S. feltiae*. This can be due to behavioural differences since infective juveniles of pathogenic nematodes use different dispersal patterns to find their hosts. *P. hermaphrodita* performs cruise foraging, i.e. moves actively in search for hosts (MacMillan *et al.* 2009) whereas host finding of *S. feltiae* follows a sit and wait strategy (Susurluk 2009). These differences are likely to affect encounter rates with predators and the outcome of predator-prey interactions. Secondly, entomopathogenic nematodes such as *S. feltiae* contain specific symbiotic bacteria, necessary to kill the insect host. The symbiont of *S. feltiae* is the gram-negative bacterium *Xenorhabdus bovienii* (Kaya & Gaugler 1993). This bacterium produces metabolites, such as

antibiotics or intercellular protein crystals, and several *Xenorhabdus* strains have been observed to be toxic to Acari, e.g. the mushroom mite *Luciaphorus* sp. (Bussaman *et al.* 2006). Unlike entomopathogenic nematodes the snail-parasitic P. hermaphrodita is not associated with symbiotic bacteria. Frequently, *Moraxella osloensis* is present but it is unknown if this bacterium affects nematode predation (Tan & Grewal 2001). Overall, the presence of mutualistic bacteria producing insect toxins likely has affected the use of *S. feltiae* as food source negatively.

4.2   Scavenging

By feeding on animal carcasses scavengers live on an easy available energy-rich resource that does not defend itself (Foltan *et al.* 2005). From a matter flux perspective scavenging and predation differ little. However, in contrast to predation, scavenging does not contribute to population regulation of the species consumed. Therefore, from a food web perspective differentiating between predation and scavenging is essential. In molecular gut content analyses predation and scavenging are difficult to separate (Juen & Traugott 2005). By using two similar prey species and offering the one dead and the other alive this problem can be resolved. Adopting this approach we separated for the first time between scavenging and predation under field conditions.

A large number of microarthropods including several typical primary detritivorous species had nematode DNA from dead nematodes in their guts indicating that scavenging is important in soil food webs and that dead animals may be an important component of their diet. Scavenging has only rarely been studied using molecular gut content analyses (Foltan *et al.* 2005). High incidence of scavenging in some species of oribatid mites may explain the high stable isotope signatures in these species (Schneider *et al.* 2004). Interestingly, dead *P. hermaphrodita* were intensively consumed whereas dead *S. feltiae* were only consumed rarely. This indicates that mites selectively feed on the two species even if they are dead, and that the defense mechanisms of *S. feltiae* against predation, likely mediated by the symbiotic bacterium *X. bovienii*, continue to function in dead specimens.

Secondary predation can be an important problem in the field when using PCR because of the high sensitivity of the method (Sheppard *et al.* 2005). However, as the two studied nematode species are rather large and the exposure in the field was short secondary predation unlikely contributed to the observed prey DNA in consumers as oribatid mites are unlikely to be able to overdo consumers of the nematode species used.

4.3 Detection time of prey DNA

Prey DNA detection was still possible after 128 h post feeding in the trials where nematodes were offered for 48 h to *S. magnus*. Previous studies also found long detection times for invertebrate prey (Zaidi *et al.* 1999; Agusti *et al.* 2003; Sheppard *et al.* 2005). This indicates that prey can still be detected in the predator with molecular methods after a rather long period of time, much longer than 10 h heat extraction and collection as in our field trial. This implies that the detection rates of prey DNA in mites from our study represent the lower limit of feeding events. Prey DNA detection was rare or impossible after 128 h post feeding in the trials where nematodes were offered for only 4 h to *S. magnus* indicating that it takes a certain period of time to handle and ingest the nematodes.

Overall, the detection time for *P. hermaphrodita* was much longer than that of *S. feltiae* indicating that either *S. feltiae* is digested faster than *P. hermaphrodita* or that *S. feltiae* is consumed less frequently than *P. hermaphrodita*. We assume the later to be more likely since *S. feltiae* DNA was not detectable after exposure to prey for 4 h but was detected in 100% of the individuals of *S. magnus* exposed to prey for 48 h. Generally, prey detection time depends on prey species, predator species, temperature, the length of the amplified fragments and other factors. Additionally, it has to be noted that DNA in dead individuals already starts degenerating before it is ingested affecting DNA detection time.

4.4 Laboratory experiment

Results of the laboratory feeding experiment indicate that some oribatid mite species (*D. riparius*, *S. magnus*, *A. longisetosus*) frequently consume nematodes, whereas others (*A. striculus*, *H. rufulus*) at least occasionally consume nematodes. *D. riparius* is known to consume fungi (Maraun *et al.* 1998); feeding on nematodes had not been observed before. *A. longisetosus* is a tropical oribatid mite that occurs in a variety of habitats and consumes different materials (Smrz & Norton 2004). This opportunistic feeding is confirmed by the results of our study. Feeding on nematodes by *A. striculus* and *S. magnus* was unexpected since Phthiracaridae have never been observed feeding on nematodes before (Pande & Berthet 1973). Additionally, ^{15}N signatures of *S. magnus* indicate that it predominantly feeds on litter (Schneider *et al.* 2004). *H. rufulus* has high stable isotope (^{15}N/^{14}N) ratios (Schneider *et al.* 2004; Pollierer *et al.* 2009) and has been observed feeding on dead Collembola and 'worms' (Riha 1951) supporting the assumption that this species at least in part lives as scavenger. Notably, the gamasid mite *H. aculeifer* consumed less nematodes than some of the oribatid mite species. This may be due to its small body size and the large size of the two nematode

species used in this study. Three oribatid mite species (*A. coleoptrata*, *C. coriaceus*, *E. plicatus*) did not consume nematodes. *A. coleoptrata* and *C. coriaceus* have low ^{15}N signatures indicating that they predominantly feed on decaying plant material (Schneider *et al.* 2004). *E. plicatus* is a soil and bark living species with specialized long and thin mouthparts that can be used for feeding on plant parenchyma and presumably also on fungi but likely not on nematodes as assumed by Erdmann *et al.* (2007). Generally, feeding on the two nematode species did not differ significantly. This suggests that consumption of nematodes by mites is little affected by nematode size. Further, the thicker cuticle of *S. feltiae* as compared to *P. hermaphrodita* did not prevent predation by mites.

4.5   *Suitability of the method*

Molecular gut content analyses of soil animals are difficult and prone to errors (King *et al.* 2008). Possible errors start with a harsh extraction of the animals from the soil, where the individuals can be injured and contamination of other individuals is possible. Therefore, in this study soil microarthropods were extracted by heat and slowly moved out of the soil and fell onto moist filter paper in cooled collection boxes. Further, they were separated quickly to avoid contamination (King *et al.* 2008). Another critical aspect is the storage of the animals before DNA extraction. In our study the mites were killed and stored at -20 °C in 96% ethanol at the laboratory study and at -80 °C in 180 ml ATL-Buffer from the DNeasy® Blood & Tissue Kit (Qiagen) in all other experiments to avoid destabilization of the DNA during thawing. Furthermore, nematodes may be attached to the surface of mites, and animals may regurgitate their gut content when they die which may contaminate the whole sample. Therefore, mites were checked for attached nematodes and afterward placed individually into microcentrifuge tubes. Although tested specific primers for the nematodes were used (Read *et al.* 2006), a PCR bias cannot be excluded. In the experiments the PCR was replicated two times to minimize false negatives. Weak bands from the field experiment were always sequenced. In the laboratory trials every sample with a band was sequenced to avoid false positives.

5. Conclusions

Results of this study suggest that dead and living nematodes are an important component of the diet of soil microarthropods which previously have been assumed to live as litter and fungal feeders. Since many nematode species feed on bacteria and fungi the bacterial and fungal food chain in soil may be more interlinked than previously assumed. Detection of

nematodes in the diet of a number of oribatid mite species suggests that the role of nematodes in the diet of detritivorous microarthropods has been underestimated. In particular, scavenging and predation may be more important for soil microarthropod nutrition than previously assumed and deserves more attention in soil food web studies in future. Further, as soil microarthropod species differentially fed on the two offered nematode species the role of nematode species identity needs to be explored in more detail. A number of other issues deserve further attention, e.g. the effect of season on microarthropod diets, the role of predator-prey body size ratios for prey consumption, and variations in DNA detection times due to predator and prey species identity. Despite these challenges molecular gut content analyses offer the unique opportunity to address one of the most challenging frontiers in ecology, the structure and functioning of belowground food webs.

Acknowledgments

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Supporting information

Table 1: 74 non-target-species were tested with the specific primers for *Steinernema feltiae* and *Phasmarhabditis hermaphrodita*. – no band occurred; + band occurred on the agarosegel.

Taxon	Supraspecific Taxon	*P. hermaphrodita*	*S. feltiae*
Acari			
Holoparasitus stramenti Oudemans, 1936	Gamasida	-	-
Hypoaspis aculeifer (Canestrini, 1883)	Gamasida	-	-
Parasitus lunulatus (J. Müller, 1859)	Gamasida	-	-
Veigaia nemorensis (C.L.Koch, 1836)	Gamasida	-	-
Zercon gurensis Mihelčič, 1962	Gamasida	-	-
Zercon vagabundus Karg, 1971	Gamasida	-	-
Achipteria coleoptrata (Linné, 1758)	Oribatida	-	-
Archegozetes longisetosus Aoki, 1965	Oribatida	-	-
Atropacarus striculus (Koch, 1835)	Oribatida	-	-
Carabodes coriaceus Koch, 1835	Oribatida	-	-
Chamobates voigtsi (Oudemans, 1902)	Oribatida	-	-
Damaeus riparius Nicolet, 1855	Oribatida	-	-
Eupelops plicatus (Koch, 1835)	Oribatida	-	-
Galumna sp.	Oribatida	-	-
Hypochthonius rufulus Koch, 1835	Oribatida	-	-
Liacarus subterraneus (Koch, 1844)	Oribatida	-	-
Nothrus palustris (C. L. Koch 1839)	Oribatida	-	-
Nothrus silvestris Nicolet, 1855	Oribatida	-	-
Oribatella calcarata (C. L. Koch, 1836)	Oribatida	-	-
Oribatella quadricornuta (Michael, 1880)	Oribatida	-	-
Platynothrus peltifer (Koch, 1839)	Oribatida	-	-
Steganacarus magnus (Nicolet, 1855)	Oribatida	-	-
Trachytes aegrota (C. L. Koch, 1841)	Uropodida	-	-
Uropoda athiasae (Hirschmann & Zirngiebl-Nicol, 1969)	Uropodida	-	-
Uropoda cassidea (Hermann, 1804)	Uropodida	-	-
Uroseius cylindricus (Berlese, 1916)	Uropodida	-	+
Araneae			
Coelotes inermis (L. Koch, 1855)	Amaurobiidae	-	-
Clubiona terrestris Westring, 1851	Clubionidae	-	-
Tropiphorus sp.	Curculionidae	-	-
Micrargus herbigradus (Blackwall, 1854)	Linyphiidae	-	-
Tapinocyba insecta (L. Koch, 1869)	Linyphiidae	-	-
Walckenaeria cucullata (C. L. Koch, 1836)	Linyphiidae	-	-
Walckenaeria obtusa Blackwall, 1836	Linyphiidae	-	-

Molecular detection of nematode predation and scavenging in oribatid mites:
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Chilopoda

Strigamia acuminata (Leach, 1815)	Geophilomorpha	-	-
Lithobius aulacopus Latzel, 1880	Lithobiidae	-	+
Lithobius lapidicola Meinert, 1872	Lithobiidae	-	+
Lithobius mutabilis L. Koch, 1862	Lithobiidae	-	-
Lithobius muticus (C.L. Koch, 1847)	Lithobiidae	-	+
Lithoibius nodupilipes Latzel, 1881	Lithobiidae	-	-
Lithobius crassipes L. Koch, 1862	Lithobiidae	-	+

Collembola

Heteromurus nitidus (Templeton, 1835)	Entomobryidae	-	-
Orchesella flavescens (Bourlet, 1839)	Entomobryidae	-	-
Ceratophysella armata (Nicolet, 1841)	Hypogastruridae	-	-
Hypogastrura burkilli (Bagnall, 1940)	Hypogastruridae	-	-
Neanura muscorum (Templeton, 1835)	Neanuridae	-	+
Protaphorura armata (Tullberg, 1869)	Onychiuridae	-	-
Supraphorura furcifera (Börner, 1901)	Onychiuridae	-	-
Pogonognathellus (*Tomocerus*) cf. *longicornis* (Müller, 1776)	Tomoceridae	-	-

Coleoptera

Malthodes sp.	Cantharidae	-	-
Abax ovalis (Duftschmid, 1812)	Carabidae	-	-
Abax parallelipipedus (Piller & Mitterbacher, 1783)	Carabidae	-	-
Notiophilus rufipes Curtis, 1829	Carabidae	-	-
Pterostichus burmeisteri Heer, 1841	Carabidae	-	-
Pterostichus oblongopunctatus (Fabricius, 1787)	Carabidae	-	-
Trechus nigrinus Putzeys, 1847	Carabidae	-	-
Athous haemorrhoidalis (Fabricius, 1801)	Elateridae	-	-
Athous subfuscus (Müller, 1764)	Elateridae	-	-
Lamprohiza splendidula (Linnaeus, 1767)	Lampyridae	-	-
Nargus anisotomoides (Spence, 1815)	Leiodidae	-	-
Scydmaenidae	Scydmaenidae	-	-
Domene scabricollis (Erichson, 1840)	Staphylinidae	-	-
Habrocerus capillaricornis (Gravenhorst, 1806)	Staphylinidae	-	-
Philonthus carbonarius (Gravenhorst, 1802)	Staphylinidae	-	+
Philonthus laevicollis (Boisd. & Lacord. 1935)	Staphylinidae	-	-
Stilicus rufipes (Germar, 1836)	Staphylinidae	-	-
Xantholinus laevihatus Jacobsen, 1849	Staphylinidae	-	-

Dermaptera

Chelidurella sp.	Dermaptera	-	-

Diplopoda

Haploporatia eremita (Verhoeff, 1909)	Diplopoda	-	-
Polydesmus complanatus (Linnaeus, 1761)	Diplopoda	-	+
Glomeris sp.	Glomeridae	-	+

Diplura			
Campodea sp.	Campodeidae	-	-
Isopoda			
Ligidium cf. *hypnorum* (Cuvier, 1792)	Isopoda	-	-
Porcellio sp.	Isopoda	-	+
Trichoniscus pusillus Brandt, 1833	Isopoda	-	+
Nematoda			
Acrobeloides buetschlii (de Man, 1884)	Cephalobidae	-	-
Turbatrix aceti (Müller, 1783)	Panagrolaimidae	-	-
Oligochaeta			
Aporrectodea caliginosa (Savigny, 1826)	Lumbricidae	-	+
Lumbricus terrestris Linnaeus, 1758	Lumbricidae	-	-
Opiliones			
Anelasmocephalus cambridgei (Westwood, 1874)	Trogulidae	-	-
Trogulus nepaeformis (Scopoli, 1763)	Trogulidae	-	-
Pseudoscorpione			
Neobisium carcinoides (Hermann, 1804)	Pseudoscorpiones	-	-

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The role of free-living nematodes as prey for higher trophic levels of forest soil food webs

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Abstract

Nematodes form the most abundant invertebrates in soils and are key prey in soil food webs. Uncovering their contribution to predator nutrition is essential to understand soil food webs and the way energy channels through soil systems. Molecular gut content analysis of soil microarthropods as consumers of nematodes using specific markers is a novel approach for studying predator-prey interactions in soil. We designed new primer pairs (partial 18S rDNA) for soil-living bacterial feeding nematode taxa (*Acrobeloides buetschlii*, *Panagrellus redivivus, Plectus velox* and *Plectus minimus*). Primer specificity against more than 100 non-target soil organisms was tested. Further, we determined how long nematode DNA can be traced in the gut of predators. Potential predators were identified in laboratory experiments including nine soil mite (Oribatida, Gamasina and Uropodina) and ten springtail (Collembola) species. Finally, the approach was tested under field conditions by analyzing five mite and three collembola species for feeding on the three target nematode species. The results proved the three primer sets to specifically amplify DNA of the respective nematode taxa. Detection time of nematode DNA in predators varied with time of prey exposure. Further, consumption of nematodes in the laboratory varied with microarthropod species. The analysis of field sampled microarthropods for the first time proved free-living nematodes to form important prey for a wide range of species including those commonly regarded as decomposers. Overall, the results highlight the eminent role of nematodes for predator populations in soil food webs and for channeling bacterial carbon to higher trophic levels.

1. Introduction

The soil food web forms a major component and provides essential functions of terrestrial ecosystems (Scheu & Setälä 2002; Bardgett & Wardle 2010). As with all food webs it is based on predator-prey interactions channelling the flux of matter and energy to higher trophic levels (Pimm 1991; Terborgh & Estes 2010). Soil food webs are often regarded as 'black box' since they are species-rich, the organisms typically are small and difficult to observe, and thus trophic relationships are little understood (Wardle & Yeates 1993; Symondson 2002; Scheu *et al.* 2005). The major energy channels of soil food webs are the bacterial, the fungal and the plant litter channel (Moore & Hunt 1988; Scheu *et al.* 2005). The transfer of carbon to higher trophic levels is assumed to be fastest via the bacterial energy channel (van Hees *et al.* 2005; Strickland *et al.* 2012), and recent studies using fatty acid analyses suggest that a wide range of predators rely on carbon out of this channel (Pollierer *et al.* 2012). However, carbon transfer from low to high trophic levels still is little understood, but one of the most important links presumably are bacterial-feeding nematodes (Freckman 1988; Crotty *et al.* 2011; Ferlian *et al.* 2012).

Free-living soil nematodes feed on a wide range of resources, e.g. dead organic material, bacteria, fungi, algae, plants or live as predators or omnivores (Yeates *et al.* 1993). They reach densities of several million individuals per square metre and nematode communities of forests often comprise more than 100 species (Yeates *et al.* 2000), thereby functioning as key players in soil food webs (Moore *et al.* 2003; Ferris 2010; Yeates 2010). Nematodes serve as prey for many soil animals including mites and collembolans (Rockett & Woodring 1966; Muraoka & Ishibashi 1976; Walter 1988a,b; Read *et al.* 2006; Heidemann *et al.* 2011). Bacterial feeding nematodes typically account for >50% of total free-living nematodes, but in hotspots of microbial activity this may rise to >90%; together with protists they form the most important bacterial grazers in soil (Griffiths 1990; Zunke & Perry 1997; Scheu *et al.* 2005; Mulder & Vonk 2011). Investigating nematodes as prey for soil animals is difficult since only solid structures such as mouthparts and grinders are left as remnants in the gut of predators (Walter 1988a; Sunderland 1988), thereby nematodes form 'invisible' prey.

Microarthropods, such as mites and collembolans, are abundant and diverse in below-ground systems (Petersen & Luxton 1982; Maraun *et al.* 1998; Rusek 1998; Schatz 2002). Most soil microarthropod species have been assumed to function as decomposers, i.e. to feed on dead organic material, bacteria and fungi but gamasid and uropodid mites are known to live as predators. However, recent studies using fatty acid and stable isotope analysis indicate that many putative decomposer species also live as predators or scavengers (Ruess *et al.* 2004; Schneider *et al.* 2004; Chahartaghi *et al.* 2005). Therefore, animal diets presumably form an

essentially component of the trophic niche of many 'decomposer' soil invertebrates thereby contributing to their high local diversity (Anderson 1975; Scheu & Setälä 2002; Maraun *et al.* 2003a).

Molecular gut content analysis is a new and promising tool for studying neglected trophic interactions (Symondson 2002; King *et al.* 2008), such as feeding of microarthropods on nematodes. It allows to sensitively detect even small amounts of DNA in the gut of consumers over long periods of time (Sheppard *et al.* 2005; King *et al.* 2008; Eitzinger & Traugott 2011). Specific primers i.e., primers that only bind to certain genera or species, allow screening of predator communities for certain prey taxa or species. For tracing bacterial feeding nematodes in soil predators we designed novel primers and tested their specificity. Until today, only three primer pairs specific for entomopathogenic nematodes species have been used to detect nematode predators in soil food webs (Read *et al.* 2006; Heidemann *et al.* 2011).

We aimed at developing tools for detecting field-living bacterial feeding nematode species in predators of soil food webs of temperate deciduous forest ecosystems by molecular gut content analysis. For achieving this goal we (1) designed specific and sensitive primer pairs for four field-living abundant bacterial feeding nematode taxa, (2) tested them for specificity, (3) investigated the detection time of prey DNA in the gut of predators, (4) identified microarthropod predators of these nematode taxa in laboratory, and (5) in the field.

2. Materials and Methods

2.1 The organisms

Four free-living bacterial feeding soil nematode species, *Acrobeloides buetschlii* (de Man*,* 1884), *Panagrellus redivivus* Goodey, 1945, *Plectus minimus* Cobb, 1893 and *Plectus velox* Bastian, 1865, were investigated as microarthropod prey. All four species have a cosmopolitan distribution and cover the size range of nematodes typically found in soil. *A. buetschlii* lives in forest soils and reaches a length of ~ 0.3-0.5 mm. *P. redivivus* has a length of 1-2 mm and lives ovoviviparously in decaying and fermenting materials. *P. velox* is larger (~ 1-1.2 mm) than *P. minimus* (~ 0.35 mm); both species live in forest soils but also occur in mosses and lichens. *P. velox* is described as being synonymous to *P. parietinus* (http://nematode.unl.edu), but we followed Holovachov (2006) keeping the validity of *P. velox*. Large numbers of nematodes were needed for design of the molecular markers, the optimization of the method and for the laboratory experiments. This is only possible with laboratory mass cultures (Goodey 1963). As these are difficult to obtain for free living nematodes, existing axenic laboratory cultures from widespread soil nematode species were used. However, this selection did not reflect their

relative abundance in the soil of the investigated field site. *P. minimus* and *P. velox* were supplied by René Seiml-Buchinger (HU Berlin, Germany), *P. redivivus* by Interaquaristik.de Shop (www.interaquaristik.de, Biedenkopf-Breidenstein, Germany) and *A. buetschlii* was taken from cultures of one of the authors (L. Ruess).

A total of 24 microarthropod species were investigated as potential predators of nematodes. In the laboratory we studied the gamasid mite *Pergamasus septentrionalis* (Oudemans, 1902), the uropodid mites *Trachytes aegrota* (C.L. Koch, 1841), *Uropoda cassidea* (Hermann, 1804) and its deutonymph, the oribatid mites *Achipteria coleoptrata* (Linné, 1758), *Carabodes* sp., *Galumna* sp., *Platynothrus peltifer* (Koch, 1839) and *Steganacarus magnus* (Nicolet, 1855) as potential predators of the four nematode species and the collembolans *Folsomia candida* Willem, 1902, *Heteromurus nitidus* Templeton, 1835, *Hypogastrura purpurescens* Lubbock, 1867, *Lepidocyrtus cyaneus* Tullberg, 1871, *Proisotoma minuta* Tullberg, 1871, *Protaphorura armata* Tullberg, 1869, *Sinella coeca* Schott, 1896, *Sinella curviseta* Brook, 1882 and *Tomocerus minor* Lubbock, 1862 as potential predators of *P. velox* and *P. minimus*. All species are inhabitants of European forest soils except *F. candida*, *S. coeca* and *S. curviseta* which typically live in disturbed habitats. In the field experiment we investigated predation on nematodes in *U. cassidea* and the oribatid mites *Chamobates subglobulus* (Oudemans, 1900), *Nothrus palustris* (C. L. Koch, 1839), *N. silvestris* Nicolet, 1855 and *S. magnus*, and the collembolans *Entomobrya muscorum* Nicolet, 1842, *Orchesella villosa* (Geoffroy, 1764) and *T. vulgaris* Tullberg, 1871. Each of them is abundant in soil food webs of deciduous forests.

2.2 Study site

Microarthropods for the laboratory experiments were sampled from the Hainich forest (near Birkungen, Thüringen, Germany), the Göttinger forest (Göttingen, Lower Saxony, Germany), and from an agricultural experimental field of the Georg August University Göttingen near the Klostergut Reinshof (Göttingen, Lower Saxony, Germany). The samples for the field experiment were taken in October 2011 from the Hainich forest.

The Hainich is the largest coherent deciduous forest of Germany and is dominated by *Fagus sylvatica* interspersed with ash (*Fraxinus excelsior*), lime (*Tilia europaea*), maple (*Acer platanoides* and *A. pseudoplatanus*) and wild service trees (*Sorbus torminalis*). Parent rock is Triassic limestone; the main soil types are Luvisols, Cambisols and Stagnosols with an average pH of 4.6. The Göttinger forest ('Göttinger Wald') is a 140 year old beech forest interspersed with maple and ash. Parent rock is limestone; Leptosols with mull humus predominate with an average pH of 5.3 (Schaefer 1990). The wheat field is located in the

Leinegraben and its dominant soil types are Cambisols, Luvisols and stagnic Luvisols. Annual precipitation at the study sites varies between 500 and 800 mm, and mean annual temperature between 6.5 and 8°C (Erdmann *et al.* 2012; Kramer *et al.* 2012).

2.3 Alignment, primer design and primer sensitivity

We assembled a dataset with 163 sequences from National Center for Biotechnology Information (NCBI; www.ncbi.nlm.nih.gov) of the 18S rDNA of bacterial feeding nematodes using a species list of the study region (Alphei 1995). The datasets included different species of the nematode genera *Acrobeloides*, *Panagrellus* and *Plectus* including the species *A. buetschlii*, *P. redivivus* and *P. minimus*. Our own sequences of the 18S rDNA gene of the species *A. buetschlii*, *P. minimus* and *P. velox* from our cultures were added. Sequence alignment and editing was carried out using BioEdit v7.0.5. The secondary alignment was carried out using the ClustalW multiple alignment function of the program (Hall 1999).

Sequence data were used to design specific primer pairs amplifying short parts of the 18S rDNA for the three nematode targets *A. buetschlii*, *P. redivivus* and *Plectus* spp. (*P. minimus* and *P. velox*; Table S1, Supporting Information) by eye following the guidelines of King *et al.* (2008). The annealing temperature was optimized by a temperature gradient PCR of the designed primers with the respective single nematode DNA. We optimized the PCR reaction, the used template DNA concentration and the PCR program for each primer set to maximize nematode detection and to avoid false negative results. Microarthropods were fed for four days with the respective nematodes and tested with the designed primers to confirm that amplification of the target species after consumption by a predator is possible.

Primer sensitivity was determined for all three primer pairs by a two-fold serial dilution of target DNA to test for amplification success using DNA of the nematodes *A. buetschlii*, *P. redivivus* and *P. velox* and the oribatid mite *S. magnus*. DNA concentration in the original extracts was measured using a Qubit® 2.0 Fluorometer (Life Technologies™, Carlsbad, California, USA), adjusted to 100 pg/µL for *A. buetschlii*, 500 pg/µL for *P. redivivus* and 50 pg/µL for *P. velox*, and twofold serially diluted by adding RNase free water. We used the serial diluted target DNA as template in the PCR assays at concentrations of 250 for *A. buetschlii* and *P. redivivus*, and 125, 62.5, 31.25, 15.62, 7.82, 3.91, 1.96, 0.98, 0.49, 0.25, 0.13, 0.05 and 0.025 pg of target DNA per µL PCR for each of the three nematode species. A further assay was performed by adding 250 pg/µL DNA of *S. magnus* to *P. redivivus* and 200 pg/µL DNA of *S. magnus* to *A. buetschlii* and *P. velox* in each PCR to check for possible inhibition of nematode DNA detection in the presence of predator DNA.

2.4 Primer specificity

We tested the specific primer pairs for *A. buetschlii*, *P. redivivus* and *Plectus* spp. (*P. minimus* and *P. velox*) in a cross-reaction test with 108 non-target species, including the respective nematode species as positive control, the twelve mite and the twelve collembola species used in this study (Table S2, Supporting Information). Therefore, the animals were starved for seven days and stored separately at -80 °C in 180 mL buffer ATL of the extraction kit DNeasy® Blood & Tissue Kit (Qiagen).

2.5 Detection time of prey DNA

Detection time of prey DNA in consumers was investigated using the oribatid mite *S. magnus* with *P. redivivus*, *P. minimus* and *P. velox* as nematode prey and the gamasid mite *P. septentrionalis* with *A. buetschlii* as nematode prey. All mites were extracted from soil and litter of the Göttinger forest and Hainich forest by heat (Kempson *et al.* 1963). We starved predator individuals for seven days. Thereafter, the four nematode species were offered separately to 20 mite individuals for 4 h. During the experiment mites were kept in darkness at 13°C. Since a longer feeding interval can increase the detection time of nematode prey (Heidemann *et al.* 2011) *S. magnus* was also fed with *P. minimus* and *P. velox* for 48 h. Thereafter, mites were checked for attached nematodes and starved for 0, 2, 4, 8, 16, 32, 64 and 128 h. Then, living mites were transferred into 180 mL buffer ATL of the extraction kit DNeasy® Blood & Tissue Kit (Qiagen) and stored at -80 °C.

2.6 Laboratory feeding experiments

In the laboratory eight mite species (*P. septentrionalis*, *T. aegrota*, *U. cassidea*, *A. coleoptrata*, *Carabodes* sp., *Galumna* sp., *P. peltifer* and *S. magnus*) and the deutonymph of *U. cassidea* were tested for feeding on the nematodes *A. buetschlii*, *P. redivivus*, *P. minimus* and *P. velox* in no-choice experiments. Additionally, we tested *P. minimus* and *P. velox* as prey for ten collembola species (*L. cyaneus*, *T. vulgaris, F. candida, H. nitidus, H. purpurescens, P. armata, P. minuta, S. coeca, S. curviseta* and *T. minor*). We starved ten individuals of each microarthropod species for seven days and offered the nematode prey species separately to the microarthropods for four days. Starving and feeding occurred in darkness at 13°C. Thereafter, we checked the mites and collembolans under a stereomicroscope for attached nematodes to avoid false positives; no nematodes were attached. Two unfed individuals of each species were used as control and tested with the specific primers to exclude

contamination during handling of the samples. Mite and collembola individuals were stored separately at -80°C in 180 mL buffer ATL of the extraction kit DNeasy® Blood & Tissue Kit (Qiagen).

2.7 Field experiment

In the field experiment we tested five mite and three collembola species for feeding on *A. buetschlii*, *P. redivivus* and *Plectus* spp. In October 2011 we collected animals in mosses (1 m²) of three sites (plot 1-3) in the Göttinger forest where *Plectus* spp. and *A. buetschlii* are abundant (Alphei 1995). We analysed the density and species composition of soil animals at each plot by taking two soil cores for microarthropods and nematodes. Microarthropod cores (5 cm Ø) were split into organic and soil layer (4 cm thickness), and the animals extracted by heat (Macfadyen 1961); for determination of densities both layers were pooled. Nematode cores (2.5 cm Ø) were extracted by modified Baermann method (see Ruess 1995), counted and the densities of *A. buetschlii*, *P. redivivus* and *Plectus* spp. were determined.

Microarthropods studied in the field experiment were extracted by heat (Kempson *et al.* 1963) with the extraction lasting for three hours, identified to species and checked for attached nematodes; no nematodes were detected. Overall, 63 individuals of eight species were analysed including *C. subglobulus* (n=4), *E. muscorum* (n=3), *N. silvestris* (n=10), *N. palustris* (n=5), *O. villosa* (n=17) *S. magnus* (n=3), *T. vulgaris* (n=19) and *U. cassidea* (n=2). The animals were placed individually in 180 μl buffer ATL of the DNeasy® Blood & Tissue Kit (Qiagen) and stored at -80°C. Animals were frozen within 7 h after sampling in the field.

2.8 DNA extraction, PCR and sequencing

DNA from whole individuals of soil microarthropods and nematodes was extracted following the protocol of the DNeasy® Blood & Tissue Kit (Qiagen). To verify the success of the DNA extractions and to exclude inhibition of the DNA amplification we performed a PCR with D3 primers amplifying a 320 bp fragment of the 28S rDNA for every sample (Maraun *et al.* 2003b). PCRs with D3 primers and thermal cycle parameters were carried out as described in Heidemann *et al.* (2011). The PCR success of the macrofauna samples used to test for primer specificity was confirmed by using the primers of Folmer *et al.* (1994) amplifying a 710 bp fragment of the cytochrome oxidase I (COI). To avoid false negative amplification results only samples with a PCR product were included in the study.

Sequences of *A. buetschlii*, *P. minimus* and *P. velox* from our cultures were obtained using general 18S rDNA primers and thermal cycle parameters of Domes *et al.* (2007). The primers amplify a ~1750 bp fragment of 18S rDNA. The sequences were added to the dataset for the design of the specific primers. Every PCR contained 1 µL (concentration 100 pmol/mL) of each primer (Eurofins MWG Operon, Ebersberg, Germany), 1 µL of BSA (3%), 1 µL of 25 mM $MgCl_2$ (Genaxxon BioScience GmbH, Ulm, Germany), 12.5 µL 2x SuperHot Mastermix (Genaxxon BioScience GmbH, Ulm, Germany) including polymerase, 5 µL template DNA and were filled up to 25 µL with RNase free water.

Nematode DNA was targeted using specific primers for *A. buetschlii* amplifying a 287 bp fragment [Acro-F-197 (forward primer) 5'- CGG CTT CGG CTG TTT CTG GTT -3'; Acro-R-484 (reverse primer) 5'- GAT GAC CGG CCT CAT AAG AGA ACG TC -3'], *P. redivivus* amplifying a 217 bp fragment [Pana-F-278 (forward primer) 5'- CCA ACG GCA GTG TAT TGT CCT GAC G -3'; Pana-R-494 (reverse primer) 5'- TAG GAA GGT TGT AAA TTC -3'] and *Plectus* spp. amplifying a 156 bp fragment [(*P. minimus* and *P. velox*); Plec-F-644 (forward primer) 5'- CTG RGA TCC AAG GCT TAT ACT GC -3'; Plec-R-799 (reverse primer) 5'- TAG ARC CGT GGT CTT ATT CT -3']. Each of the three primer pairs amplifies parts of the 18S rDNA. Every PCR contained 2 µL (concentration 100 pmol/mL) of each primer (Eurofins MWG Operon, Ebersberg, Germany), 1 µL of BSA (3%) for *A. buetschlii* (2 µL for *P. redivivus* and for *Plectus* spp.), 1 µL of 25 mM $MgCl_2$ for *A. buetschlii* (2 µL for *P. redivivus* and for *Plectus* spp.; Genaxxon BioScience GmbH, Ulm, Germany), 12.5 µL 2x SuperHot Mastermix (Genaxxon BioScience GmbH, Ulm, Germany) including polymerase, 2.5 µL template DNA for *A. buetschlii* (0.5 µL for *P. redivivus* and 2.5 µL for *Plectus* spp.) and were filled up to 25 µL with RNase free water. Thermal cycle parameters included an initial denaturation step of 10 min at 95°C followed by 35 cycles of 30 s at 95°C, 60 s at 62°C for *A. buetschlii* (45 s at 63°C for *P. redivivus* and 45 s at 62°C for *Plectus* spp.) and 30 s at 72°C, and a final extension stage of 10 min at 72°C. PCR products were visualised using the capillary electrophoresis system QIAxcel (Qiagen). Fifty percent of all positive samples of the laboratory trials and all positive samples of the field trial were sequenced by Macrogen Inc. (Seoul, South Korea) or by the Göttingen Genomics Laboratory at the Institute of Microbiology and Genetics of the Georg August University Göttingen and compared to NCBI (www.ncbi.nlm.nih.gov) to confirm the species identity of amplified fragments.

2.9 Statistical analysis

We calculated means and standard derivations of nematode detection rates by using the package 'simpleboot' in R (Simple Bootstrap Routines. R-package version 1.1-3; Roger D.

Peng 2008; R Development Core Team 2009). Additionally, 95% confidence limits were calculated by 9999 bootstrap resamples for each predator feeding on a certain prey.

We used logistic regression (Hosmer & Lemeshow 1989; Sokal & Rohlf 1995) for the laboratory and field experiment to analyze if (i) the microarthropod species differently consumed the nematode prey offered, (ii) the offered nematode species were differently consumed, and (iii) the detection of each nematode species varied between different microarthropod species. The analyses were carried out for each treatment separately using SAS 9.3 (SAS Institute Inc., Cary, USA).

3. Results

3.1 Primer design and sensitivity

Fragments of the 18S rDNA between 1556 and 1760 bp were successfully amplified from 10 individuals per sample of each *A. buetschlii*, *P. minimus* and *P. velox,* sequenced (GenBank accession numbers KC206039, KC206040 and KC206041, respectively) and included in our dataset to the 163 sequences from NCBI of the 18S rDNA of bacterial feeding nematodes. Three primer pairs were designed amplifying taxon-specific DNA fragments (inclusive the primer region) of 287 bp from *A. buetschlii*, 217 bp from *P. redivivus* and 156 bp from *Plectus* spp. (*P. minimus* and *P. velox*; Figures S1 and S3 and Table S1, Supporting Information) of the 18S rDNA in the guts of microarthropods after feeding on the respective nematode species. If the primer sets for *A. buetschlii* and *P. redivivus* amplify their target on species or genus level remains unclear since no other species of these two genera were available for non-target tests. However, *P. minimus* and *P. velox* were amplified by one primer pair, therefore we defined these primers as specific to *Plectus* spp. (*P. minimus* and *P. velox*). Often, the primer set for *P. redivivus* amplified a second fragment of ~160 bp which was missing when DNA of *P. redivivus* was absent. However, as the target fragment could be easily recognised this did not pose any problem for the analysis.

The primer set for *A. buetschlii* had a minimum detection limit of 0.05 pg/µL of target DNA; with 200 pg/µL of DNA of *S. magnus* present the minimum amount detected was 0.025 pg/µL (Figure S2, Supporting Information). For *P. redivivus* the minimum amount nematode DNA detected was 0.05 pg/µL which holds in presence of 250 pg/µL DNA of *S. magnus.* For *P. velox* the minimum amount of nematode DNA detected was 0.025 pg/µL and this was also true in presence of 200 pg/µl DNA of *S. magnus.*

3.2 Primer specificity

A total of 108 non-target taxa including the nematode species *A. buetschlii*, *P. redivivus*, *P. minimus* and *P. velox*, were tested with the respective primer sets to check for cross-reactions (Table S2, Supporting Information). The primer set for *A. buetschlii* showed one cross-reaction to *Octolasion cyaneum* (Savigny, 1826).

Cross-reactions of the primer set for *P. redivivus* occurred for 16 non-target species: *Clubiona terrestris* Westring, 1851, *Microneta viaria* (Blackwall, 1841), *Strigamia acuminata* (Leach, 1815), *Lithoibius nodupilipes* Latzel, 1881, *Abax parallelepipedus* (Piller & Mitterbacher, 1783), *Hypogastrura burkilli* (Bagnall, 1940), *Haploporatia eremita* (Verhoeff, 1909), *Musca domestica* Linnaeus, 1758, *Ligidium hypnorum* (Cuvier, 1792), *Porcellio* sp., *Trichoniscus pusillus* Brandt, 1833, *Galleria* sp., *Steinernema feltiae* (Filipjev, 1934), *Aporrectodea caliginosa* (Savigny, 1826), *Lumbricus terrestris* Linnaeus, 1758 and *Octolasion cyaneum* (Savigny, 1826).

The non-target test with the primers for *Plectus* spp. showed cross-reactions for ten taxa: *Dendrobaena octaedra* (Savigny, 1826), *Eupelops* sp., *Galleria* sp., *Hypogastrura burkilli* (Bagnall, 1940), *Liacarus xylariae* (Schrank, 1803), *Lithobius lapidicola* Meinert, 1872, *Lophopilio palpinalis* (Herbst, 1799), *Lumbricus terrestris* Linnaeus, 1758, *Pratylenchus zeae* Graham, 1951 and *Trichorhina tomentosa* (Budde-Lund, 1893). However, not all individuals of *Eupelops* sp. showed cross-reactions indicating that cross-reactivity differs between species of that genus.

All three primer sets did not show cross-reactions with the mite and collembola species used in our study. We sequenced about 50% of the positive prey bands of the laboratory experiments and all of the field trial and never found any of the above or other species in the gut of the microarthropod species. We always only detected *Plectus* spp. and *A. buetschlii* or *Acrobeloides* sp. with the respective primer pair. We therefore assume that the positive cross-reactions are not relevant for our study.

In some cases the primer set for *A. buetschlii* amplified a shorter fragment of ~90 bp which was clearly distinguishable from the target and occurred as single or second band. NCBI blast search identified this fragment as short fragment of the 18S rDNA of *A. buetschlii*; amplifications of this fragment were ignored.

3.3 Detection time of prey DNA

We determined the detection time of nematode prey in the gamasid mite *P. septentrionalis* after feeding on *A. buetschlii* and in the oribatid mite *S. magnus* after feeding on *P. redivivus*, *P. minimus* and *P. velox* for 4 h. Immediately post feeding on *A. buetschlii* prey DNA was detected in 10% of the individuals of *P. septentrionalis* and this increased to 30% and 50% 2 and 4 h later but then dropped to 0-20% 8 to 128 h post feeding (Figure 1a). In contrast, only 10% of *S. magnus* were tested positive for *P. redivivus* directly post feeding, which further decreased to 0-5% with time (Figure 1b). For *P. minimus* 80% of the individuals of *S. magnus* were tested positive immediately post feeding which dropped continuously to 0% after 32 and 64 h but increased to 30% at 128 h (Figure 1c). *P. velox* was detected in only 0-10% of *S. magnus* throughout the 128 h of the experiment (Figure 1d).

When feeding on nematodes for 48 h 35% of the individuals of *S. magnus* were tested positive for DNA of *P. minimus* immediately and 2 h post feeding; thereafter, prey detection varied between 5 and 30% and apparently increased until 64 h post feeding (Figure 1e). When feeding on *P. velox* for 48 h detection of prey DNA in *S. magnus* was high and varied between 55 and 100% immediately and 2 h post feeding, respectively; detection remained high until 128 h with 35 to 60% (Figure 1f).
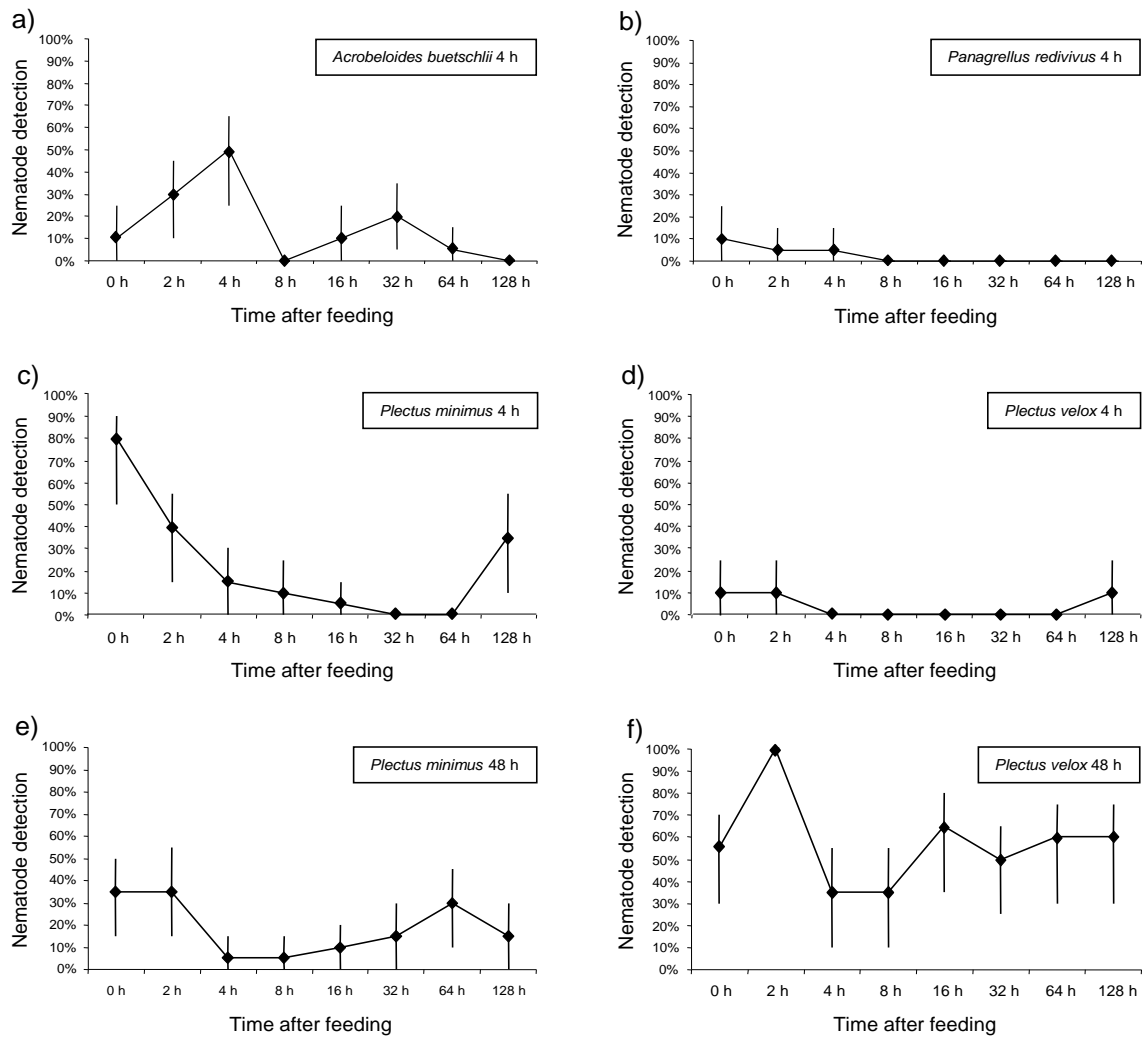
Figure 1: Prey DNA detection at different time points post feeding of *Pergamasus septentrionalis* for 4 h on (a) *Acrobeloides buetschlii*, and of *S. magnus* on (b) *Panagrellus redivivus*, (c) *Plectus minimus* and (d) *Plectus velox* and post feeding of *Steganacarus magnus* for 48 h on (e) *P. velox* and (f) *P. minimus*. Data represent percentages of 20 mite individuals tested positive per time point. Error bars represent confidence limits; for statistical analysis see text.

3.4 Laboratory feeding experiments

Detection of DNA of the four nematode species *A. buetschlii*, *P. redivivus*, *P. minimus* and *P. velox* in the eight species of mites and the deutonymph of *U. cassidea* studied varied between mite species (χ^2 = 38.97, df = 8, P< 0.0001) and also between nematode species (χ^2 = 15.04, df = 3, P = 0.0018). Moreover, feeding on individual nematode species also differed between mite species (significant mite species × nematode species interaction; χ^2 = 57.72, df = 24, P = 0.0001; Figure 2a). On average, detection of nematode DNA in mites declined from *P. minimus* (42%) to *P. velox* (29%) to *A. buetschlii* (27%) to *P. redivivus* (18%).

In uropodid mites 50% of the individuals of *T. aegrota* were tested positive for *A. buetschlii*, 20% for *P. redivivus*, 40% for *P. minimus* and 90% for *P. velox*. In *U. cassidea* DNA of *A. buetschlii* and *P. minimus* (both 60%) were more often detected than DNA of *P. redivivus* (40%) and *P. velox* (30%). Almost all individuals of deutonymphs of *U. cassidea* were tested positive for *P. minimus* (90%), less for *P. velox* (40%) and only 10% for *A. buetschlii* and *P. redivivus*. In the gamasid mite *P. septentrionalis* detection of prey DNA was high for *P. velox* (80%) and *A. buetschlii* (50%) but low for *P. redivivus* (20%) and *P. minimus* (0%). In oribatid mites 70% of *S. magnus* were tested positive for DNA of *P. minimus*, 40% for *P. redivivus,* 20% for *A. buetschlii* and only 10% for *P. velox*. Detection of *A. buetschlii* and *P. redivivus* was similar in *Galumna* spp., *P. peltifer* and *Carabodes* spp. (10% each). As in *S. magnus* more individuals were tested positive for *P. minimus* (40%) than for *P. velox* (10%) in *Galumna* spp. Only few individuals of *P. peltifer, Carabodes* spp. and *A. coleoptrata* were tested positive for nematode DNA, in each of the species most frequently for *P. minimus* (30%, 20% and 30%, respectively).

Similar to mites detection of nematode DNA in the eight collembola species studied also significantly varied with collembola species (χ^2 = 48.17, df = 9, P < 0.0001; Figure 2b). However, in contrast to mites nematode DNA detection did not differ significantly between the two nematode species investigated (*P. minimus* and *P. velox;* χ^2 = 2.63, df = 1, P = 0.105) and prey detection in collembola species did not vary with nematode species (χ^2 = 7.80, df = 9, P = 0.554). On average, 29% of the studied collembola were tested positive for nematode DNA. Detection frequency declined from *S. coeca* and *H. nitidus* (both on average 65%) to *T. vulgaris* (45%) to *P. armata* (35%) to *T. minor* (25%) to *S. curviseta* and *F. candida* (both 20%), and *H. purpurescens* (15%). No nematode DNA was detected in *L. cyaneus* and *P. minuta*.
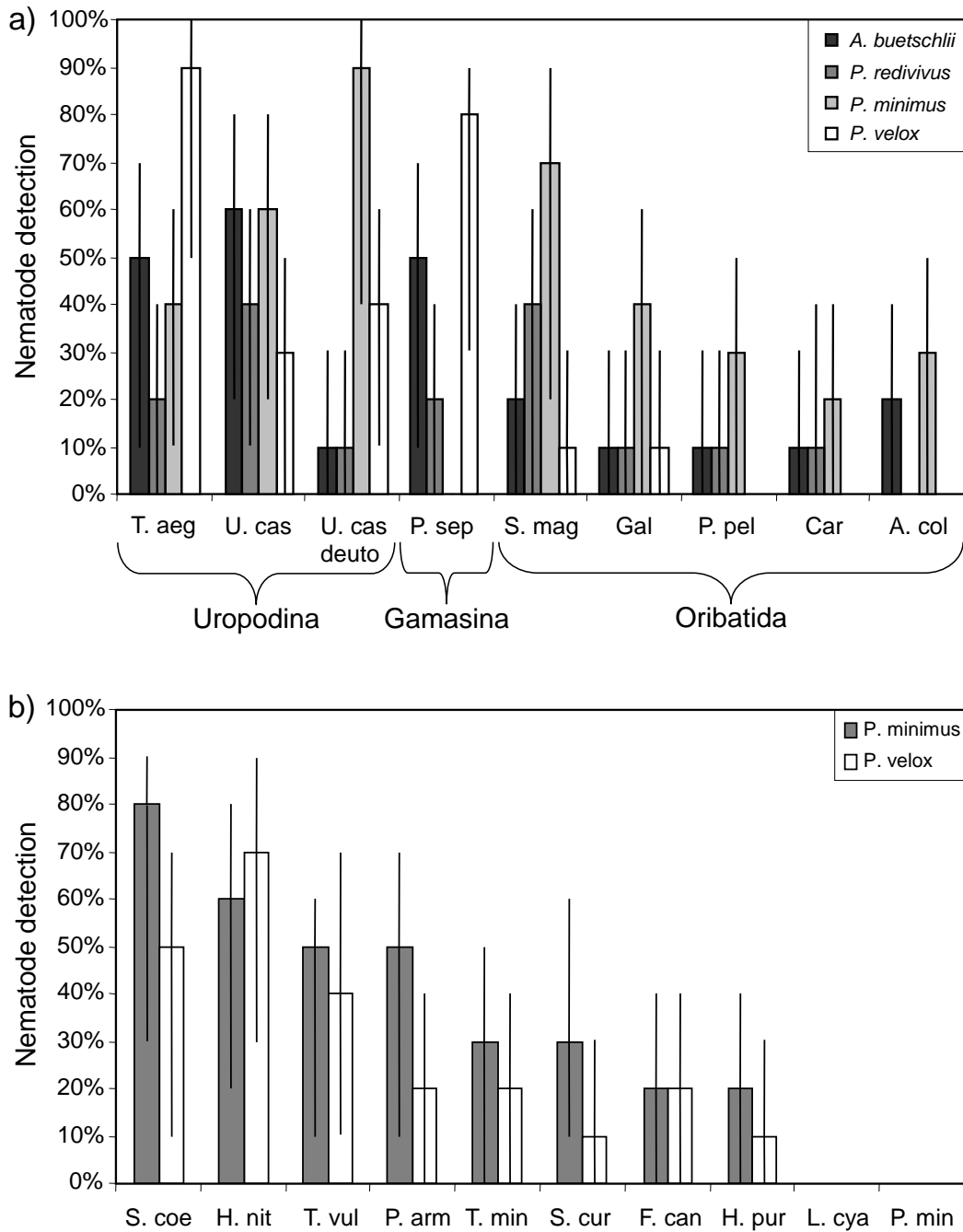
Figure 2: Frequency of detection of (a) *Acrobeloides buetschlii*, *Panagrellus redivivus*, *Plectus minimus* and *Plectus velox* in soil mite species, and (b) *P. minimus* and *P. velox* in collembola species by molecular gut content analysis in no-choice laboratory experiments. T. aeg = *Trachytes aegrota*, U. cas = *Uropoda cassidea*, U. cas. deuto = deutonymph of *U. cassidea*, P. sep = *Pergamasus septentrionalis*, S. mag = *Steganacarus magnus*, Gal = *Galumna* sp., P. pel = *Platynothrus peltifer*, Car = *Carabodes* sp., A. col = *Achipteria coleoptrata*, S. coe = *Sinella coeca*, H. nit = *Heteromurus nitidus*, T. vul = *Tomocerus vulgaris*, P. arm = *Protaphorura armata*, T. min = *Tomocerus minor*, S. cur = *Sinella curviseta*, F. can = *Folsomia candida*, H. pur = *Hypogastrura purpurescens*, L. cya = *Lepidocyrtus cyaneus*, P. min = *Proisotoma minuta*. Error bars represent upper and lower confidence limits; for statistical analysis see text.

3.5 Field study

The frequency of detection of nematode DNA in microarthropods differed significantly between the eight studied microarthropod species (χ^2 = 29.16, df = 7, P= 0.0001), and varied between the three studied nematode species (χ^2 = 71.14, df = 2, P< 0.0001). Further, detection of nematode DNA in microarthropod species varied among nematode species (significant mite species × nematode species interaction; χ^2 = 60.02, df = 14; P< 0.0001; Figure 3). On average, 70% of the studied microarthropods were tested positive for *Plectus* spp., 12% for *A. buetschlii* and none for *P. redivivus*.

In detail, DNA of *A. buetschlii* was detected in 33% of the investigated individuals of *S. magnus* and *E. muscorum*, and in 20% and 10% of the specimens of *N. palustris* and *N. silvestris*, respectively. DNA of *Plectus* spp. was detected in all of the studied individuals of *U. cassidea*, *S. magnus*, *N. silvestris* and *C. subglobulus*, in 80, 42 and 35% of the specimens of *N. palustris*, *T. vulgaris* and *O. villosa,* respectively, but in none of the individuals of *E. muscorum*. *P. redivivus* was detected in none of the tested microarthropod individuals.

Nematode density at the study site was 543,200 ind/m², thereof *Plectus* spp. contributed 12,894 ind/m² and *Acrobeloides* spp. 6,498 ind/m²; no individuals of the genus *Panagrellus* spp. were observed. Collembola density was 12,220 ind/m² and that of Acari (predominantly Oribatida) 8,850 ind/m².
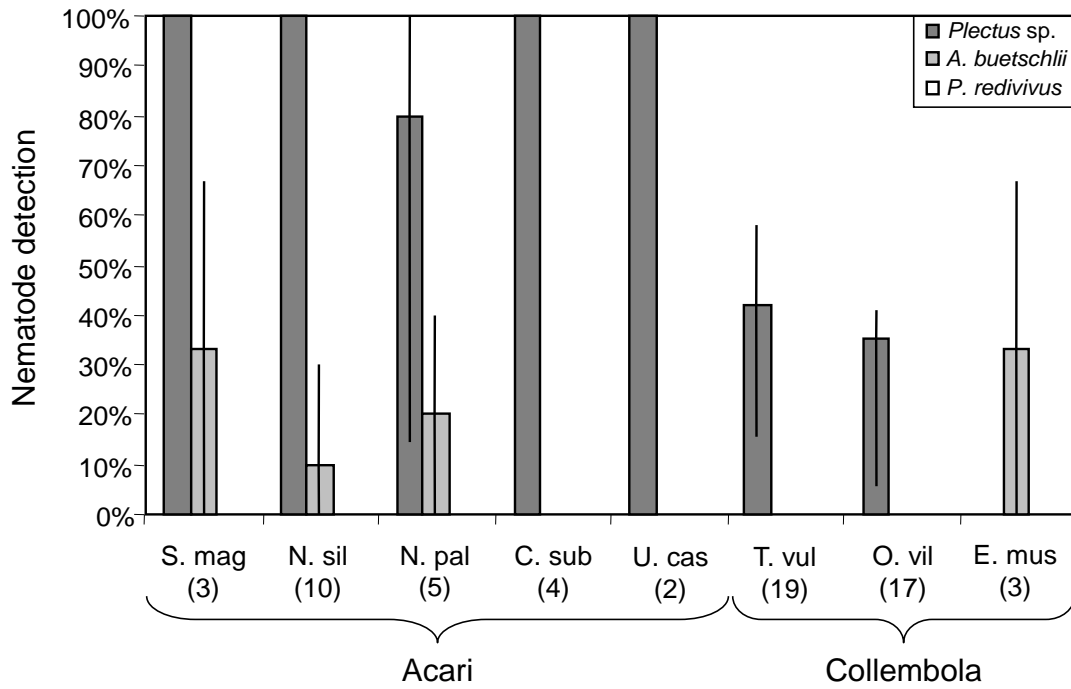
Figure 3: Frequency of detection of *Acrobeloides buetschlii*, *Panagrellus redivivus* and *Plectus* spp. in soil mite and collembola species by molecular gut content analyses in the field. U. cas = *Uropoda cassidea*, S. mag = *Steganacarus magnus*, N. sil = *Nothrus silvestris*, N. pal = *Nothrus palustris*, C. sub = *Chamobates subglobulus*, T. vul = *Tomocerus vulgaris*, O. vil = *Orchesella villosa*, E. mus = *Entomobrya muscorum*. Error bars represent upper and lower confidence limits; for statistical analysis see text.

4. **Discussion**

4.1 Primer design

For nematode detection in microarthropod predators we designed and optimized specific primers that amplify short fragments of the multi-copy 18S rDNA. The designed primers operated well and sensitively detected DNA of nematode prey species in the gut of microarthropods in the laboratory and the field. We choose 18S rDNA for designing primers as it is a conserved gene which allowed us to develop primers for prey taxa at different taxonomic resolution (Hoogendoorn & Heimpel 2001; Jarman *et al.* 2004; King *et al.* 2008; Kuusk & Agustí 2008) and has been used for nematode barcoding (Floyd *et al.* 2002; Pompanon *et al.* 2011). We aimed at designing species-specific nematode primers; however, proving specificity at the level of species is difficult and needs extensive checking for cross-reactions of related and other taxa. The developed primers may be partly genus- rather than species-specific. In fact, NCBI blast search indicated that our primers for *Plectus* spp. (*P. minimus* and *P. velox*) amplify a conserved fraction of the 18S rDNA of this genus suggesting resolution at genus level. In

contrast, the primers for *A. buetschlii* and *P. redivivus* amplify a more variable fraction of the nematode 18S rDNA indicating that these are indeed species-specific.

Detection of gut content is only possible when the primers detect tiny amounts of prey DNA as the amount of DNA decreases during digestion (Sint *et al.* 2011). Further, detection of prey DNA may be hampered by the large amount of predator DNA (King *et al.* 2008). We therefore performed a twofold serial dilution with and without fixed amounts of predator DNA in every reaction using our new primers (Eitzinger & Traugott 2011). Very small quantities of prey DNA were detected in these PCR reactions ranging from 0.025 to 0.050 pg/µg with and without predator DNA, indicating that the presence of predator DNA did not affect the PCR reaction.

4.2   Primer specificity

For investigation of trophic interactions the designed primers need to be prey specific; especially when used in the field where they may bind to DNA of a large number of prey species. We tested our primers against more than 100 potential prey species in laboratory trials. Only few cross-reactions with non-target organisms were detected and no cross-reactions occurred to potential predators used in this study. Moreover, primer suitability was tested by sequencing every positive reaction from the field experiment to check for potential non-target sequences. NCBI blast search of these sequences confirmed that only target species were amplified. Overall, this indicates that the detected cross-reactions of non-target species were irrelevant in our study and that our primers are suitable to assign predator-prey relationships for nematodes in the field.

4.3   Detection time of nematode prey in predators

After feeding on nematodes for 4 h each of the four nematode species was detected in predators but detection frequency varied between prey species. We minimized variations in prey detection due to different temperatures (Hoogendorn & Heimpel 2001; von Berg *et al.* 2008a; Sint *et al.* 2011) by using the same ambient temperature during the feeding and starving of the microarthropods, and also similar annealing temperature of the three primer sets. Different detection frequencies therefore likely reflect differences in digestion of nematode species. However, prey detection may also be affected by predator behaviour, e.g. active or passive foraging or voracity (Read *et al.* 2006). Fed with *A. buetschlii* detection rates for *P. septentrionalis* differed from those of *S. magnus* feeding on *Plectus velox*, *P. minimus* and *Panagrellus redivivus*. Presumably, fast and aggressive predators such as gamasid mites

ingest prey faster than slow moving species such as oribatid mites. Digestion rates of prey species have been reported to vary little among predator species (Agusti *et al.* 2003; Sheppard *et al.* 2004; Read *et al.* 2006), but we found detection rates to vary markedly in oribatids (but not in collembolans) fed the closely related nematode species *P. minimus* and *P. velox* indicating that prey digestion may also vary for similar prey species.

Moreover, the exposure time of nematode prey to predators had a major impact on its detection time. Overall, there was no clear difference of nematode detection in the gut of the respective predators 4 and 48 h after feeding on nematodes. However, generally detection frequency was higher in *S. magnus* when feeding for 48 h on *P. velox* compared to 4 h, but this did not hold for *P. minimus*. This indicates that the duration of feeding on nematodes strongly impacts prey detection time in predators. Potentially, prey detection also varies with prey size as *P. minimus* is much smaller than *P. velox* and therefore may be easier to handle by oribatid mites.

Detection efficiency of nematode DNA in the gut of predators declined little and inconsistently with time; even 128 h post feeding prey DNA in predators was detected. This indicates that even long after feeding prey DNA can be detected in the gut of predators which is advantageous for prey screening in predators in the field (Sheppard & Harwood 2005).

A shortcoming of this study is that we could not visually prove feeding of predators on prey. Therefore, failure to detect prey in predators may have been due to predators that did not feed on the offered prey rather than failure to detect prey present in the gut. The fact that detection rates always were below 100% suggests that some of the predators did not feed on the nematodes offered.

Overall, our results indicate that rates of detection and detection time of nematodes in mite predators vary strongly and depend on both prey species and the duration predators fed on prey. Similar to these findings prey detection frequency has been found to vary in every predator-prey combination (Zaidi *et al.* 1999; Hoogendorn & Heimpel 2001; Harwood *et al.* 2004; Sheppard *et al.* 2005; Hosseini *et al.* 2008; Traugott & Symondson 2008; von Berg *et al.* 2008a,b; Gagnon et al. 2011; Sint *et al.* 2011). Therefore, data on detection frequencies of prey in predators need careful interpretation (Greenstone *et al.* 2007).

4.4   Trophic links as indicated by laboratory studies

In our laboratory studies many mite and collembola species were detected positive for DNA of one or more of the four nematode species studied (in the following termed 'feeding' or

'consumption'). Despite this may not necessarily reflect the situation in the field, the results suggest that gamasid and uropodid mites heavily feed on nematode species which is conform to previous findings (Karg 1993; Scheu & Falca 2000; Klarner *et al.* 2013). Gamasid mites are mobile predators of the upper soil layer (Koehler 1999). *P. septentrionalis* was tested positive for each of the nematode species except the small *P. minimus* indicating that this large gamasid predator preferentially feeds on large nematode prey. Indeed, *P. septentrionalis* is known to hunt for bigger nematodes, collembolans, and little sclerotized mites (Karg 1993; Peschel *et al.* 2006, Heidemann *et al.* 2011); the small *P. minimus* therefore presumably is beyond the size range of *P. septentrionalis*.

Uropodid mites reach high densities at sites rich in organic matter which typically are colonized heavily by nematodes (Koehler 1999). *T. aegrota* was detected to feed on each of the four nematode species offered, but to preferentially feed on *P. velox.* This suggests that *T. aegrota* feeds on a wide range of nematode taxa including predatory nematode species of different body size explaining why small mesostigmate predators occupy high trophic positions in the soil food web (Klarner *et al.* 2013). *U. cassidea* also fed on each of the nematode species with small nematodes, i.e. *P. minimus*, being particularly important for deutonymphs. This indicates that the size spectrum of prey differs between developmental stages in predators which also has been suggested from data on stable isotope analyses (Klarner *et al.* 2013).

Similar to gamasid and uropodid mites each of the oribatid mite species fed on nematodes, however, prey detection frequency was lower than in gamasids an uropodids. Oribatid mites have been assumed to mainly feed on dead organic material (Behan-Pelletier 1999; Maraun & Scheu 2000). However, adult *S. magnus* heavily fed on *P. minimus* and also *P. redivivus*, and of the oribatid mite species studied most frequently was tested positive for nematode prey. The results support earlier findings based on parasitic nematodes used as model nematode prey (Heidemann *et al.* 2011) and suggests that *S. magnus* indeed feeds on free-living soil nematodes. Each of the oribatid mite species studied, including *Galumna* spp., preferentially fed on small nematodes confirming earlier suggestions that if feeding on nematodes oribatid mites prefer to feed on small nematode species (Rockett & Woodring 1966; Muraoka & Ishibashi 1976; Rockett, 1980; Oliveira *et al.* 2007). Low frequency of prey detection in *P. peltifer*, *A. coleoptrata* and *Carabodes* spp. suggests that these species only occasionally feed on nematodes and predominantly act as decomposers ingesting dead organic matter (Siepel 1990, Schneider *et al.* 2004, Heidemann et al. 2011).

Feeding of collembolans on nematodes was only investigated for two nematode species but the results resemble those for oribatid mites. Collembolans are known to feed on dead organic material, plants and fungi (Ruess *et al.* 2004; Chamberlaine *et al.* 2006a,b), however,

some are known to also feed on nematodes (Visser *et al.* 1987; Chen *et al.* 1995, Ruess *et al.* 2005; Read *et al.* 2006). Nematode detection frequency was high in *S. coeca, H. nitidus, T. vulgaris* and *P. armata* indicating that nematodes form an important part of their diet. In contrast, detection frequency of nematodes was low in *T. minor, S. curviseta, F. candida* and *H. purpurescens* indicating that these species only occasionally feed on nematodes (Lee & Widden 1996; Chamberlain *et al.* 2006a,b; Read *et al.* 2006). This supports earlier suggestions on the opportunistic feeding mode and high degree of omnivory in collembolans (Chahartaghi *et al.* 2005). However, the differences in nematode detection rates also point to niche differentiation in collembolans, e.g., the fact that no nematode DNA was detected in *L. cyaneus* and *P. minuta* suggest that these species occupy distinctly different niches. Overall, the results indicate that nematodes form a significant fraction of the diet of collembolans.

4.5 Nematodes as prey in the field

At least one of the nematode species studied was detected in each of the microarthropod species analysed from field samples indicating that feeding on nematodes is common in both collembolans and mites including oribatid mites. The nematode genus *Plectus* was detected most frequently, followed by *A. buetschlii* whereas none of the studied microarthropod species fed on *P. redivivus*. Of the studied nematode species *Plectus* species are most abundant at the study site suggesting that microarthropods opportunistically feed on the most frequent nematodes. However, in the studied forest *Plectus* spp. contributed only about 2% to total nematode density suggesting that either the microarthropods feed preferentially on these nematodes or feeding on *Plectus* spp. represents only a small fraction of the nematodes fed by microarthropods.

Among oribatid mites *S. magnus, N. silvestris* and *N. palustris* frequently fed on *Plectus* spp., but occasionally also consumed *A. buetschlii*. Feeding on nematodes by *S. magnus* and *N. palustris* is rather surprising as natural variations in $^{15}N/^{14}N$ ratios suggest them to feed on litter materials, i.e. to function as primary decomposers (Schneider *et al.* 2004). In contrast, stable isotope signatures of *N. silvestris* indicated that this species at least in part lives on an animal diet. The results therefore suggest that even oribatid mite species which predominantly feed on litter occasionally also ingest nematodes. The oribatid mite *C. subglobulus*, the uropodid mite *U. cassidea* and the collembola *T. villosa* und *O. vulgaris* also were tested positive for *Plectus* spp., indicating that they also feed on nematodes. In addition to the mite species *S. magnus, N. silvestris* and *N. palustris* only the collembola species *E. muscorum* fed on *A. buetschlii*, however, due to high variation in detection rates it remains unclear whether this is due to selective feeding on certain nematode species.

Overall, our findings indicate that many oribatid mite and collembola species, previously thought to predominantly live on litter material and microorganisms particularly fungi, also feed on free-living nematodes. Although nematodes may only comprise a small fraction of the diet of these species they likely contribute significantly to their nutrition as nematodes are of high nutritional value (Chamberlain *et al.* 2006a). Thus, many soil microarthropods live on a mixed diet, supporting previous assumptions that omnivory is common in soil animal food webs (Scheu and Setälä 2002). Future studies need to investigate to what extend different fractions of animal prey in decomposer animals contribute to niche separation thereby contributing to the astounding diversity of microarthropods in soil.

5. Conclusions

Overall, the results suggest that nematodes are an important but underestimated component of the diet of soil microarthropods previously assumed to predominantly feed on plant litter and microorganisms. Feeding on bacterial feeding nematodes by oribatid mites and collembolans, the most abundant microarthropod taxa in soil, presumably contributes significantly to the flux of energy from root exudates via bacteria to higher trophic levels including large predator species such as centipedes (Crotty *et al.* 2011; Ferlian *et al.* 2012; Pollierer *et al.* 2012). Notably, we only analyzed few nematode taxa and they only represented a small fraction of the nematode community at the study site. Nevertheless, they were frequently detected as prey in a range of microarthropod species suggesting that nematodes form an important part of the diet of collembola and mite species including those previously thought to live as detritivores or fungivores. The results therefore highlight that the decomposer food web is more complex than previously assumed with trophic relationships being exceptionally diverse. Novel tools such as molecular gut content analysis are indispensible if we are to understand the structure and functioning of these food webs.

Acknowledgements

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Supporting Information

Table S1: Designed primers, target organism, primer sequence, size of amplified fragments from the 18S rDNA gene and optimized annealing temperature to detect the nematodes *Acrobeloides buetschlii*, *Panagrellus redivivus* and *Plectus* spp. (*P. minimus* and *P. velox*).

Target organism	Primer name	Sequence 5'-3'	Size [bp]	Annealing temp. [°C]
Acrobeloides buetschlii	Acro-F-197	CGG CTT CGG CTG TTT CTG GTT	287 bp	62°C
	Acro-R-484	GAT GAC CGG CCT CAT AAG AGA ACG GTC TC		
Panagrellus redivivus	Pana-F-278	CCA ACG GCA GTG TAT TGT CCT GAC G	217 bp	63°C
	Pana-R-494	TAG GAA GGT TGT AAA TTC		
Plectus spp. (*P. minimus* and P. velox)	Plec-F-644	CTG RGA TCC AAG GCT TAT ACT GC	156 bp	62°C
	Plec-R-799	TAG ARC CGT GGT CTT ATT CT		

Table S2: Cross-reaction test of 108 non-target-species (including the respective target nematode species) with the specific primers for *Acrobeloides buetschlii*, *Panagrellus redivivus* and *Plectus* spp. (*P. minimus* and *P. velox*).

– no band occurred; + band occurred on the agarose gel; (+) not all species of the genus were tested positive / no detection in predator's gut.

Species	Family	*Acrobeloides buetschlii*	*Panagrellus redivivus*	*Plectus* spp.
Acari				
Dinychus perforatus Kramer, 1882	Gamasida	-	-	-
Eviphis ostrinus (C.L. Koch, 1838)	Gamasida	-	-	-
Geholaspis aeneus Krauss, 1970	Gamasida	-	-	-
Lysigamasus sp.	Gamasida	-	-	-
Parasitus lunulatus (J. Müller, 1859)	Gamasida	-	-	-
Pergamasus septentrionalis (Oudemans, 1902)	Gamasida	-	-	-
Veigaia nemorensis (C.L. Koch, 1836)	Gamasida	-	-	-
Zercon sp.	Gamasida	-	-	-
Zerconopsis remiger (Kramer, 1876)	Gamasida	-	-	-
Achipteria coleoptrata (Linné, 1758)	Oribatida	-	-	-
Belbidae	Oribatida	-	-	-
Carabodes femoralis (Nicolet, 1855)	Oribatida	-	-	-
Chamobates subglobulus (Oudemans, 1900)	Oribatida	-	-	-
Eupelops sp.	Oribatida	-	-	(+)
Euzetes globulus (Nicolet, 1855)	Oribatida	-	-	-
Galumna sp.	Oribatida	-	-	-
Hermannia gibba (C.L, Koch, 1836)	Oribatida	-	-	-
Hypochthonius rufulus Koch, 1835	Oribatida	-	-	-
Liacarus xylariae (Schrank, 1803)	Oribatida	-	-	+
Nothrus palustris (C. L. Koch 1839)	Oribatida	-	-	-
Nothrus silvestris Nicolet, 1855	Oribatida	-	-	-
Oribatella calcarata (C. L. Koch, 1836)	Oribatida	-	-	-

Oribatella quadricornuta (Michael, 1880)	Oribatida	-	-	-
Platynothrus peltifer (Koch, 1839)	Oribatida	-	-	-
Steganacarus magnus (Nicolet, 1855)	Oribatida	-	-	-
Tritegeus bisulcatus Grandjean, 1953	Oribatida	-	-	-
Xenillus tegeocranus (Hermann, 1804)	Oribatida	-	-	-
Trachytes aegrota (C. L. Koch, 1841)	Uropodida	-	-	-
Urodiaspis tecta (Kramer, 1876)	Uropodida	-	-	-
Uropoda cassidea (Hermann, 1804)	Uropodida	-	-	-
Uroseius cylindricus (Berlese, 1916)	Uropodida	-	-	-

Araneae

Coelotes inermis (L. Koch, 1855)	Amaurobiidae	-	-	-
Clubiona terrestris Westring, 1851	Clubionidae	-	+	-
Harpactea lepida (C.L. Koch, 1838)	Dysderidae	-	-	-
Micrargus herbigradus (Blackwall, 1854)	Linyphiidae	-	-	-
Microneta viaria (Blackwall, 1841)	Linyphiidae	-	+	-
Tapinocyba insecta (L. Koch, 1869)	Linyphiidae	-	-	-
Walckenaeria cucullata (C. L. Koch, 1836)	Linyphiidae	-	-	-
Walckenaeria obtusa Blackwall, 1836	Linyphiidae	-	-	-
Robertus lividus (Blackwall, 1836)	Theridiidae	-	-	-

Chilopoda

Strigamia acuminata (Leach, 1815)	Geophilomorpha	-	+	-
Lithobius lapidicola Meinert, 1872	Lithobiidae	-	-	+
Lithobius mutabilis L. Koch, 1862	Lithobiidae	-	-	-
Lithobius muticus (C.L. Koch, 1847)	Lithobiidae	-	-	-
Lithoibius nodupilipes Latzel, 1881	Lithobiidae	-	+	-
Lithobius crassipes L. Koch, 1862	Lithobiidae	-	-	-

Coleoptera

Abax parallelipipedus (Piller & Mitterbacher, 1783)	Carabidae	-	+	-
Pterostichus burmeisteri Heer**,** 1841	Carabidae	-	-	-
Pterostichus oblongopunctatus (Fabricius, 1787)	Carabidae	-	-	-
Trechus nigrinus Putzeys, 1847	Carabidae	-	-	-
Tropiphorus sp.	Curculionidae	-	-	-
Athous haemorrhoidalis (Fabricius, 1801)	Elateridae	-	-	-
Athous subfuscus (Müller, 1764)	Elateridae	-	-	-
Lamprohiza splendidula (Linnaeus, 1767)	Lampyridae	-	-	-
Nargus anisotomoides (Spence, 1815)	Leiodidae	-	-	-
Domene scabricollis (Erichson, 1840)	Staphylinidae	-	-	-
Habrocerus capillaricornis (Gravenhorst, 1806)	Staphylinidae	-	-	-
Philonthus sp.	Staphylinidae	-	-	-
Stilicus rufipes (Germar, 1836)	Staphylinidae	-	-	-
Xantholinus laevihatus Jacobsen, 1849	Staphylinidae	-	-	-
Xantholinus tricolor (Fabricius 1787)	Staphylinidae	-	-	-

Collembola

Entomobrya muscorum (Nicolet, 1841)	Entomobryidae	-	-	-
Heteromurus nitidus (Templeton, 1835)	Entomobryidae	-	-	-
Lepidocyrtus cyaneus Tullberg, 1871	Entomobryidae	-	-	-
Sinella coeca (Schott, 1896)	Entomobryidae	-	-	-
Sinella curviseta Brook, 1882	Entomobryidae	-	-	-
Orchesella villosa (Geoffroy, 1764)	Entomobryidae	-	-	-

Ceratophysella cf. denticulata (Bagnall, 1941)	Hypogastruridae	-	-	-
Hypogastrura burkilli (Bagnall, 1940)	Hypogastruridae	-	+	+
Folsomia candida Willem, 1902	Isotomidae	-	-	-
Isotoma viridis Bourlet, 1839	Isotomidae	-	-	-
Proisotoma minuta (Tullberg 1871)	Isotomidae	-	-	-
Protaphorura armata (Tullberg, 1869)	Onychiuridae	-	-	-
Tomocerus minor (Lubbock, 1862)	Tomoceridae	-	-	-

Dermaptera

Chelidurella sp.	Dermaptera	-	-	-

Diplopoda

Haploporatia eremita (Verhoeff, 1909)	Diplopoda	-	+	-
Polydesmus complanatus (Linnaeus, 1761)	Diplopoda	-	-	-
Glomeris sp.	Glomeridae	-	-	-

Diplura

Campodea sp.	Campodeidae	-	-	-

Diptera

Drosophila melanogaster Meigen, 1830	Drosophilidae	-	-	-
Musca domestica Linnaeus, 1758	Muscidae	-	+	-

Isopoda

Ligidium cf. *hypnorum* (Cuvier, 1792)	Isopoda	-	+	-
Porcellio sp.	Isopoda	-	+	-
Trichoniscus pusillus Brandt, 1833	Isopoda	-	+	-
Trichorhina tomentosa (Budde-Lund, 1893)	Platyarthridae	-	-	+

Lepidoptera

Galleria sp.	Pyralidae	-	+	+

Mollusca

Deroceras sp.	Gastropoda	-	-	-

Myriapoda

Symphyla	Myriapoda	-	-	-

Nematoda

Acrobeloides buetschlii (de Man, 1884)	Cephalobidae	+	-	-
Heterorhabditis megidis Poinar, Jackson & Klein, 1987	Heterorhabditidae	-	-	-
Panagrellus redivivus Goodey, 1945	Panagrolaimidae	-	+	-
Turbatrix aceti (Müller, 1783)	Panagrolaimidae	-	-	-
Plectus minimus Cobb, 1893	Plectidae	-	-	+
Plectus velox Bastian, 1865	Plectidae	-	-	+
Pratylenchus zeae Graham, 1951	Pratylenchidae	-	-	(+)
Caenorhabditis elegans (Maupas, 1900)	Rhabditidae	-	-	-
Phasmarhabditis hermaphrodita (Schneider, 1859)	Rhabditidae	-	-	-
Steinernema feltiae (Filipjev, 1934)	Steinernematidae	-	+	-

Oligochaeta

Enchytraeidae	Enchytraeidae	-	-	-
Aporrectodea caliginosa (Savigny, 1826)	Lumbricidae	-	+	-

Dendrobaena octaedra (Savigny, 1826)	Lumbricidae	-	-	+
Lumbrius terrestris Linnaeus, 1758	Lumbricidae	-	+	+
Octolasion cyaneum (Savigny 1826)	Lumbricidae	+	+	-
Opiliones				
Lophopilio palpinalis (Herbst, 1799)	Phalangiidae	-	-	+
Anelasmocephalus cambridgei (Westwood, 1874)	Trogulidae	-	-	-
Trogulus nepaeformis (Scopoli, 1763)	Trogulidae	-	-	-
Pseudoscorpione				
Neobisium carcinoides (Hermann, 1804)	Pseudoscorpione	-	-	-
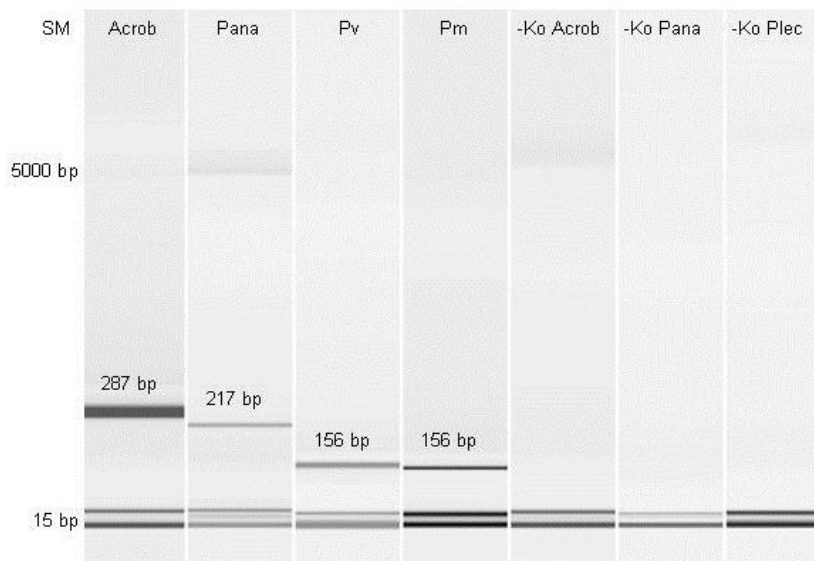
Figure S1: Visualisation of amplified 18S rDNA fragments of *Acrobeloides buetschlii, Panagrellus redivivus*, *Plectus velox* and *Plectus minimus* using specific primer sets in the QIAxcel system (with the QX Alignment Marker 15bp/5kb). Lane 1 Acrob: *A. buetschlii* (Acrob-F-247 + Acrob-R-554); Lane 2 Pana: *P. redivivus* (Pana-F-379 + Pana-R-611); Lane 3 Pv and Lane 4 Pm: *P. velox* and *P. minimus* (Plec-F-789 + Plec-R-961); Lane 5 – 7: Negative control using H$_2$O instead of DNA tested with the respective primer pairs. SM: with QIAxcel, an internal marker (15 and 5000 bp) is used (QX Alignment Marker 15 bp/ 5kb; Qiagen, Hilden, Germany).

Figure S2: Visualisation of primer sensitivity in the QIAxcel system (with the QX Alignment Marker 15 bp/5 kb) using two-fold serial dilution of DNA from *Acrobeloides buetschlii* as template in a PCR with specific primer sets. Lane 1 –Ko: Negative control using H_2O instead of DNA tested with the primer pair for *A. buetschlii* (Acrob-F-247 + Acrob-R-554); Lane 2 +Ko: Positive control; Lane 3-16: different concentrations of template DNA (pg/µl) in the PCR reaction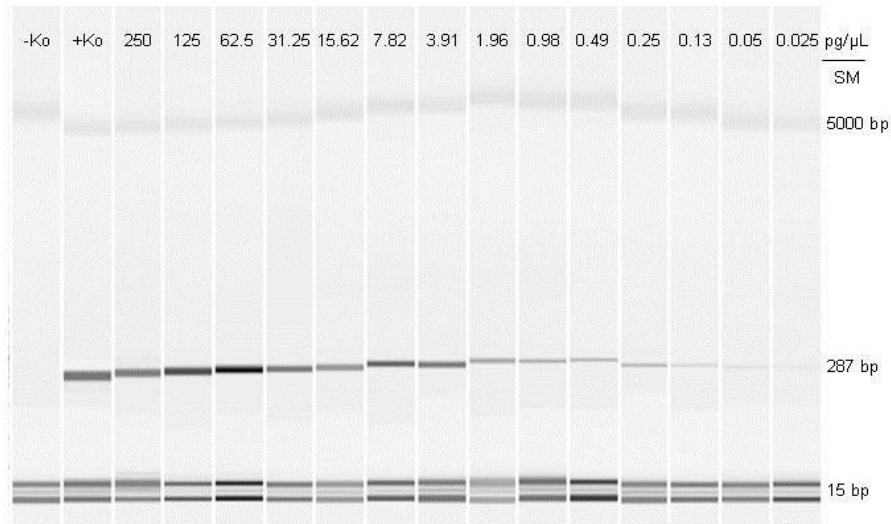 in presence of 200 pg/µl DNA of *Steganacarus magnus.* SM: with QIAxcel, an internal marker (15 and 5000 bp) is used (QX Alignment Marker 15 bp/ 5kb; Qiagen, Hilden, Germany).


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                                    10        20        30        40        50        60        70        80
                               ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Acrobeloides buetschlii   GACTCATAGTAACTGAGCTGATCGCACGGTCTTGCACCGGCGACGTGTCTTTCAAGTATCTGCCGTATCAACTTTCGATGGTAGT
Panagrellus redivivus     TGGTTGTGACGGGTAACGGAGAATTAGGGTTCGACTCCGGAGAAATAGCCTGAGAAACGGCTTTTACATCCAAGGAAGGCAGCAG
Plectus spp.              TGGTTTTCCCTTGATGCTCTTTACTGGGTGTCTTGGGTGGCTAGCGAGTTTACTTTGAAAAAATTAGAGTGCTTAACACAGGCTA

                                    90       100       110       120       130       140       150       160       170
                               ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Acrobeloides buetschlii   GTATCTGACTACCATGGTGATCACGCGTAACGGAGAATAAGGGTTCGGCTCCGGAGAGTTCGCCTGAGAAATCGCGAACACATCT
Panagrellus redivivus     GCGCGAAAATTACCCACTCTTAGTACGAGGAGGTAGTGACGAGAAGTGACAAGATCTGTCCCTTCGGGGGCGGGTCATTGGAATG
Plectus spp.              ACGCCTGAATACTTGTGCATGGAATAAT

                                   180       190       200       210       220       230       240       250
                               ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Acrobeloides buetschlii   AAGGAAGGCAGCAGGCGCGCGAAAATTACCCACTCTCAGCGACGAGGAGGTAGTGACGAAAAATAAC
Panagrellus redivivus     GTTT
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Figure S3: Sequence of the fragments (without primer region) amplified with specific primers for *Acrobeloides buetschlii*, *Panagrellus redivivus* and *Plectus* spp.

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Consumption of nematodes by soil microarthropods varies between microhabitats of deciduous forests as indicated by molecular gut content analyses

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Submitted:

Heidemann K, Ruess L, Scheu S, Maraun M. Consumption of nematodes by soil microarthropods varies between microhabitats of deciduous forests as indicated by molecular gut content analyses.

Abstract

Soils are complex and heterogeneous habitats including not only litter and soil but also microhabitats such as mosses, fungal mats and grass patches. Soil animal food webs in such microhabitats have been separated in a slow fungal and a fast bacterial energy channel. Bacterial feeding nematodes are an important component of the bacterial energy channel by consuming bacteria and forming prey for higher trophic levels such as soil microarthropods. Investigating the role of nematodes as prey for higher trophic level consumers has been hampered by methodological problems related to their small body size and lack in skeletal structures which can be traced in the gut of consumers. Recent results from molecular gut content analyses suggest that nematodes form major prey of soil microarthropods including those previously thought to live as detritivores. Using molecular markers we traced nematode prey (*Plectus* spp. and *A. buetschlii*) in fourteen abundant soil microarthropod species including Mesostigmata and Oribatida (Acari) from three different habitats (litter, grass and moss). We hypothesized that (1) mite species differentially consume different nematode species, (2) mite feeding differs between the three habitats, and (3) feeding on nematodes differs between mite species. Mites indeed differentially consumed the two nematode taxa with consumption of individual species / genera being related to their density. As expected the consumption of nematodes differed between habitats indicating shifts in trophic niches with changing habitat characteristics. Also as expected the different mite species differentially consumed nematodes indicating differences in trophic niches which likely contribute to the high diversity of microarthropods in deciduous forests. Overall, the results suggest that free-living bacterial feeding nematodes form an important prey for soil microarthropods including those previously assumed to live as detritivores.

1. Introduction

The soil system is patchy and comprises different habitats, such as litter, patches of mosses and grass, and fine and coarse woody debris (Mikola & Sulvaka 2001; Proctor *et al.* 2002). These habitats significantly affect the density and distribution of species, but also contribute to variations in interactions between soil animal species (Sulkava & Huhta 1998; Hohberg & Traunspurger 2005). Microhabitats and associated spatial heterogeneity cause species to be patchily distributed (Nielsen *et al.* 2012; Caruso et al. 2012a,b). Thereby microhabitats contribute to the turnover of species at small spatial scales and to high diversity of soil animal species (Anderson 1975; Scheu & Setälä 2002; Maraun *et al.* 2003a).

Soil food webs form an essential component of terrestrial ecosystems (Scheu & Setälä 2002; Bardgett & Wardle 2010). Based on predator-prey interactions they are responsible for the flux of matter and energy through ecological systems (Pimm 1991; Terborgh & Estes 2010). In soil carbon and nutrients are channelled to higher trophic levels via the bacterial, fungal and plant litter energy channel (Moore & Hunt 1988; Scheu *et al.* 2005). Depending on forest type and microhabitat the relative contribution of the different energy channels to carbon and nutrient cycling varies (Ruess 2003). Further, carbon processing is assumed to vary between the different energy channels with the bacterial energy channel being the fastest (van Hees *et al.* 2005; Strickland *et al.* 2012). However, the role of different consumer organisms for channelling carbon to higher trophic levels remains little understood, but it is commonly assumed that bacterial feeding nematodes and their microarthropod predators form a major link of the bacterial energy channel (Freckman 1988; Crotty *et al.* 2011; Ferlian *et al.* 2012).

Free-living soil nematodes hold a key position in soil food webs (Bongers & Ferris 1999; Moore *et al.* 2003). They comprise a large diversity of species differing in feeding nodes thereby consuming a wide range of resources (Yeates *et al.* 1993). Further, nematodes represent the most abundant metazoan animals of terrestrial ecosystems with densities in soil reaching millions of individuals per square metre (Yeates *et al.* 2000). Notably, bacterial feeding nematodes typically account for >50% of total free-living nematodes but in hot spots of microbial activity they may account for 90-99% (Zunke & Perry 1997; Mulder & Vonk 2011). Together with protists they form the main bacterial consumers in virtually any ecosystem including soils (Griffiths 1990; Zunke & Perry 1997; Scheu *et al.* 2005). Due to their high abundance and the fact that they are little defended, nematodes are assumed to form abundant prey of many soil arthropods, in particular predatory soil microarthropods such as gamasid mites (Walter & Ikonen 1989; Walter & Proctor 1998). However, due to the scarcity of skeletal elements only the mouthparts can be traced in the gut of nematode predators and therefore nematodes are regarded 'invisible' prey (Walter 1988; Sunderland 1988). Due to the difficulties

in tracing nematode prey in predators their role in soil food webs likely has been underestimated (Walter 1988; Read *et al.* 2006; Heidemann *et al.* 2011).

Mites (Acari) are abundant, diverse and ubiquitous microarthropods in soil (Maraun & Scheu 2000). Mesostigmatid mites (Mesostigmata: Gamasina and Uropodina) are known to live as predators and many species feed on nematodes (Karg 1993; Koehler 1997; 1999; Klarner *et al.* 2013). In contrast, most oribatid mite species (Oribatida) are assumed to feed on bacteria, fungi and/or dead organic material. However, recent studies using stable isotopes and molecular markers indicate that many oribatid mite species live as predators or scavengers (Schneider *et al.* 2004; Heidemann *et al.* 2011; Maraun *et al.* 2011). Due to the high density of soil microarthropods feeding on nematodes is likely to contribute substantially to the flux of energy from bacteria via bacterial feeding nematodes through mites and collembolans to top trophic levels (Crotty *et al.* 2011; Ferlian *et al.* 2012; Pollierer *et al.* 2012). Including nematodes as high quality food in their diet is likely to contribute to the high local density of microarthropods and may explain the high trophic position of some detritivore species as indicated by stable isotope analysis (Scheu & Falca 2000; Pollierer *et al.* 2009; Maraun *et al.* 2011).

Studying nematodes as prey for mites is difficult in the field since both are small and difficult to observe in the porous habitat of the soil. Further, mites typically are only ingesting prey liquids preventing detection of prey fragments in their guts (Wardle & Yeates 1993; Symondson 2002; Scheu *et al.* 2005). Molecular gut content analysis using molecular markers specific for nematodes i.e., primers that only bind to certain genera or species of nematodes, is a promising tool for investigating predator-prey interactions in soil and is expected to allow fundamental progress in soil food web research, including the opening of hitherto neglected trophic interactions (Read *et al.* 2006; Heidemann *et al.* 2011).

The present study aimed at investigating the role of free-living bacterial feeding nematode species as prey for soil mites in deciduous forests and their variation between microhabitats. We used primers for two common and abundant bacterial feeding nematode species/ genera (*A. buetschlii* and *Plectus* spp.) developed recently (see Chapter 3) and screened 14 soil mite species for feeding on these nematodes. Mites from different forest habitats including litter, and patches of moss and grass were investigated to test whether the food spectrum of these mites varies in space. We hypothesized that (1) mite species differentially consume nematode species, (2) feeding of mites on nematodes varies in space, i.e. differs between forest microhabitats, and (3) mites previously thought to predominantly live as fungivores, i.e. oribatid mites, frequently consume nematodes irrespective of forest microhabitat.

2. Material and Methods

2.1 The organisms

Acrobeloides buetschlii (de Man, 1884) and *Plectus* spp. are free-living bacterial feeding nematodes and were investigated as prey for soil mites. Both have a cosmopolitan distribution and are abundant in the litter layer of forest soils as well as in patches of moss and grass. *A. buetschlii* reaches a length of 0.3-0.5 mm. Species of the genus *Plectus* are among the most abundant nematodes of the Plectidae, the most widespread nematodes of the world (Wu *et al.* 2011), and even occur in the Antarctic (Freckman & Virginia 1993; Powers *et al.* 1998). *Plectus* spp. reach a length of 0.35-1.2 mm.

We investigated 14 soil mite species as potential predators of nematodes, including three mesostigmatid mite species, i.e. *Dinychus perforatus* Kramer, 1882 (Uropodina), *Pergamasus septentrionalis* (Oudemans, 1902) (Gamasina) and *Uropoda cassidea* (Hermann, 1804) (Uropodina), and eleven oribatid mite species, i.e. *Achipteria coleoptrata* (Linné, 1758), Belbidae, *Carabodes femoralis* (Nicolet, 1855), *Eupelops* spp., *Galumna* spp., *Hermannia gibba* (C.L. Koch, 1836), *Nothrus palustris* (C.L. Koch 1839), *Nothrus silvestris* Nicolet, 1855, *Oribatella calcarata* (C.L. Koch, 1836), *Platynothrus peltifer* (C.L. Koch, 1839) and *Steganacarus magnus* (Nicolet, 1855). Specimens from different microhabitats were screened for feeding on *A. buetschlii* and *Plectus* spp. using specific primers.

2.2 Study site

Samples were taken from the litter layer, and patches of moss and grass from an area of 80 m^2 of the Hainich forest (near Birkungen, Thüringen, Germany) in May 2010. The Hainich is the largest cohesive deciduous forest of Germany with a mean annual temperature of 6.5-8 °C, a mean annual precipitation of 500-800 mm and an average soil pH at the study sites of 4.6. It is dominated by beech (*Fagus sylvatica*) interspersed with ash (*Fraxinus excelsior*), lime (*Tilia europaea*), maple (*Acer platanoides* and *A. pseudoplatanus*) and wild service trees (*Sorbus torminalis*). The parent rock is Triassic limestone; the main soil types are Luvisols, Cambisols and Stagnosols (Erdmann *et al.* 2012).

2.3 Field sampling

In May 2010 we collected animals in the litter layer, and in patches of moss and grass of a minimum size of 1 m^2. In an area of 80 m^2 in the forest three sites were identified and at each

of these sites samples from each of the three habitats were taken. Mites for molecular gut content analysis were sampled from plots of ~ 1 m^2 by heat for three hours (Kempson *et al.* 1963) and identified to species level; the species were selected based on their abundance and presence in the three microhabitats. Individuals were checked for attached nematodes; no nematodes were attached. Handling of the specimens followed the recommendations of King *et al.* (2008) and guidelines given in Heidemann *et al.* (2011). Three species of mesostigmatid mites and eleven species of oribatid mites were investigated: *D. perforatus* (0-31-0 individuals from litter-moss-grass sites, respectively) [Mesostigmata, Uropodina], *P. septentrionalis* (12-8-0) [Mesostigmata, Gamasina], *U. cassidea* (25-23-10) [Mesostigmata, Uropodina], *A. coleoptrata* (87-8-17), Belbidae (37-78-25), *C. femoralis* (0-10-0), *Eupelops* spp. (37-11-23), *Galumna* spp. (52-36-15), *H. gibba* (0-10-0), *N. palustris* (0-10-21), *N. silvestris* (0-45-45), *O. calcarata* (11-0-0), *P. peltifer* (12-21-0), *S. magnus* (0-32-0) [all Oribatida]. Individual mites were placed in 180 µl buffer ATL of the DNeasy® Blood & Tissue Kit (Qiagen GmbH, Hilden, Germany) and placed in the freezer at -80°C. The time between sampling in the field and freezing of the animals was on average 7 h with a maximum of 10 h.

For investigating nematode and soil mite communities at the study sites additional samples were taken in close vicinity of the sites the mites for the molecular gut content analysis were taken. Nematodes were extracted from soil cores of a diameter of 2.5 cm using a modified Baermann method (see Ruess 1995). Mites were extracted from soil cores of a diameter of 20 cm and extracted by heat (Macfadyen 1961). Nematodes were sorted to trophic groups and within bacterial feeding species the densities of *Acrobeloides* spp*.* and *Plectus* spp. were determined.

2.4   Molecular gut content analysis

DNA from whole mite individuals was extracted following the protocol of the DNeasy® Blood & Tissue Kit (Qiagen GmbH, Hilden, Germany). To verify the success of the DNA extractions and to exclude inhibition of the DNA amplification we performed a PCR with D3 primers amplifying a 320 bp fragment of the 28S rDNA for every sample (Maraun *et al.* 2003b). PCRs with D3 primers and thermal cycle parameters were carried out as described in Heidemann *et al.* (2011). To avoid false negative amplifications only samples with a PCR product were included in the analyses.

We used specific primers for *A. buetschlii* and *Plectus* spp. (the latter primer pair was tested positive for *P. velox* and *P. minimus*; we therefore assume it to be specific for the genus

Plectus) amplifying 287 bp and 156 bp fragments of the 18S rDNA gene, respectively. These primers have been established recently and used successfully for tracing the target nematodes in microarthropod predators (see Chapter 3). Prey DNA was amplified as described in Chapter 3. PCR products were visualised using the capillary electrophoresis system QIAxcel (Qiagen GmbH, Hilden, Germany). About half of the positive samples were sequenced by Macrogen Inc. (Seoul, South Korea) or by the Göttingen Genomics Laboratory at the Institute of Microbiology and Genetics of the Georg August University Göttingen and compared to NCBI (www.ncbi.nlm.nih.gov) to confirm the species identity of amplified fragments.

2.5 Statistical analysis

We calculated means and standard derivations of nematode detection rates by using the package 'simpleboot' in R (Simple Bootstrap Routines. R-package version 1.1-3; Roger D. Peng, 2008; R Development Core Team, 2009). Additionally, 95% confidence limits were calculated by 9999 bootstrap resamples for each predator feeding on a certain prey.

Since *A. buetschlii* was only rarely consumed we excluded data on *A. buetschlii* from the analyses. With the dataset on the detection of *Plectus* spp. we tested (i) if feeding on *Plectus* spp. differed between microhabitats, (ii) if the mite species differentially consumed *Plectus* spp., and (iii) if differential consumption varies between microhabitats (mite species × microhabitat interaction). The analyses were carried out using logistic regression (Hosmer & Lemeshow 1989; Sokal & Rohlf 1995) in SAS 9.13 (SAS Institute Inc., Cary, USA).

3. Results

Nematode DNA was detected in the gut from 13 of the 14 studied mite species (in the following termed 'consumption' or 'feeding'). Generally, *Plectus* spp. was more frequently consumed than *A. buetschlii* ($\chi^2 = 75.75$, df = 1, P< 0.0001). Of the 752 individuals studied in total DNA of *A. buetschlii* was only detected in four mite individuals (in *U. cassidea* from litter and in Belbidae, *C. femoralis* and *H. gibba* from moss).

On average *Plectus* spp. was detected in 23.6% of the total mite individuals studied. *Plectus* spp. was detected more frequently in mites from litter (16.9%) than in those from grass (9.0%) and from moss patches (8.1%) with the effect of microhabitat being significant ($\chi^2 = 11.71$, df = 2, P= 0.0029). Detection of nematode DNA differed significantly between the studied mite species ($\chi^2 = 80.47$, df = 13, P< 0.0001), with the differences between mite species varying

among microhabitats (mite species × microhabitat interaction; $\chi^2 = 29.71$, df = 12, P= 0.0031; Fig. 1).

Among the three mesostigmatid mite species studied feeding on *Plectus* spp. was most pronounced in *P. septentrionalis* (Fig. 1). Most individuals of this species from litter (83.3%) were tested positive for *Plectus* spp., whereas none of the eight individuals studied from moss were tested positive; in grass patches no *P. septentrionalis* were found. The gamasid mite *D. perforates* was only found in moss patches were it fed on *Plectus* spp., however, detection frequency was low (9.7%). In contrast, in the uropodid mite *U. cassidea* about half of the individuals from litter were tested positive for *Plectus* spp., 17.4% from moss and 30% from grass. In oribatid mites feeding on *Plectus* spp. was most pronounced in Belbidae with on average 14-24% of the individuals tested positive and the frequency of detection differing little between microhabitats. Three oribatid mite species were tested positive for *Plectus* spp. in two of the three studied microhabitats with the detection frequency being generally <20% (*Eupelops* spp., *N. palustris* and *N. silvestris.*). Four oribatid mite species (*A. coleoptrata*, *Galumna* spp., *O. calcarata* and *P. peltifer*) were tested positive for *Plectus* spp. in one of the three microhabitats with the detection frequency again being generally <20% and a maximum in *P. peltifer* in moss (19.1%) Only three of the eleven oribatid mite species studied were not detected to feed on *Plectus* spp. (*C. femoralis*, *H. gibba* and *S. magnus*).

Half of the samples positive for nematode DNA were sequenced and the sequences blasted for matches in the NCBI database (www.ncbi.nlm.nih.gov). The hits indicated that virtually all (99%) of the bands indeed originated from *Plectus* or *Acrobeloides* indicating high specificity of the primers used and confirming that cross-reactions of non-target species are negligible.

Average nematode densities in the litter, grass and moss microhabitats were 2,277,366±423,016, 898,317±207,557 and 1,027,599±515,451 ind./m^2, respectively. Thereof *Plectus* spp. contributed 7.4, 9.3 and 11.9% in litter, grass and moss microhabitats, respectively. Respective values for *Acrobeloides* spp. were 5.4, 2.4 and 0.8%.
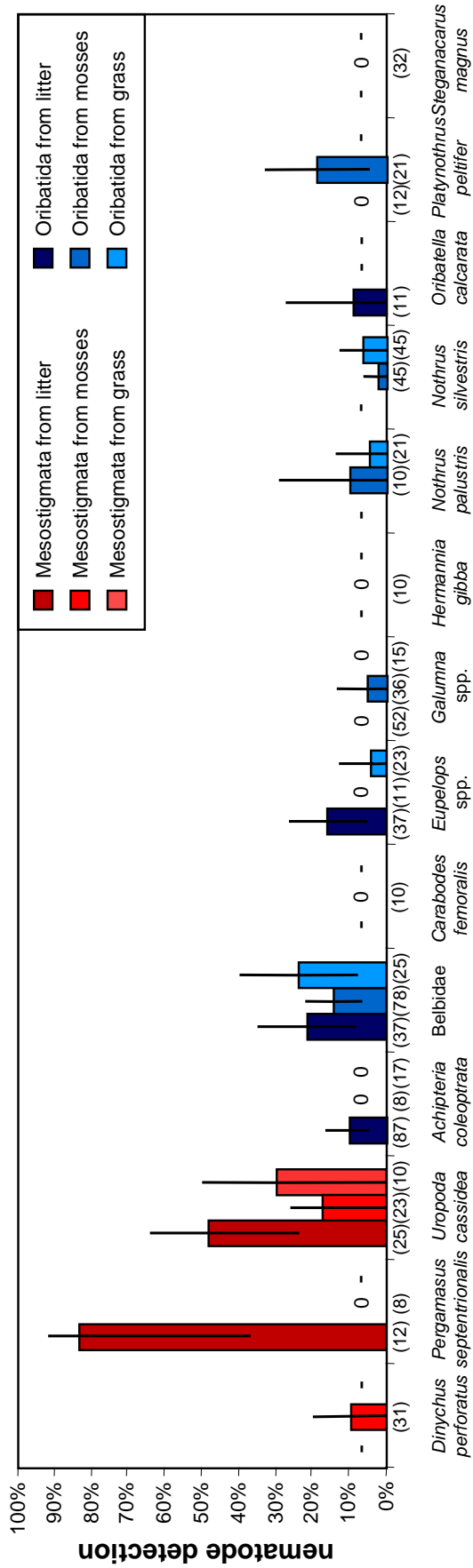
Consumption of nematodes by soil microarthropods varies between microhabitats of deciduous forests as indicated by molecular gut content analyses

Figure 1: Detection of *Plectus* spp. in the gut of 14 mite species from three microhabitats (litter, grass and moss patches) using specific molecular markers amplifying part of the 18S rDNA. Data represent percentages of mite individuals in which nematodes were detected with the number of individuals analyzed given in brackets. The upper and lower confidence limits are indicated as error bars; for statistical analysis see text.

4. Discussion

Overall, 13 of the 14 mite species studied were detected to feed on nematodes with feeding on *Plectus* spp. being more important than feeding on *A. buetschlii.* This suggests that free-living bacterial-feeding nematodes form an important component of the diet of soil mites including species that previously have been assumed to live as detritivores or fungal feeders, i.e. oribatid mites.

More frequent detection of *Plectus* spp. than *A. buetschlii* indicates that either *A. buetschlii* is avoided as prey, e.g. due to defense mechanism, or that it was less consumed because it was less abundant than *Plectus* spp. Additionally, *A. buetschlii* may also have been detected less frequently than *Plectus* spp. since the primers for *A. buetschlii* were species-specific whereas the primers for *Plectus* spp. presumably detect all species from the genus. Notably, the body size of both *A. buetschlii* and *Plectus* spp. overlapped suggesting that differential detection of the two target species/genera was not due to differences in body size.

Detection frequency of *Plectus* spp. as prey of mites in litter exceeded that in moss and grass patches indicating that feeding on nematodes varies between microhabitats of the forest floor. This suggests that trophic niches of soil microarthropods vary in space and indicates that mites adjust their prey spectrum if environmental conditions and prey availability change. Notably, differences in detection frequency of *Plectus* spp. in litter as compared to grass and moss corresponded to differences in density of *Plectus* spp. in these habitats. This suggests that nematodes were fed according to their availability as prey and indicates that mites opportunistically feed on nematodes.

The mite species studied differentially consumed *Plectus* spp. As expected feeding on nematodes in the two mesostigmatid mites *P. septentrionalis* and *U. cassidea* generally exceeded that in the studied oribatid mite species. Notably, feeding on *Plectus* spp. in the studied microhabitats by *U. cassidea* was more balanced than by *P. septentrionalis*. *P. septentrionalis* has been assumed to predominantly feed on collembolans and other little sklerotized mites (Karg 1993; Peschel *et al.* 2006). High detection frequency of *Plectus* spp. in *P. septentrionalis* in litter (>80%) identifies this mite species as ferocious predator of nematodes and supports results of recent studies based on variations in stable isotope ratios suggesting this species to predominantly feed on nematodes (Scheu & Falca 2000; Klarner *et al.* 2013). High detection frequency is remarkable considering that *Plectus* spp. contributed only 7.4% to total nematode density in litter. Failure to detect *P. septentrionalis* to feed on *Plectus* spp. in moss suggests that nematodes are less important prey for *P. septentrionalis* in moss as compared to litter, however, it may also be related to the low number of individuals

studied (eight).

Uropodid mites have been assumed to predominantly feed on fungi but natural variations in stable isotope ratios suggested them to predominantly feed on nematodes. Assuming that they not only feed on *Plectus* spp. but on a range of nematode species results of the present study support this conclusion. Notably, uropodid mites reach high densities at sites rich in organic matter which typically are also colonized heavily by nematodes (Koehler 1999). *D. perforatus* (Mesostigmata) was only found in mosses where it occasionally fed on *Plectus* spp. This species is known to frequently occur in mosses (Karg 1989) where it has been assumed to predominantly feed on nematodes (Karg 1993). Considering that *Plectus* spp. contributed only 11.9% to total nematode density in moss and *D. perforatus* was tested positive for *Plectus* spp. in 9.7% of the studied individuals this may well be the case.

Oribatid mites have been assumed to mainly feed on litter, dead organic material, lichens or fungi (Behan-Pelletier 1999; Maraun & Scheu 2000; Schneider *et al.* 2004), but some studies suggested them to also feed on nematodes (Rockett & Woodring 1966; Muraoka & Ishibashi 1976; Rockett 1980; Oliveira *et al.* 2007). Recent molecular studies highlighted that feeding on nematodes is common in oribatid mites (Heidemann *et al.* 2011). Results of the present study suggest that irrespective of forest floor microhabitat feeding on nematodes is common in Belbidae and this has been suggested earlier using parasitic nematodes as model nematode prey (Heidemann *et al.* 2011). Since Belbidae also feed on fungi as indicated by laboratory observations and stable isotopes (Maraun *et al.* 1998; 2011) they likely function as trophic level omnivores. *Eupelops* spp.*, N. palustris, N. silvestris, A. coleoptrata, O. calcarata, P. peltifer* and *Galumna* spp. occasionally consumed *Plectus* spp. indicating that nematodes form part of their diet. The typical pincette-like 'peloptoid' mouthparts of *Eupelops* spp. have been assumed to serve for parenchyma feeding although nematode consumption is also conceivable. *Eupelops* spp. has been assumed to predominantly feed on fungi; however, Erdmann *et al.* (2007) suggested this species to feed on a mixture of litter, fungi and nematodes. *N. palustris* and *N. silvestris* were both tested positive for nematode prey in grass and moss patches, confirming earlier observations that both feed on nematodes (Heidemann *et al.* 2011). Stable isotope analysis suggested *N. palustris* to predominantly feed on litter but *N. silvestris* to feed on animal diet (Schneider *et al.* 2004). Similar detection frequency for *Plectus* spp. in *N. palustris* and *N. silvestris* in the present study does not support niche differentiation in respect to nematode prey in these two species. *A. coleoptrata, O. calcarata* and *P. peltifer* in litter occasionally consumed *Plectus* spp. indicating that these species regularly feed on nematodes but predominantly feed on other food sources. In fact, natural variations in stable isotope ratios suggest them to mainly feed on litter (Schneider *et al.* 2004).

Galumna spp. consumed *Plectus* spp. only in moss not in litter and grass patches which was unexpected. Nematode feeding of Galumnidae (especially species of the genus *Pergalumna*) has been observed before (Rockett & Woodring 1966; Muraoka & Ishibashi 1976; Oliveira *et al.* 2007; Norton & Behan-Pelletier 2009) and stable isotope signatures also indicate them to live as predators or scavengers (Schneider *et al.* 2004). Possibly, *Galumna* spp. mainly feed on other nematode species than the ones tested in our study. *S. magnus*, *C. femoralis* and *H. gibba* were not detected to feed on *Plectus* spp. indicating that they live on other diets than nematodes; indeed, based on natural variations in stable isotope ratios these species have been assumed to predominantly feed on litter and fungi (Schneider *et al.* 2004). However, in previous studies using molecular gut content analysis *S. magnus* has also been found to feed on nematodes in the litter layer of deciduous forests (Heidemann *et al.* 2011). As *S. magnus* was only found in moss patches in the present study the diet of this species may vary between microhabitats.

5. Conclusions

Overall, results of the study suggest that feeding of mites on nematodes is more common than previously assumed. By consuming bacterial feeding nematodes mites form an important link channelling carbon from bacteria to higher trophic levels. As many bacteria live on easily available carbon resources, such as root exudates and dissolved organic carbon leaching from litter, mites integrate rhizosphere and litter resources thereby contributing to the channelling of both above- and belowground resources to higher trophic levels of soil food webs. Differential feeding on nematodes by mite species suggests niche differentiation of mite species thereby contributing to the coexistence of the many mite species in the seemingly homogeneous soil habitat. Differences in the fraction of nematodes in the diet of mites of different microhabitats suggest generalistic feeding and high plasticity in prey capture. Notably, these conclusions not only apply to mites previously viewed as predators, such as mesostigmatid mites, but also to mites assumed to predominantly live as detritivores, such as oribatid mites. This highlights that mite-nematode interactions are more widespread than previously assumed and form a hitherto understudied link in soil food webs.

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Chapter 5

General Discussion

Results of this thesis represent major advances in the understanding of the soil animal food web which is more complex and trophically diverse than previously assumed. It provides promising tools to investigate soil animal food webs pointing to the role of bacterial-feeding nematodes as prey for microarthropods thereby channelling carbon from the rhizosphere to higher trophic levels.

The first part of this thesis, which used specific molecular markers for two entomopathogenic nematode species (*Phasmarhabditis hermaphrodita* and *Steinernema feltiae*; Read *et al.* 2006), showed that soil mites indeed consume nematodes and include dead and living prey in their diet (Chapter 2). Mite species, which previously were assumed to be decomposers, preyed on nematodes, although both feeding modes (predation and scavenging) seem to be common in microarthropods.

In the second part of this thesis newly designed molecular markers were used to investigate the role of free-living bacterial-feeding nematodes (*A. buetschlii, Panagrellus redivivus, Plectus minimus* and *Plectus velox*) as prey for microarthropods (Chapter 3). Nematode DNA was detectable in the gut of a predator up to 128 h; however, the detection time varied in each of the studied nematode-mite combination indicating that detection times vary individually for every predator-prey interaction. Although only few nematode taxa were analyzed, they were frequently detected as prey in a range of microarthropod species. This indicates that in the field nematodes form an important part of the diet of collembola and mite species including those previously thought to live as detritivores or fungivores.

The third part of this thesis showed that soil mites consume the investigated free- living soil nematodes to a different degree depending on their microhabitat indicating that the trophic niche of mites varies in space (Chapter 4). Overall, I conclude that trophic niche differentiation contributes to the coexistence of the large number of mite species in the seemingly homogeneous soil habitat.

Results of this thesis clearly show that several collembolan, mesostigmatid and oribatid mite species frequently consume nematodes in the field. Mesostigmatid mites have been assumed to feed on nematodes before (Karg 1993; Klarner *et al.* 2013) which was confirmed by results of this thesis indicating that especially *Uropoda cassidea* consumes nematodes frequently. Most microarthropods such as collembolans and oribatid mites were assumed to be 'classical decomposers' feeding mainly on litter, dead organic material or fungi (Behan-Pelletier 1999; Maraun & Scheu 2000). However, results of this thesis support earlier studies suggesting nematode consumption for e.g., *Folsomia candida*, *Hypochthonius rufulus* and *Nothrus silvestris* (Read *et al.* 2006; Schneider et al. 2004; Pollierer *et al.* 2009), whereas for some species, such as *Entomobrya muscorum*, *Nothrus palustris*, *Platynothrus peltifer* and

Steganacarus magnus, nematode consumption has never been observed or proposed before (Siepel 1990; Schneider et al. 2004; Chahartaghi *et al.* 2005;).

Whether feeding on nematodes is based on scavenging or predation remains unclear, although scavenging has been proposed to be frequent in microarthropods (Schneider et al. 2004; Chahartaghi *et al.* 2005). Predators have to attack, subdue and kill their prey whereas scavengers live on an easily available energy-rich resource that does not defend itself (Foltan *et al.* 2005). In contrast to scavenging, predation has a top-down effect since it contributes to population regulation of the species consumed. Therefore, from a food web perspective differentiating between predation and scavenging is essential. Results of this thesis showed that in the field dead and living prey is available as food for microarthropods, and frequent consumption of dead and living nematodes by mite species suggests that both feeding modes, predation and scavenging, are common in microarthropods. Therefore, soil microarthropods not only benefit from the high nutritional values of nematodes but likely also exert top-down forces on nematode populations. Unfortunately, molecular gut content analysis determines only the presence or absence of nematode DNA and can not discriminate between dead and living prey. However, the approach of adding model nematodes to the field uncovered that scavenging occurs in the field and needs more attention in the future.

Not only the feeding mode differs between microarthropod species, also nematode species were consumed differentially by the mites pointing to differences in the predator-prey interactions at the species level. This may be due to body size ratios, life stages, defense mechanisms, behavioural or physical resistance of the prey (Mulder *et al.* 2011). Results of this thesis indicate that the small *P. minimus* was avoided whereas the large *P. velox* was preferred by *P. septentrionalis* suggesting that body size ratios are likely to determine the prey choice of this mesostigmatid mite (Karg 1993). *P. minimus* was particularly important for deutonymphs of *U. cassidea* supporting the view that the size spectrum of prey differs between developmental stages in predators (Klarner *et al.* 2013). Results of this thesis also showed differences between the rate of consumption of *A. buetschlii* and *Plectus* spp. in the field. However, the body sizes of these nematodes overlapped suggesting that differential detection of the two target taxa in microarthropods was not due to differences in body size but rather due to differences in density of these nematode taxa at the study site indicating that mites opportunistically feed on nematodes.

Moreover, the model nematode *P. hermaphrodita* was more frequently consumed than *S. feltiae* and the latter was preferred as carrion over living individuals. This may be due to behavioural differences which are likely to affect encounter rates between prey and predators and the outcome of predator-prey interactions, e.g. *S. feltiae* follows a sit and wait strategy (Susurluk 2009) and *P. hermaphrodita* moves actively in search for hosts (MacMillan *et al.*

2009). Furthermore, *S. feltiae* contains the specific symbiotic bacterium *Xenorhabdus bovienii* (Kaya and Gaugler 1993) which is necessary to kill insect hosts and has been observed to be toxic to Acari (Bussaman *et al.* 2006). This defence against consumption likely continues to function in dead specimens – at least temporarily.

Results of this thesis indicate that the detection frequency of *Plectus* spp. as prey of mites in litter exceeds that in moss and grass patches indicating that microarthropods consume nematodes to a different degree depending on the respective microhabitat. For instance, *Galumna* spp. and *P. septentrionalis* are known to feed on nematodes (Rockett & Woodring 1966; Muraoka & Ishibashi 1976; Scheu & Falca 2000; Schneider *et al.* 2004; Oliveira *et al.* 2007; Norton & Behan-Pelletier 2009; Klarner *et al.* 2013). However, in litter detection frequency of *Plectus* spp. in *P. septentrionalis* was high, but the nematode was not detected in mite individuals from patches of moss. In contrast, *Galumna* spp. consumed *Plectus* spp. in moss but not in litter and grass patches. This indicates that nematodes are less important prey for *P. septentrionalis* in the respective habitats. *P. septentrionalis* possibly relies on other nematode species as prey suggesting that they can switch their trophic niche and adjust their prey spectrum to changes of environmental conditions and prey availability. Differential feeding on nematodes by putative decomposer mite species of different microhabitats suggests generalist feeding mode and high plasticity in their prey capture ability thereby contributing to the coexistence of the many mite species in the soil habitat. Microarthropods may have a major influence on nematode populations as they typically reach high densities, have wide diet range and since only few free-living nematode species were investigated but frequently detected as prey.

Overall, nematode-microarthropod interactions in soil need more consideration. Molecular gut content analyses using specific nematode markers are a promising method to study neglected predator-prey interactions at the species or genus level (Symondson 2002; Read *et al.* 2006; King *et al.* 2008) and has been used earlier to study a wide range of trophic interactions in the soil system, mainly that of macrofauna (Agusti *et al.* 2003; Admassu *et al.* 2006; Juen & Traugott 2007; Eitzinger & Traugott 2011). Although it is eligible to calibrate feeding events in the field, the results of this thesis indicate strong variation of detection rate and time of nematode DNA in mite predators. Together with earlier findings results of my thesis indicate that the prey detection frequency varies in every predator-prey combination (Zaidi *et al.* 1999; Hoogendorn & Heimpel 2001; Harwood *et al.* 2004; Sheppard *et al.* 2005; Hosseini *et al.* 2008; Traugott & Symondson 2008; von Berg *et al.* 2008a,b; Gagnon et al. 2011; Sint *et al.* 2011). In the case of microarthropods as predators of nematodes the feeding is difficult to prove. Therefore, the detection frequency additionally depends on the duration predators fed

on prey. Therefore, data on detection frequencies of prey in predators to evaluate feeding events in the field remain a goal for future studies (Greenstone *et al.* 2007).

Nevertheless, as demonstrated in this thesis nematode DNA is detectable up to 128 h in the gut of microarthropods. This long detection time is advantageous for prey screening in predators in the field (Sheppard & Harwood 2005). Therefore, the presented method in this thesis will contribute to identifying nematode predators, tracing the role of nematodes as prey and the better understanding of mechanisms influencing predator-prey interactions in soil.

A number of methodological challenges remain, e.g. the establishment of a multiplex PCR approach would be beneficial to save costs and time in screening experiments in future studies. As a further goal to evaluate feeding events of nematodes by microarthropods in the field more work is necessary to optimize prey exposure time for a wider range of predator-prey combinations and real-time quantitative PCR assay (qPCR) needs to be developed for quantifying the consumed prey DNA and track its decline during digestion. Further, more experimental work is needed for uncovering factors influencing predator-prey interactions in the field, such as body size of prey and predator, prey density, forest type, season, humidity and other biotic and abiotic factors. Therefore, the new designed molecular markers and the methodology adopted in this thesis opens promising perspectives to better understand the structure and functioning of the nematode-based food chain as major component of belowground food webs.

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List of Publications

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(Please note that for this publication I have used my maiden name Peschel)

Peschel K, Norton RA, Scheu S, Maraun M (2006) Do oribatid mites live in enemy-free space? Evidence from feeding experiments with the predatory mite *Pergamasus septentrionalis*. *Soil Biology & Biochemistry*, **38**, 2985–2989.

Submitted

Kerstin Heidemann, Annika Hennies, Johanna Schakowske, Lars Blumenberg, Liliane Ruess, Stefan Scheu, Mark Maraun. The role of free-living nematodes as prey for higher trophic levels of forest soil food webs. Submitted

Kerstin Heidemann, Liliane Ruess, Stefan Scheu, Mark Maraun. Consumption of nematodes by soil microarthropods varies between microhabitats of deciduous forests as indicated by molecular gut content analyses. Submitted

Thesis declarations

Declaration of the author's own contribution to manuscripts with multiple authors

Chapter 2 comprises a manuscript that have been published in a peer-reviewed journal; Chapter 3 and 4 comprise manuscripts that are currently submitted to peer reviewed journals. In all manuscripts I am the first author and I have collected and analyzed the data, written the manuscripts, developed the main ideas, created tables, figures and appendices and contributed significantly to the study design. Annika Hennies, Johanna Schakowske and Lars Blumenberg performed their Bachelor thesis as part of this project. They contributed to Chapter 2 by collecting and analyzing the data, creating tables and figures, and contributed significantly to the no-choice experiment with collembolans (Annika Hennies) and the evaluation of the detection time of *P. minimus* (Johanna Schakowske) and *P. velox* (Lars Blumenberg) after 48 h of nematode exposure. All persons contributing to the manuscripts have been named so. All co-authors contributed to finalising the manuscripts.

Plagiarism declaration

I declare that I have written this doctoral thesis independently. All persons contributing to the manuscripts have been named so. All sentences or passages quoted from other people's work have been specifically acknowledged by clear cross-referencing. I have not submitted this thesis in any form for another degree at any university or institution.

Kerstin Heidemann

Göttingen, February 2013