ZENTRUM
FÜR BIODIVERSITÄT UND NACHHALTIGE LANDNUTZUNG
SEKTION
BIODIVERSITÄT, ÖKOLOGIE UND NATURSCHUTZ

– CENTRE OF BIODIVERSITY AND SUSTAINABLE LAND USE –
SECTION: BIODIVERSITY, ECOLOGY AND NATURE CONSERVATION

Molecular Analysis of Centipede Predation

Dissertation zur Erlangung des Doktorgrades der
Mathematisch-Naturwissenschaftlichen Fakultäten der
Georg-August-Universität Göttingen

vorgelegt von

Mag. rer. nat.

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aus
Innsbruck

Göttingen, Juli 2013

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Tag der mündlichen Prüfung:	19. Juli 2013

Probieren geht über Studieren

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Summary

Soil food webs are an essential part of terrestrial ecosystem functioning and characterised by a high degree of cross linkage between the members of a highly diverse soil community. Centipedes are abundant predators in the litter and soil layers of temperate forests. They are assumed to be generalist predators, feeding on a wide range of prey such as collembola and earthworms. However, knowledge of their feeding ecology is scarce, as the opaque habitat, the high diversity of prey, and extra-oral digestion hamper analysis of their feeding behaviour. Molecular gut content analysis, however, allows their trophic interactions to be studied even under these unfavourable conditions and consequently allows assessment of prey choice and of the strength of the predator-prey interactions. I therefore used group and species-specific PCR assays to track the DNA of abundant prey in guts of lithobiid and geophilomorph centipedes to illustrate feeding interactions. Based on these results I examined the effects of a variety of biological and environmental factors on centipede prey choice and the strength of predator-prey interaction. In addition, I conducted experiments to study the effect of two factors on prey DNA detection success which could lead to over-or underestimation of feeding link strength.

In **Chapter 2** I report on the design and optimization of group- and species- specific PCR assays to screen soil predators for twelve abundant prey including extra- and intraguild prey. Cross-reactivity tests against a wide spectrum of non-target animals and prey DNA dilution tests confirmed high specificity and sensitivity of assays. An initial screen of the gut contents of 50 lithobiid individuals revealed that centipedes feed on many prey species though showing preferences for collembolan and dipteran prey.

Prey DNA detection success can be affected by a variety of factors some of which lead to over- or underestimation of the strength of predator-prey interactions. In **Chapter 3** I tested whether the success with which prey can be detected in a predator's gut is positively correlated with decreasing predator body mass and with prey quality. I used singleplex PCR and quantitative real-time PCR to analyse the time-dependent reduction in DNA detection success for three qualitatively different prey (Collembola, Diptera, Lumbricida) which were fed to lithobiid predators. Likewise I analysed singleplex detection rates of collembolan prey in three centipede size classes. Contrary to my expectations, prey DNA detection success is not affected by predator body size nor prey quality but by PCR assay sensitivity and prey DNA copy number. This suggests that both DNA concentration and assay sensitivity need to

be considered when assessing prey quality effects on prey DNA detection success.

Land-use change can affect feeding ecologies of soil predators by changing resource availability and habitat structure. In **Chapter 4** I therefore studied prey choice of staphylinid and centipede predators along a land-use gradient in forest ecosystems of two regions. Predators were screened for collembolan, dipteran and lumbricid prey and the results were tested against a set of environmental and biological parameters. In fact, forest management does not affect prey choice but the depth of the litter layer and soil pH are important factors. Likewise, trophic interactions varied with prey abundance. In all cases I observed that the direction of effect depends on predator body mass. Large predators feed more in low-structured habitats and at high prey densities, while the opposite is true for small predators.

Functional response models are used to predict the strength of trophic interactions, which can be affected by predator-prey body mass ratios. In **Chapter 5** I analysed the feeding behaviour of lithobiid and geophilomorph centipedes and compared the results with the body-size-dependent functional response for eight different prey groups. I showed that calculated feeding rates of most prey are significantly correlated with prey DNA detection rates for lithobiid predators. Intraguild prey and lumbricids however correlated negatively, indicating that prey-specific traits must be taken into account to fully explain feeding interactions in soil food webs.

Overall, I show that molecular gut content analysis resolves trophic interactions between centipedes and their prey. These interactions are defined by a variety of factors of which body size is the most important.

Chapter 1 General introduction

Belowground systems

Soil is an essential part of most terrestrial ecosystems. The highly diverse structure of the soil environment provides a stable microclimate and nutrient supply and is home to a large variety of organisms that coexist with a high degree of possible interactions (Brusaard *et al.* 1997, Wardle 2006). These soil organisms play a crucial role in many ecological processes, such as nutrient turnover, and can consequently affect the productivity and stability of communities above and below ground (Bardgett & Wardle 2010). Despite this enormous importance for ecosystem functioning and human well being, there is still a lack of knowledge of the biology and function of this system.

Soil animals play key roles as decomposers, bioturbators, predators and root feeders. The strength and direction of their impact depends on a wide range of factors such as habitat structure, community composition and species abundance (Lavelle *et al.* 2006, Coleman 2008). This has particular consequences for disturbed areas such as managed forest sites, where change of tree diversity or age structure can create unstable or unfavourable conditions, eventually resulting in a loss of biodiversity (Niemelä 1997). The resilience and stability of these systems depend on the cross-linking of the many species involved (Bardgett & Cook 1998, Lavelle *et al.* 2006).

Studying these links can reveal the processes driving ecosystem function and aid in explaining the evolution, structure and diversity of soil systems. Interactions in the soil system include different processes such as the creation of habitat by ecosystem engineers as do earthworms (Lavelle *et al.* 1997), phoretic relationships between mites and centipedes (Bloszyk *et al.* 2006), and seed dispersal by slugs (Türke *et al.* 2010).

Feeding interactions are possibly the single most important relationship connecting soil organisms. Soil animals are, indeed, highly connected, even in simple soil environments (Pimm 1982). Studying food webs allows the tracking of energy and nutrient fluxes and can also highlight many other interactions that are directly or indirectly associated with consumption. For example, the strength of intraguild predation between two soil predators is responsible for top-down pressure on mutual prey but has also important implications for predator diversity and food web stability (Schneider *et al.* 2012). Furthermore, the analysis of trophic interactions may explain specific patterns and traits, such as high reproduction rates or

evolution of predator defence mechanisms.

Many soil animals feed on more than one trophic level, complicating the traditional classification of feeding preferences into phytophagous, saprophagous, and zoophagous. The high variety of possible prey suggests that soil predators are rather generalist and feed on a wide prey spectrum, including intraguild prey (Scheu & Setälä 2002).

Analysis of trophic interactions in soil

Until recently soil food webs could not be sufficiently resolved. This was because traditional methods (*e.g.* microscopic gut dissection) are not sensitive enough to disentangle species-specific feeding interactions. The opaque character of soil, as well as the small size of soil animals, does not allow direct observation of feeding events without disturbing the system (King *et al.* 2008). Laboratory feeding trials however do permit monitoring of trophic interactions, but due to their simplified set-up are prone to produce results that do not represent natural processes.

Over the most recent twenty years new techniques have been developed and optimised which have helped to push forward food web analysis. The flow of nitrogen and carbon in soil systems can be tracked using stable isotope analysis of ^{15}N and ^{13}C, revealing compartmentalisation of soil food webs in the bacterial and fungal pathways (Pollierer *et al.* 2009). As ^{15}N becomes enriched along a food chain, animals can be assigned to distinct trophic levels on the basis of ^{15}N thereby distinguishing decomposers from root feeders, microbivores and predators. The analysis of neutral fatty acids (NLFAs) allows even more detailed study of feeding history, as it enables tracking of bacteria, fungi and plant prey in the consumer's body (Ruess *et al.* 2004). Both methods however, despite being advantageous in displaying long-term feeding, are not specific enough to identify species-specific trophic links and in particular animal-animal interactions.

Molecular gut content analysis (MGCA), *i.e.* tracking prey molecules in a predator's gut, helps to fill this gap. In contrast to conventional microscopic gut dissection or faecal analysis, where ingested prey is identified by their hard remains, MGCA can even be used to study the feeding of predators with extra-oral digestion and of small consumers such as mites and collembola. Following the pioneering approaches using protein electrophoresis (Traugott 2003), using polyclonal and monoclonal antibodies (Sunderland & Sutton 1980; Harwood *et*

al. 2004) to detect prey proteins, the development of specific polymerase chain reaction (PCR) assays targeting prey DNA significantly increased the specificity of results. Species or group specific PCR assays amplify even small remnants of prey DNA in the predator gut (or in faeces and regurgitates) that are then visualized and identified using gel or capillary electrophoresis. Due to the high sensitivity of the assays, it is possible to detect prey DNA in a predator's gut up to several days after the predator has fed, therefore identifying trophic interactions in unprecedented detail. To analyse trophic interactions of generalist predators, which feed on a wide spectrum of prey organism, multiplex PCR assays have become an important tool. In multiplex PCR assays, a set of prey-specific primers allow simultaneous screening for up to 12 prey organisms, providing a cost and time-effective method (Harper *et al.* 2005).

Design and optimisation of singleplex and particularly multiplex PCR assays requires careful preparation. Primers should preferably target multi-copy genes, such as mitochondrial cytochrome *c* oxidase subunit I (COI), to enhance amplification success of semidigested prey. Therefore, the long propagated use of PCR fragments < 300 bp may be of minor importance. Sensitivity tests using DNA dilution series determine the least amount of prey DNA to start DNA amplification and should be used to standardize the amplification success of different PCR assays (Sint *et al.* 2011, 2012). In multiplex PCRs the product size has to be chosen well as it should allow different prey DNA fragments to be distinguished by their length. Additionally, cross reactivity tests of organisms that can serve as alternative prey or gut parasites (e.g. Nematomorpha) are necessary to confirm the specificity of a primer.

In contrast to stable isotope and fatty acid analyses, MGCA only provides snapshots of feeding activity. While this allows very fine scale resolution over time of feeding activity, many predators will contain no amplifiable prey DNA, necessitating screening large numbers of consumers. Furthermore, results only yield data on prey DNA presence or absence, preventing any assessment of the amount of prey ingested. Hence, MGCA cannot assess predation impact so screening results should be interpreted carefully. For example, different sensitivity of PCR assays or prey type can significantly affect the probability of DNA amplification. It is therefore highly recommended to compare MGCA data with results from feeding experiments to help to assess predation impact.

The functional response is the feeding rate of a predator as a function of prey density (Holling 1959, Rall *et al.* 2012) and is an important determinant of trophic interaction strength (Berlow *et al.* 2004). Conducting extensive feeding trials in which predators are confronted with

different prey abundances, a predator's intake rate can be decribed based on the instantaneous rate of successful attacks, handling time and predator-prey body mass ratios (Vucic-Pestic *et al.* 2010, Kalinkat *et al.* 2013). The results can then be used to identify key predators and are also able to predict diet switching, therefore explaining the feeding ecologies of generalist predators (Kalinkat *et al*. 2011). Functional response models are particularly popular among community ecologists who use the feeding rates of a wide spectrum of consumers to assess the stability of food webs and whole animal communities. Feeding interactions in terrestrial food webs are most usually described by a Holling type II functional response, indicating that predators are limited in their feeding due to limited gut size. However, habitat structure, switching between different prey groups, and high predator-prey body size ratio can be responsible for a shift from a type II to a type III functional response, which possibly explains low feeding rates on small invertebrates such as oribatid mites in the soil system (Vucic-Pestic *et al.* 2010, Kalinkat *et al*. 2013). In particular, predator body size might account for a large part of prey choice and help explain the stability of food webs as well as species richness of prey and predator communities in the soil system through specific size-dependant prey choice (Otto *et al*. 2007, Schneider et al. 2012).

Centipedes and their prey

Centipedes are among the most prominent invertebrate predators in the litter and soil of temperate forests. They reach densities up to 650 individuals per m² with body masses up to 50 mg and consequently are supposed to have an important topdown effect on soil invertebrates such as collembola (Lewis 1981, Jabin *et al.* 2007). In Central Europe, two groups of centipedes are most abundant: the mainly soil-living Geophilomorpha and the litter-dwelling Lithobiomorpha. In my study sites the genus *Lithobius* is represented by nine species that predominantly differ in body size and preferred habitat. These are *L. aulacopus* Latzel 1880, *L. crassipes* L. Koch, 1862, *L. curtipes* C.L. Koch, 1847, *L. dentatus* C.L. Koch, 1844, *L. melanops* Newport, 1845, *L. muticus* C.L. Koch, 1847, *L. mutabilis* L. Koch, 1862, *L. nodulipes* Latzel, 1880 and *L. piceus* L. Koch, 1862. The larger species, such as *L. dentatus, L. muticus and L. mutabilis* are more resistant against desiccation and can be found in the upper litter layers, while the smaller *L. crassipes* and *L. curtipes* seek shelter in the interface between the soil and litter layer but can also be found under dead wood (Fründ 1987, Jabin *et al.* 2007). The geophilomorphs in my study, *Geophilus* spp.,

Schendyla nemorensis (C.L.Koch 1837) and *Strigamia acuminata* (Leach 1815) are characterised by their worm-like body which facilitates movement in densely packed soil and litter layers. The habitat also defines the preferred prey of these generalist predators. Geophilomorphs prey primarily on earthworms, and a group will sometimes attack a single worm, while lithobiids prefer collembola, employing a sit-and-wait strategy (Poser 1988, Rosenberg 2009). However, feeding analyses indicated that both groups exhibit a broad range of possible prey including plant material and intraguild prey (Lewis 1981). Centipedes use their maxillipedes (poison claws) to kill and presumably predigest their prey, allowing them to kill prey larger than themselves. However, feeding experiments, including functional response trials, showed that lithobiids preferably feed on prey sizes which are two orders of magnitudes smaller than themselves thereby exhibiting specific predator-prey size ratios (Rall *et al.* 2011). Centipedes show gradual development, undergoing four larval and four subadult stages before becoming a sexually mature adult. Despite their smaller size, subadult specimens utilise the same feeding strategy as adults, which makes centipedes an ideal model to study body-size related prey choice.

The study site

This study forms part of the Biodiversity Exploratories project (DFG priority program 1374), an integrated long term project to study the effect of land-use change on biodiversity and ecosystem processes (Fischer et al. 2010). The study sites are located in three regions across Germany: The National park Hainich and its surroundings in Thuringia (exploratory Hainich), the biosphere reserve Schorfheide-Chorin in Brandenburg (exploratory Schorfheide) and the biosphere reserve Schwäbische Alb in Baden-Württemberg (exploratory Schwäbische Alb).

Field studies were conducted in forest plots of 100 × 100 m in the Hainich and Schorfheide that represent four differently managed forest types: managed coniferous forests, 30 year old managed beech forests, 70 year old managed beech forests and unmanaged natural beech forests. The coniferous forests consist of Norway spruce (*Picea abies* H. Karst.) (Hainich) and Scots pine (*Pinus silvestris* L.) (Schorfheide), while beech forests are dominated by (*Fagus sylvatica* L.), with ash (*Fraxinus excelsior* L.), sycamore (*Acer pseudoplatanoides* L.), hornbeam (*Carpinus betulus* L.), and lime (*Tilia cordata* Mill., *T. platyphyllos* Scop.).

Differences in the bedrock of the Hainich and Schorfheide may account for most disparities between the two exploratories. Despite similar topsoils in both exploratories (cambisols and luvisols), pH values are lower in the sandy Schorfheide (3.30-6.65) while the limestone region

of Hainich is characterized by higher values (pH 5.64 -7.23; Klarner *et al*. in prep). In Hainich leaf litter densities range from 0.15 (young beech) to 0.37 g/cm^2 (coniferous forests), while in Schorfheide leaf litter ranges from 0.18 (coniferous forests) to 0.59 g/cm^2 (young beech). The study sites therefore provide an excellent opportunity to study the effect of decomposition, which affects centipede predation by providing habitat structure as well as resources for their prey.

Objectives of the thesis and chapter outline

This thesis focuses on the trophic interactions between centipede predators and their prey and the factors which drive prey choice. Chapter 2 describes the design and optimisation of species- and group-specific PCR assays to identify the DNA of twelve prey species in the gut of centipede predators. In feeding experiments, the effect of body size and prey quality on prey DNA detection success was studied (Chapter 3). In Chapter 4 and 5 I analyse the environmental and biological factors affecting centipede prey choice and compare the predictability of functional response models with data from molecular gut content analysis (Chapter 5).

In the following the main hypotheses are outlined and an overview about every chapter is given.

Main hypotheses

(1) Predator body size and prey quality influence prey DNA detection intervals in a predator's gut, thus affecting DNA detection success (**Chapter 3**).

(2) Prey DNA detection rates in centipedes decrease with increasing habitat structure by providing refuge for prey; forest type is of negligible importance because forest type has no influence on the factors affecting feeding ecology in centipedes (**Chapter 4**).

(3) Prey DNA detection rates are positively correlated with prey abundance as encounter rates are then higher (**Chapter 4 and 5**).

(4) As centipede species of same size show similar feeding behaviour, centipede feeding is driven by allometric rather than taxonomic constraints (**Chapter 5**).

In Chapter 2 we describe the design and optimisation of singleplex PCR assays targeting twelve extra-and intraguild prey of soil invertebrate predators. Based on 18S rDNA sequences we created group specific PCR primers for amplification of dipterans, gamasid and oribatid mites, staphylinid beetles, spiders and woodlice. Additionally we developed species-specific markers targeting the cytochrome *c* oxidase subunit I (COI) gene of abundant collembolan species *Ceratophysella denticulata* (Bagnall, 1941), *Folsomia quadrioculata* (Tullberg, 1871), *Lepidocyrtus lanuginosus* (Gmelin, 1788), *Pogonognathellus longicornis* (Müller, 1776), and *Protaphorura armata* (Tullberg, 1869) as well as *Lithobius* spp. Cross reactivity testing against up to 119 non-target organism and sensitivity tests revealed high sensitivity and specificity of the new assays. A first test of gut content of lithobiid predators displayed generalist feeding behaviour, amplifying five out of eleven target organisms. Predators fed primarily on collembolan and dipteran prey, while levels of intraguild predation were low.

Chapter 3 investigates the impact of predator body size and prey quality on prey DNA detection success, as those factors might lead to over- or underestimation of feeding rates. We hypothesize that prey DNA detection intervals will be significantly shorter in large predators and prey of high quality will be digested faster. Using singleplex PCR and quantitative PCR (qPCR) we studied time-dependent decreases in DNA detection of three qualitatively different prey (collembola, diptera, earthworms). Likewise we analysed singleplex detection rates of collembolan prey in three centipede size classes. Time trials lasted for as long as 168 h, including 13 intervals to illustrate DNA breakdown. Results showed that body size and prey quality do not significantly affect PCR success. However, qPCR revealed that PCR assay sensitivity and prey DNA copy number are important factors and should be considered more closely when interpreting field-derived MGCA results.

In Chapter 4 we study feeding of stapylinid beetles and two lithobiid predators, *L. crassipes* and *L. mutabilis* on three abundant prey along a land-use gradient in forest ecosystems. Predators were sampled in four differently managed sites in the Hainich and Schorfheide exploratories and screened in single and multiplex PCRs for collembolan, dipteran and lumbricid prey. The results indicate that forest management does not affect prey choice, but point to predator body mass, prey density, litter mass and soil pH as driving factors in prey choice. Interestingly, the two lithobiid species are affected differently by high litter mass. The smaller *L. crassipes* benefited from high litter as it then had a larger effective foraging area,

while predation rates of the larger *L. mutabilis* decreased as it was less able to hunt in a thick litter layer. In contrast, high prey densities increased predation by large centipedes, but reduced it for small centipedes due to prey defence mechanisms. The results point to predator body size as the driving factor for lithobiid trophic interactions, while taxonomic constraints may be negligible.

Chapter 5 compares functional response models with molecular gut content analysis to predict prey choice and predation impact of lithobiid and geophilomorph centipedes in natural forest systems. Screening results of 597 centipede predators, which were tested for 14 abundant prey groups, indicated that predator body size and prey identity were the two factors driving prey choice while prey abundance had no effect. Likewise, feeding rates were calculated using a functional response model and these rates were highly significantly correlated with MGCA results. Predator-prey body size ratios therefore account for a large part of centipede prey choice but species-specific traits, such as defence mechanisms must be taken into account to fully explain feeding interactions in soil food webs.

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Chapter 2

Unveiling soil food web links: New PCR assays for detection of prey DNA in the gut of soil arthropod predators

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Published in

Eitzinger, B., Micic, A., Körner, M., Traugott, M. & Scheu, S. (2013). Unveiling soil food web links: New PCR assays for detection of prey DNA in the gut of soil arthropod predators. *Soil Biology and Biochemistry*, 57, 943–945. http://dx.doi.org/10.1016/j.soilbio.2012.09.001

Soil Biology & Biochemistry 57 (2013) 943–945

Contents lists available at SciVerse ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Short communication

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ARTICLE INFO

Article history:
Received 10 May 2012
Received in revised form
23 July 2012
Available online 21 September 2012

Keywords:
Molecular gut content analysis
Predator–prey interactions
Araneae
Chilopoda
Collembola
Diptera
Gamasina
Oribatida
Isopoda
Staphylinidae
18S rDNA
COI

ABSTRACT

Molecular gut content analysis provides a highly specific and sensitive tool to examine the diet of soil invertebrates. Here, we present new polymerase chain reaction (PCR) assays for the detection of twelve prey taxa common in Central European forest soils. The assays target five species of collembolans as well as dipterans, gamasid and oribatid mites, lithobiid centipedes, spiders, staphylinid beetles and woodlice at the group level, amplifying 123–299 bp long DNA fragments. Cross-reactivity tests against 119 soil invertebrate taxa confirm their specificity. These new PCR assays were found to be highly sensitive, revealing the consumption of five different prey taxa in field-collected centipedes. Thus they provide a ready-to-use approach for unravelling trophic interactions among soil arthropods.

Molecular techniques have become increasingly popular to study predator–prey interactions under natural conditions (King et al., 2008; Pompanon et al., 2012) including those below-ground (e.g. Juen and Traugott, 2007; Heidemann et al., 2011). This is because they allow tracking feeding interactions which are inaccessible with conventional methodology (Symondson, 2002). Using polymerase chain reaction (PCR) assays it is possible to detect DNA of animal prey (including carrion; Juen and Traugott, 2005; Foltan et al., 2005) and of plant food sources (Staudacher et al., 2011) in a consumer's gut. This offers a new means to study the trophic linkages among soil-dwelling animals as well as between plants and root feeding animals, addressing an important compartment of the soil food web.

Most predators in soil are supposed to be generalists (Scheu and Setälä, 2002). Therefore, we intended to address trophic links on higher taxonomic levels (i.e., family and order rather than species level), to enable for a broad characterization of the predators' dietary spectrum.

The goal was to establish ready-to-use PCR assays which allow targeting a variety of prey groups which regularly might fall within the prey range of soil-dwelling generalist predators. Twelve new assays were designed and tested on field-collected specimens of *Lithobius* spp. Leach, 1814. Within these assays we target five species of collembolans using species-specific primers, whereas dipterans, gamasid and oribatid mites, lithobiid centipedes, staphylinid beetles, spiders, and woodlice are targeted by group-specific primers.

Invertebrates were collected in summers of 2008–2010 in beech forests of the national park Hainich (Thuringia, Germany) by sieving of litter and heat extraction of soil samples. To avoid amplification of ingested food DNA, all specimens were starved for 7–10 days before freeze-killing them. After identification to species level, total DNA was extracted using the blood & tissue kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. In dipterans, gamasid mites, spiders and staphylinid beetles the nuclear 18S rDNA gene was sequenced; in collembolans part of the cytochrome C oxidase subunit I gene (COI) was sequenced (for DNA sequencing protocols see Supplementary material S1). All

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http://dx.doi.org/10.1016/j.soilbio.2012.09.001

sequences were corrected manually and checked for similarity with sequences from GenBank using the BLAST algorithm (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi). The new sequences were deposited in GenBank (JQ801570–JQ801608).

Based on these sequences and additional 18S rDNA sequences from GenBank (Table S1; Supplementary material), PCR primers, including ones with degenerated bases, were designed using PrimerPremier 5 (PREMIER Biosoft International, Palo Alto, CA, USA) following the guidelines of King et al. (2008).

The optimal annealing temperatures of each primer pair was determined by gradient PCR whereas the specificity was evaluated by cross-reactivity testing using ten individuals of each target taxon and up to 119 non-target taxa. The latter represent all major invertebrate groups at the study site (Table S2; Supplementary material). To test sensitivity of the PCR assays we employed a dilution series of DNA extracts: the DNA concentration of each target taxon (two individuals each) was measured using a Qubit 2.0 Fluorometer (Invitrogen, Carlsbad, CA, USA), adjusted to 200 pg μL^{-1}, two-fold serially diluted and then mixed with DNA of *Lithobius mutabilis* L. Koch, 1862 (200 pg μL^{-1}; for testing primers LIT S13/LIT A8, DNA of *Strigamia acuminata* (Leach, 1815) was used). This resulted in final prey DNA concentrations of 30,000, 15,000, 7500, 3750, 1875, 937.5, 468.75, 234.38, 117.19, 58.59, 29.29, 14.65, 7.32, 3.66, and 1.83 fg target taxa DNA per µL PCR and predator-to-prey DNA ratios of 1:1 to 20,000:1. Each 10 µL PCR consisted of 5 µL SuperHot Mastermix (2×), 1.25 mM $MgCl_2$ (both Geneaxxon, Ulm, Germany), 0.5 µL bovine serum albumin (3%; Roth, Karlsruhe, Germany), 0.5 µM of each primer and 3 µL of DNA extract. Thermocycling included 95 °C for 10 min, 35 cycles of 95 °C for 30 s, the primer-specific annealing temperature (see Table 1) for 30 s, 72 °C for 45 s, and a final step of 72 °C for 3 min. PCR products were separated using the capillary electrophoresis system QIAxcel (Qiagen, Hilden, Germany); fragments of the expected size and a relative fluorescent value of or above 0.1 RFU were scored as positive.

To test the new PCR assays on field-caught lithobiid predators, 50 *Lithobius* spp., collected in November 2008 at the beech forest sites mentioned above, were subjected to a CTAB-based DNA extraction protocol (Juen and Traugott, 2005). DNA extracts were purified using the Geneclean Turbo Kit (MP Biomedicals, Solon, OH, USA) yielding 150 µL of final DNA extract. One blank sample was included per 24 extracts to check for DNA carry-over contamination (none was found testing them with general COI primer (Folmer et al., 1994)).

We established specific COI primers for *Lithobius* spp. and the springtail species *Ceratophysella denticulata* (Bagnall, 1941), *Folsomia quadrioculata* (Tullberg, 1871), *Lepidocyrtus lanuginosus* (Gmelin, 1788), *Pogonognathellus longicornis* (Müller, 1776) and *Protaphorura armata* (Tullberg, 1869) as well as group-specific primers targeting the 18S rDNA gene of dipterans, gamasid and oribatid mites, spiders, staphylinid beetles and woodlice. The PCR assays amplified DNA fragments of the expected length in all targeted taxa. Only the woodlice primers ISO S6/ISO A3 showed a species-specific variation in amplicon size: *Trichoniscus pusillus* Brandt, 1833 was 123 bp, *Armadillidium vulgare* (Latreille, 1804) and *Ligidium hypnorum* (Cuvier, 1792) were 152 bp, *Oniscus asellus* Linnaeus, 1758 was 159 bp, *Philoscia muscorum* (Scopoli, 1763) was 160 bp, and *Porcellio scaber* Latreille, 1804 was 192 bp. The assays were highly specific as they exclusively amplified DNA of the target taxa. The only exception was the assay which targeted *P. armata,* which also amplified DNA of *Supraphorura furcifera* (Borner, 1901), another onychiurid springtail. Assay sensitivity was high across all twelve PCR systems: successful amplification ranged between 1875 and 1.83 fg target DNA per μL^{-1} PCR. Primers containing degenerated bases, however, were generally less sensitive (Table 1). Assay sensitivity was not adversely affected in the presence of excess predator DNA.

Fifty field-collected *Lithobius* spp. were tested for prey DNA using the newly established PCR assays. Five out of the eleven targeted prey taxa could be detected in 22 centipedes and 28 individuals had no amplifiable prey DNA in their guts. Most specimens (40%) had consumed *L. lanuginosus* followed by dipterans (16%), *F. quadrioculata* (6%), spiders (4%) and gamasid mites (2%). Simultaneous detection of two prey taxa in one predator was observed in 10 cases.

The present set of PCR assays allow testing for DNA of a wide range of possible prey of soil arthropod predators at a high level of

Table 1
Targeted taxa and genes, primer names and sequences, PCR product size, optimal annealing temperature (T_a), and PCR amplification threshold for the detection of common prey taxa within the gut content of soil-dwelling invertebrate predators. All primer pairs are used in singleplex PCR assays.

Taxon	Gene	Name	Sequence 5′–3′	Size (bp)	T_a (°C)	Detection threshold (fg μl^{-1} PCR)
Ceratophysella denticulata (Bagnall, 1941)	COI	CERDEN S5	ACTTCTTCCCCCCTCCTTAACCCTA	227	68	7.32
		CERDEN A3	CCCAGGATATTCCGGGGGC			
Folsomia quadrioculata (Tullberg, 1871)	COI	FOLQUA S4	CTGAACCGTTTATCCACCTCTC	169	62	29.29
		FOLQUA A1	AGTTCGGTCTCAAGTTATACCTACTGTG			
Lepidocyrtus lanuginosus (Gmelin, 1788)	COI	LEPLAN S3	CGATATAGCCTTTCCTCGTATAAAC	250	62	117.19
		LEPLAN A1	GGTTCGTATGTTAATGATAGTTGTG			
Pogonognathellus longicornis (Müller, 1776)	COI	POGLON S4	GATCAAATTTATAACGTTTTAGTAACC	202	62	7.32
		POGLON A4	CTAAACCTCCTGACAAGAGAAGC			
Protaphorura armata (Tullberg, 1869)[a]	COI	PROARM S3	GTAGAAAGAGGTGCAGGAACTGGC	268	68	3.66
		PROARM A3	TAATGGCTCCAGCAAGAACAGGTAAG			
Araneae group	18S	ARA S5	TAACRATACGGGACTCTTTYGAGA	255	68	468.75
		ARA A5	AGACAACCGGTGAAGATCATC			
Diptera group	18S	DIP S16	CACTTGCTTCTTAAATrGACAAATT	198	60	1.83
		DIP A17	TTyATGTGAACAGTTTCAGTyCA			
Gamasina group	18S	GAM S7	TTGGGGGCATTCGTATTGTT	230	63	29.29
		GAM A8	ATAACCCTACTTwGGTTTCCCGT			
Isopoda group	18S	ISO S6	GCwTTTrTTAGACCAAAAACCG	123–192	60	117.19
		ISO A3	CAGACACTyGrArGATACGG			
Lithobius Leach, 1814, group	COI	LIT S13	TGTTCwGCvGCwGTwGAAAG	293	54	1875.00
		LIT A8	GTdArkArTATdGTAATTGCTCC			
Oribatida group	18S	ORI S14	GCGCGCTACACTGAAGTG	299	68	29.29
		ORI A16	TCCTCTAAATGWTCAAGKTTGGG			
Staphylinidae group	18S	STA S6	TGCGGTTAAAAAGCTCGTAGTC	152	65	1.83
		STA A3	TCAATrAAGAGCACCGsGAT			

[a] PROARM S3/PROARM A3 are specific to onychiurid collembolans *P. armata* and *Supraphorura furcifera* (Borner, 1901).

specificity and sensitivity. The current screening results on centipede predators suggest high consumption rates of decomposer prey, particularly collembolans while intra-guild prey may only be accepted occasionally. This fits to findings on the diet of other soil-dwelling generalist predators such as predatory beetle larvae (Eitzinger and Traugott, 2011). Still, a larger set of individuals would need to be tested to better characterize the lithobiids' feeding preferences. Note, however, that gut content analysis cannot discriminate between active predation, secondary predation and scavenging (King et al., 2008), necessitating additional feeding experiments to clarify centipede feeding strategies. Moreover, prey DNA digestion rates might differ between prey species (e.g., Greenstone et al., 2010) and depend on other factors such as meal size, physiological status of the predators or temperature (von Berg et al., 2008) which needs to be considered when interpreting the field-derived data. The new PCR assays complement already published assays targeting other important prey groups of soil-dwelling generalist predators (e.g. Harper et al., 2005; Kuusk and Agusti, 2007; King et al., 2011), which allows shedding light on complex animal–animal feeding interactions in soil food webs. The COI and 18S rDNA sequences generated in this study will also help extending DNA-libraries of soil organism to study the diversity of life in below-ground systems.

Acknowledgements

We thank Irene Schatz, Klaus Hövemeyer, Nicole Scheunemann and Kerstin Heidemann for species identification and Roswitha Ehnes, Olivera Vucic-Pestic and Gregor Kalinkat for help with field work. Funding by the DFG Priority Program 1374 "Infrastructure – Biodiversity Exploratories" (SCHE 376/22-2) is gratefully acknowledged. Field work permits were given by state environmental offices according to § 72 BbgNatSchG.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2012.09.001.

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Chapter 3

Effects of prey quality and predator body size on prey DNA detection success in a centipede predator

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Abstract

Predator body size and prey quality are important factors driving prey choice and consumption rates. Both factors might affect prey detection success in PCR-based gut content analysis, potentially resulting in over- or underestimation of feeding rates. Experimental evidence, however, is scarce. We examined how body size and prey quality affect prey DNA detection success in centipede predators. Due to metabolic rates increasing with body size, we hypothesized that prey DNA detection intervals will be significantly shorter in large predators than in smaller ones. Moreover, we hypothesized that prey detection intervals of high-quality prey will be shorter than in low-quality prey due to faster assimilation. Small, medium and large individuals of *Lithobius* spp., abundant generalist predators in forest litter layers, were fed with Collembola and allowed to digest their prey for up to 168 h post-feeding. Additionally, medium-sized lithobiids were fed with either Diptera or Lumbricidae prey. No significant differences in 50% prey DNA detection success time intervals for a 272 bp prey DNA fragment were found between the predator size groups, indicating that predator body size does not affect prey DNA detection success. Post-feeding detection intervals were significantly shorter in Lumbricidae and Diptera compared to Collembola prey, apparently supporting the second hypothesis. However, sensitivity of diagnostic PCR differed between prey types and quantitative PCR revealed that concentration of targeted DNA varied significantly between prey types. This suggests that both DNA concentration and assay sensitivity need to be considered when assessing prey quality effects on prey DNA detection success.

Keywords: Predator-Prey, qPCR, gut content, feeding experiment, Collembola, Diptera

Introduction

DNA-based gut content analysis has become a widely used tool to unravel trophic interactions in the field (King *et al.* 2008; Pompanon *et al.* 2012). This applies in particular to soil food webs where direct observation is hindered by the minute size of the animals, a wide spectrum of possible prey which often provide no microscopically discernible remains for gut content analysis and the opaque characteristics of the habitat (Juen & Traugott 2007; Weber & Lundgren 2011; Heidemann *et al.* 2011). Analysing prey DNA in the gut of soil animals *post mortem* allows studying the feeding history under field conditions, omitting effects of disturbances or limitations of laboratory experiments.

When analysing data and interpreting results from gut content analyses, methodological (e.g., sensitivity and specificity of PCR assays), environmental (e.g., ambient temperature) and biological/physiological factors (e.g., feeding mode, body size) need to be considered. While there are several studies addressing the influence of these factors (Greenstone *et al.* 2007; Hosseini *et al.* 2008; Sint *et al.* 2011), we lack knowledge how body size within a predator species affects prey DNA detection success.

Body size of animals has major implications for biological processes including those associated with feeding and metabolism (Peters 1983; Cohen *et al.* 1993; Otto *et al.* 2007). Predators are usually larger than their prey and large predators are able to feed on a wider range of prey sizes than small ones, exploiting possible prey communities more efficiently (Cohen *et al.* 1993; Brose *et al.* 2006). Throughout the animal kingdom metabolic rate scales to the ¾ power of animal body mass (Kleiber & Rogers 1961). The increase in metabolism with body size also holds true for digestive processes, suggesting that large individuals digest their food faster than small ones. Thus larger predators are able to consume more prey per unite time than small individuals. This, however, has implications for molecular gut content analysis, where binary data indicate the presence or absence of prey DNA but do not reflect the amount of ingested prey. Feeding experiments with predator taxa of varying body masses shed some light on body-size induced variation in prey DNA detection success (Greenstone *et al.* 2007; Lundgren & Weber 2010; Waldner *et al.* 2013), however, the effect of body size cannot be separated from the impact of taxon-specific characteristics, such as feeding mode and the efficiency of the alimentary canal.

Prey identity is another factor potentially influencing prey DNA detection in predators. Generalist predators select prey depending on factors such as body size, abundance, palatability or the nutritional requirements of predators (Eitzinger & Traugott 2011; Kalinkat *et al.* 2011; Schmidt *et al.* 2012). The quality of prey tissue is likely to also affect prey DNA amplification success and consequently the molecular assessment of consumption rates. Prey of high quality, indicated e.g., by a high protein and low chitin content, is assimilated faster than low quality food sources (Jaeger & Barnard 1981; Mitra & Flynn 2007). This results in shorter gut passage times of high quality food, eventually shortening post-feeding prey DNA detection intervals.

In the present study we assessed the influence of predator body size and prey identity on prey DNA detection success in the generalist centipede predator *Lithobius* spp. (Lithobiidae, Chilopoda). We hypothesize that (1) predator body size negatively affects post-feeding prey DNA detection intervals and that (2) high quality prey will be digested faster than those of low quality, i.e. prey DNA detection periods will be significantly shorter in the former than in the latter prey type.

We tested these hypotheses conducting two feeding experiments using taxon-specific PCR assays: to assess the effect of predator body size, three size classes of *Lithobius* spp. were fed with the Collembola *Sinella curviseta* (Brook, 1882). In the second experiment, testing the effect of prey type, centipedes were fed with either Collembola (*S. curviseta*), Diptera (*Drosophila melanogaster* Meigen, 1830) or Lumbricidae (*Lumbricus terrestris* Linnaeus, 1758). For both experiments and each prey type a standardized mass of prey tissue was used, allowing to identify prey and predator body-size dependent effects on prey DNA amplification rates. To examine how prey detection in diagnostic PCR correlates with prey DNA quantity, we additionally measured the amount of prey DNA present in the gut content of the centipedes by real-time PCR (qPCR).

Centipedes within the genus *Lithobius* were used as model predators as they are widespread and occur in high numbers in the litter layer of temperate forests (Lewis, 1981). Up to eight species may coexist in one habitat, differing in body size and preference for microhabitats. Lithobiids perform a sit-and-wait hunting strategy, using their poison fangs to kill a wide spectrum of prey particularly Collembola, Diptera larvae and Lumbricidae (Lewis 1981; Poser 1988; Eitzinger *et al.* 2013). Due to similar hunting modes in small and large species as

well as in juvenile and adult individuals, lithobiids represent ideal model organisms to study effects of body size on prey DNA detection success.

Materials and Methods

Feeding experiments

Specimens of adult and juvenile *Lithobius aulacopus* Latzel, 1880; *L. crassipes* L. Koch, 1862; *L. dentatus* C.L. Koch, 1844; *L. mutabilis* L. Koch, 1862; *L. nodulipes* Latzel, 1880 and *L. piceus* L. Koch, 1862 were collected by sieving of litter in beech forests in the vicinity of Göttingen, Germany, in summer and autumn 2011. The animals were starved for one week and separated into three size classes (small, 0.1-5 mg; medium, 5.1-15 mg; large, 20-30 mg). Individuals with body masses between these size classes were not used for this experiment. They were kept in transparent glass vessels (7 cm diameter) with a moist bottom of plaster-of-Paris, rumpled tissue serving as refuge at constant 15 °C and a day/night cycle of 12:12 h. This temperature and light regime represents field conditions in central Germany in September/October and March/April. A mix of freeze-killed mealworms *Tenebrio molitor* Linnaeus, 1758 and larvae of honeycomb moths *Galleria mellonella* (Linnaeus, 1758) served as food. One week prior to the feeding experiments, the predators were starved to ensure that no prey DNA was present in their guts and that the centipedes will readily accept prey. Before start of the experiment the body mass of each predator was determined to the nearest 0.01 mg.

Two different feeding experiments were conducted to investigate (1) the effect of predator body mass and (2) prey identity on prey DNA detection success. We used three prey types of different prey quality: *S. curviseta* (Collembola, Entomobryidae) and *D. melanogaster* (Diptera, Drosophilidae) as sclerotized prey of low quality, i.e. high carbon-to-nitrogen (C:N) ratio of 6.36 and 6.64 respectively and *L. terrestris* (Oligochaeta, Lumbricidae) as soft-tissued prey with low C:N ratio (4.92), i.e. high protein content. In the first feeding trial lithobiids of the three size classes (minimum number of 130 individuals each) were offered three dead individuals of *S. curviseta*. In the second experiment, only medium-sized predators (minimum number of 130 centipedes for each predator-prey combination) were fed with two individuals of *D. melanogaster* and small pieces of *L. terrestris*. Prey in each of the two experiments was

killed by freezing and served in portions of approximately 0.8 ± 0.1 mg. The lithobiids were allowed to feed for 2 h in the climate chamber, thereafter, their biomass was determined again and they were placed in a new glass vessel. Predators which had not or only partially consumed the prey were excluded from the experiment.

For each of the three prey types and for each predator size-class, batches of a minimum of ten medium-sized centipedes were individually frozen at -20 °C in 1.5 ml reaction tubes after digesting their meal for 0, 16, 24, 32, 40, 48, 56, 72, 88, 104, 120, 144 and 168 h.

To avoid hunger to artificially affect of prey DNA digestion rates, we offered dead specimens of the isopod *Trichorhina tomentosa* (Budde-Lund, 1893) *ad libitum* to the centipedes as additional prey after the second measurement of body mass.

DNA extraction and diagnostic PCR

Prior to DNA extraction, the frozen predators were checked for attached prey remains and phoretic mites using a dissecting microscope; additionally their body length was measured. Whole lithobiids were subjected to a CTAB-based DNA extraction protocol (Juen & Traugott 2005) and purified using Geneclean Turbo Kit (MP Biomedicals, Solon, OH, USA) yielding 150 ml of final DNA extract. One blank sample was included per 47 extracts to check for DNA carry-over contamination. Successful DNA extraction was confirmed by PCR using universal invertebrate primers LCO1490 and HCO2198 (Folmer *et al.* 1994). Each 10 µL PCR contained 5 µL PCR SuperHot Mastermix (2×), 1.25 mM $MgCl_2$ (both Geneaxxon, Ulm, Germany), 0.5 µL bovine serum albumin (BSA, 3%; Roth, Karlsruhe, Germany), 0.5 µM of each primer and 3 µL of DNA extract. Thermocycling conditions were 95 °C for 10 min followed by 35 cycles of 95 °C for 30 s, 48 °C for 30 s, 72 °C for 90 s and a final elongation of 10 min at 72 °C. PCR products were separated in 1% ethidium bromide-stained agarose gels and visualized under UV light. Samples testing positive with the universal invertebrate primers were then screened for DNA of the respective prey: for Collembola, Diptera and Lumbricidae group-specific primers Col3F/Col5R (272 bp; Kuusk & Agusti 2007), DIP S16/DIP A17 (198 bp; Eitzinger *et al.* 2013) and 185F/14233R (225-236 bp; Harper *et al.* 2005) were used, respectively. PCR mixes and thermocycling conditions were the same as above only differing in the primers used, the elongation step at 72 °C for 45 s and

the following annealing temperatures: Col3F/Col5R 60 °C, DIP S16/DIP A17 60 °C, and 185F/14233R 65 °C. PCR products were separated using the capillary electrophoresis system QIAxcel (Qiagen, Hilden, Germany); fragments of the expected size and a relative fluorescent value ≥ 0.1 RFU were scored positive. Samples yielding no band in these PCR were re-tested once.

To investigate the sensitivity of singleplex PCR assays we employed a dilution series for each of the three prey species. We determined template DNA copy number of purified PCR-products of prey DNA following guidelines by Sint *et al.* (2012). The number of copies was adjusted to 100,000 amplicon copies per microlitre and then two-fold serially diluted. The serially diluted target DNA was then used as template in the singleplex PCR assays at concentrations of 20,000/ 10,000/ 5,000/ 2,500/ 1,250/ 625/ 313/ 156/ 79/ 40/ 20/ 10/ 5/ 3 and 2 copies of target DNA per reaction.

Quantitative real-time PCR (qPCR)

To quantify the amount of prey DNA present before and after different time points post-feeding in the gut content in medium-sized predators, we established a qPCR protocol using the same primers as for the diagnostic PCR described above. The PCR mix consisted of 7 µL PCR water, 0.25 µM of each primer, 10 µL Kapa SYBR FAST Mix (Kapa Biosystems Inc., Woburn, MA, USA) and 2 µL of DNA extract. Thermocycling in Stratagene Mx3005P (Agilent Technologies Inc., Santa Clara, CA, USA) started with 95 °C for 5 min followed by 40 main cycles of 95 °C for 30 s, the primer-specific annealing temperature (see above) for 30 s and 72 °C for 45 s. Subsequent dissociation curve analysis consisted of 95 °C for 60 s, 55 °C for 30 s and 95 °C for 30 s. In order to standardize the DNA quantification eight steps of a 10-fold dilution series of target DNA of *S. curviseta* (1.54 to 1.54×10^{-7} ng/µL), *L. terrestris* (6.54×10^{-1} to 6.54×10^{-8} ng/µL) and *D. melanogaster* (2.43 to 2.43×10^{-7}), along with two negative controls (PCR water instead of DNA) was run with every batch of 38 samples. Only samples showing a single peak of the expected PCR product in the dissociation curve were counted as positive and extracts which tested negative were re-tested once.

Statistical analysis

LOGIT analyses were carried out to describe the changes in prey DNA detection success over time (Field 2005). The time point for 50% prey detection probability and the corresponding 95% confidence limits were determined; non-overlapping confidence intervals were interpreted as being significantly different. All analyses were performed using SPSS (version 18). The relationship between digestion time and prey DNA quantity as measured by qPCR was calculated using non-linear regression in Sigmaplot 11.0 (Systat Software, Chicago, IL, USA). To analyse relationships between prey DNA detection success and prey type, overall DNA quantity (predator + prey) and quantity of prey DNA, we calculated a generalized linear model (GLM) in R 2.12.2 (R Development Core Team, 2011) using the function glm {stats} with subsequent step function. Prey DNA detection success was coded as binary 1/0 (prey DNA present or absent). DNA quantity was $\log_{10}$-transformed prior to the calculation.

Results

Effect of feeding on predator body mass

After feeding predator body mass increased by 0.18 ± 0.83 (SD) mg (large lithobiids), 0.40 ± 0.44 (medium lithobiids), 0.20 ± 0.24 (small lithobiids), 0.70 ± 0.53 mg (medium lithobiids fed with Diptera), and 0.40 ± 0.49 mg (medium lithobiids fed with Lumbricidae). Predator biomass increase was significant in each of the experiments except for the combination of Collembola with large centipedes (Table 1). However, 104 individuals (14.9% of the fed lithobiids) lost weight, while in 50 specimens (7.2%) body mass did not change.

Table 1. Mean lithobiid body mass and standard deviation (SD) before and after feeding on standardized (0.8 mg) prey items for 2 h. Significant effects are highlighted in bold.

Feeding trial	**Mean body mass before feeding ± SD (mg)**	**Mean body mass after feeding ± SD (mg)**	***P*-value of paired Student's t-test**
Collembola - large predator (*n*=141)	25.1±4.42	25.3±4.41	0.159 n.s.
Collembola - small predator (*n*=142)	3.5 ±1.05	3.7±1.05	**<0.001**
Collembola - medium predator (*n*=152)	8.9±2.78	9.2±2.89	**<0.001**
Diptera - medium predator (*n*=130)	10.2±3.38	10.8±3.37	**<0.001**
Lumbricidae - medium predator (*n*=132)	9.8±2.96	10.2±3.00	**<0.001**

Prey DNA detection success

The singleplex PCR assays proved to be specific and highly sensitive, amplifying DNA of the target prey species only and at low template concentrations of 625 (Diptera), 20 (Collembola) and 10 (Lumbricidae) copies of template DNA per PCR.

After feeding for 2 h, 100% of the lithobiids fed with Collembola or Diptera and 80% of the predators fed with Lumbricidae tested positive for prey DNA. DNA detection success decreased with digestion time in each of the prey taxa and predator size classes (Fig. 1 a-e). The decline in prey detection in medium-sized lithobiids was reflected by Pearson's chi-squares for Collembola (χ^2 = 18.98, P = 0.062), Diptera (χ^2 = 33.70, P < 0.0001) and Lumbricidae (χ^2 = 11.96, P = 0.367) as well as in large (χ^2 = 18.50, P = 0.071) and small lithobiids (χ^2 = 15.22, P = 0.173). Collembola DNA was detected at all time points until 168 h post feeding in each of the three predator size classes, while Diptera DNA was detected up to 144 h post-feeding. Prey DNA detection rates of Lumbricidae decreased fastest over time; their DNA could only be detected up to 40 h post-feeding.

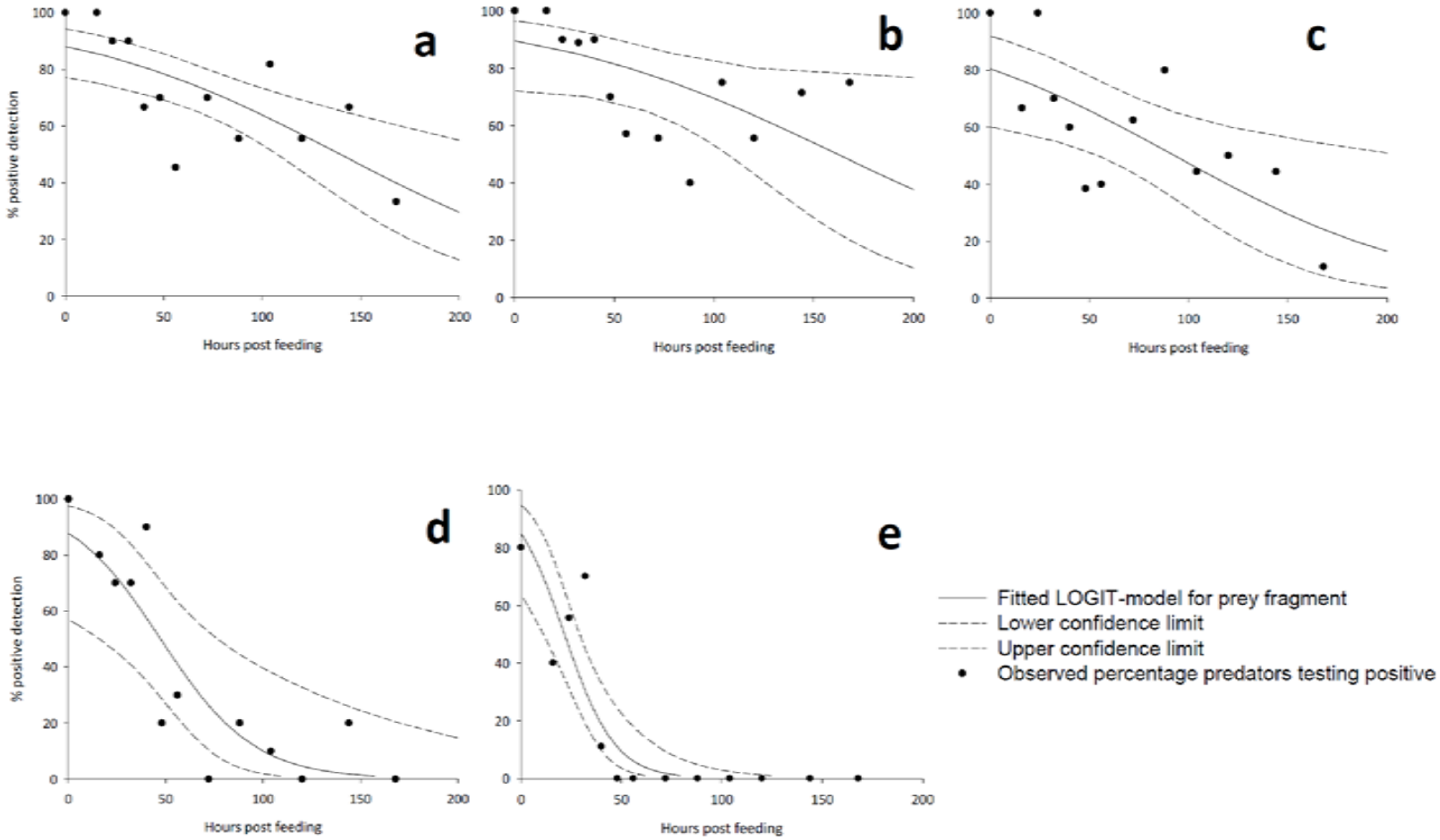

Figure 1. Fitted model of prey DNA detection rates in lithobiid predators at 13 times points post feeding: **a** small predator (0.1-5 mg), **b/d/e** medium predator (5.1-10 mg) and **c** large predator (20-30 mg). **a/b/c** Collembola prey (*Sinella curviseta*), **d** Diptera prey (*Drosophila melanogaster*) and **e** Lumbricidae prey (*Lumbricus terrestris*). Dashed lines represent 95% confidence limits.

The time span for 50% prey detection probability differed between each of the three prey types (Fig. 1 a,d,e): it was shortest in Lumbricidae with 21.6 h [lower (lCL) and upper 95% confidence limits (uCL) of 11.1 and 29.1 h, respectively], medium in Diptera with 47.4 h (lCL 15.9 h, uCL 76.4 h) and significantly longer in Collembola with 161.8 h (lCL 106.5 h, uCL 1632.9 h). In contrast, the 50% prey detection probability did not differ significantly between the three size classes of lithobiids fed with Collembola (large centipedes 92.6 h, lCL 54.2 h, uCL 208.6 h; small centipedes 139.6 h, lCL 107.5 h, uCL230.1 h).

Table 2. Mean prey DNA quantity and prey DNA copy number (±SD) of Collembola, Diptera and Lumbricidae prey items before feeding to the centipedes and after 2 h of digestion.

	Before feeding		**After feeding (2h)**		**Mean Difference DNA quantity/copies (before-after feeding)**
Prey type	Mean DNA quantity ± SD (ng/µL)	Mean DNA copy number ± SD	Mean DNA quantity ± SD (ng/µL)	Mean DNA copy number ± SD	DNA quantity/DNA copies
Collembola	5.60×10^{-3} $\pm4.97\times10^{-3}$	2.00×10^{7} $\pm1.78\times10^{7}$	6.86×10^{-4} $\pm1.42\times10^{-3}$	2.46×10^{6} $\pm5.09\times10^{6}$	$4.91\times10^{-3}/1.76\times10^{7}$
Diptera	2.45×10^{-1} $\pm5.38\times10^{-2}$	1.19×10^{9} $\pm2.62\times10^{9}$	6.90×10^{-4} $\pm8.04\times10^{-4}$	3.36×10^{6} $\pm3.92\times10^{6}$	$2.44\times10^{-1}/1.19\times10^{9}$
Lumbricidae	4.21×10^{-5} $\pm3.72\times10^{-5}$	1.76×10^{5} $\pm1.56\times10^{5}$	9.60×10^{-7} $\pm1.17\times10^{-6}$	4.01×10^{3} $\pm4.88\times10^{3}$	$4.11\times10^{-5}/1.72\times10^{5}$

Quantification of prey DNA

Compared to the DNA concentration in the prey before it was offered to the predators the quantity of prey DNA in lithobiids examined after the 2 h feeding phase was markedly lower (Table 2). For each of the three prey taxa the quantity of prey DNA decreased rapidly with the duration of digestion yielding low prey DNA concentrations at time points beyond 32 h (Fig. 2 a-c).

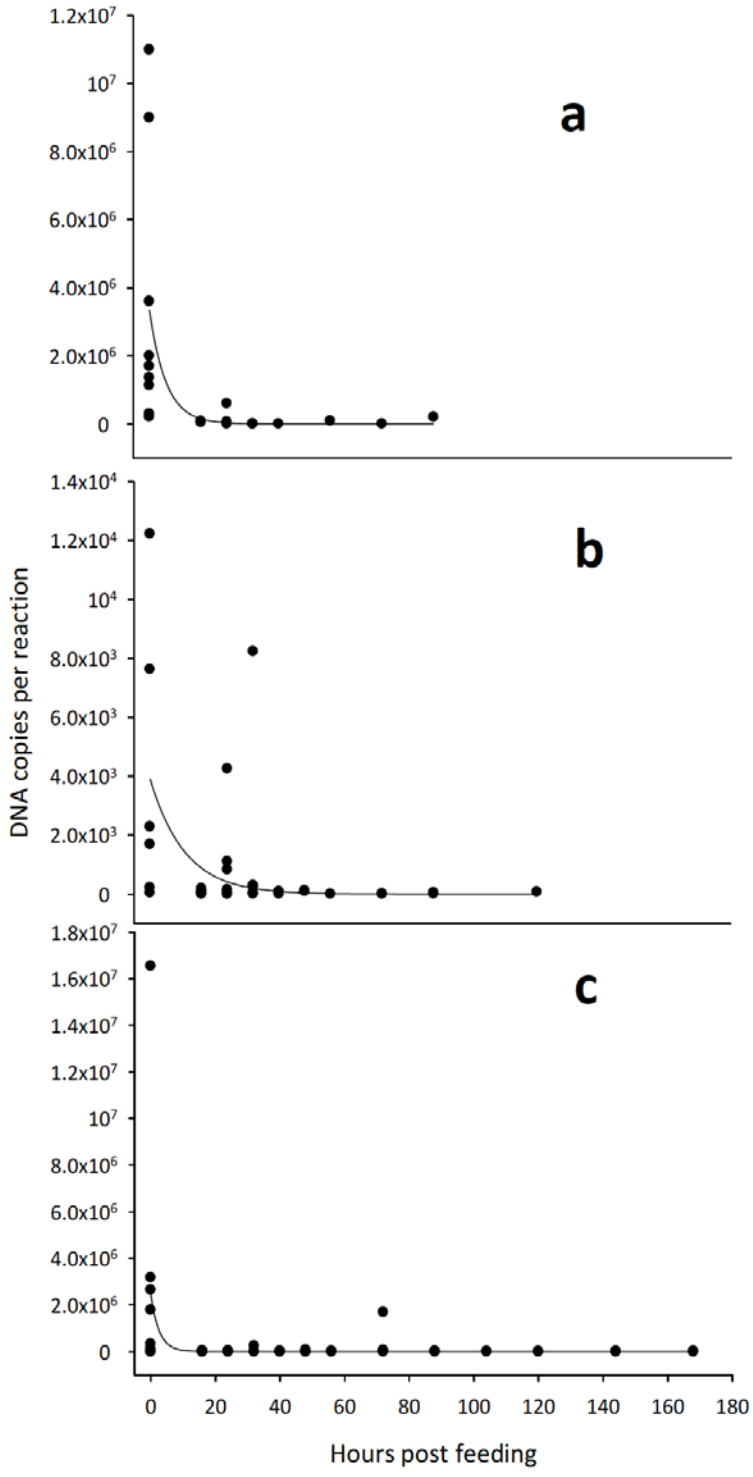

Figure 2. Quantification of prey DNA of **a** Diptera (*Drosophila melanogaster*), **b** Lumbricidae (*Lumbricus terrestris*) and **c** Collembola (*Sinella curviseta*) in medium-sized lithobiid predators at 13 time points post-feeding. Trend line indicates the decay of prey DNA calculated by linear regression.

Remarkably, DNA quantities scattered widely at each of the time points post feeding, (Fig. 2a-c; non-linear regressions for Collembola $R^2 = 0.1651$, $P = 0.9483$, Diptera $R^2 = 0.2605$, $P = 0.7897$ and Lumbricidae $R^2 = 0.1932$, $P = 0.0659$). Many samples had to be excluded from the analysis due to multiple and non-specific amplification and/or production of primer dimers. Nevertheless, GLM analysis of the pooled qPCR data showed prey DNA amplification success to be significantly correlated with prey DNA quantity and prey type with particularly high detection success in Diptera (Table 3 and 4).

Table 3. Generalized linear model (GLM) on the effect of predator body mass, DNA quantity, prey type and the two-way interactions on the detection of prey DNA in *Lithobius* predators. Significant effects are highlighted in bold.

Variable	**Df**	**Deviance**	**Resid. Df**	**Resid. Dev**	**P(>|Chi|)**
NULL		121	136.10		
Log_{10} DNA quantity overall	1	1.9287	120	134.17	0.164
Log_{10} prey DNA quantity	1	4.1990	119	129.97	**0.04**
Prey type	2	10.7440	117	119.23	**0.005**
Log_{10} DNA quantity overall × prey type	2	6.4338	115	112.8	**0.04**
Log_{10} prey DNA quantity × prey type	2	5.5666	113	107.23	0.062

Table 4. Generalized linear model (GLM) on the effect of predator body mass, prey DNA quantity, digestion time (0-130 h post feeding), prey type (Collembola, Diptera, Lumbricidae) and their two-way interactions on the detection of prey DNA in lithobiid predators via diagnostic PCR. SE represents the standard error of the estimated coefficient of the model. Significant effects are highlighted in bold.

Variable	**Estimate**	**SE**	**z-Value**	***P-Value***
(Intercept)	2.2864	1.9757	1.157	0.247
Log_{10} DNA quantity overall	1.2111	0.8003	1.513	0.130
Log_{10} prey DNA quantity	0.4559	0.2666	1.710	0.087
Diptera prey	9.3333	6.7299	1.387	0.166
Lumbricidae prey	9.7608	6.8451	1.426	0.154
Log_{10} DNA quantity overall × Diptera prey	-3.6752	1.7717	-2.074	**0.038**
Log_{10} DNA quantity overall × Lumbricidae prey	1.3835	1.7283	0.800	0.423
Log_{10} prey DNA quantity × Diptera prey	1.0138	0.8239	1.230	0.219
Log_{10} prey DNA quantity × Lumbricidae prey	1.6684	0.9280	1.798	0.072

Discussion

We investigated two hypotheses, i.e. that post-feeding prey DNA detection intervals are prolonged in low-quality prey and in small predator individuals. In the *Lithobius*-Collembola predator-prey system investigated here, predator body size did not significantly affect prey DNA detection success, conflicting with our first hypothesis. To our knowledge, only Lundgren & Weber (2010) also examined the effect of predator body size on prey DNA detection success using larvae of the coccinellid beetle *Coleomegilla maculata*. In these experiments large late instar larvae digested their prey, eggs of Colorado potato beetle *Leptinotarsa decemlineata*, more efficiently than smaller instar larvae, as indicated by a more rapid decline of prey DNA quantity in the former compared to the latter. However, when these ladybird beetle larvae were fed with *Aphis glycines*, predator body size did not affect prey DNA recovery success. The current and the findings by Lundgren & Weber (2010)

indicate that effects of predator size on prey DNA detection success are affected by the identity of both the predator and the prey.

The long post-feeding prey DNA detection intervals for Collembola in the current experiment which were derived by amplification of a 272 bp DNA fragment complicated the analysis of the body-size experiment as the 50% prey detection probabilities were characterized by wide 95% confidence limits. It has been shown that targeting long prey DNA fragments allow more detailed characterization of post-feeding detection intervals (Waldner *et al.* 2013), which probably would also have been useful in this study. Overall, post-feeding prey DNA detection times in lithobiid predators are long compared to predatory insects and spiders (Greenstone & Shufran 2003; Gagnon *et al.*, 2011; Waldner *et al.* 2013), allowing successful amplification of prey DNA up to 168 h (7 days) post-feeding. This long post-feeding detection intervals are important to be considered when interpreting prey DNA detection in field-collected lithobiids, as feeding frequency and consequently predation impact could be overestimated (McMillan *et al.* 2007; Gagnon *et al.* 2011). Waldner *et al.* (2013) detected prey DNA of the scarabaeid beetle *Amphimallon solstitiale* in lithobiid predators up to 60 h post feeding, which is on average three times longer than for the beetle predators tested in this study. Long prey retention times also have been reported in the feeding studies conducted by Poser (1990), in which an ELISA-based approach allowed tracking prey protein for even 20 days after the feeding event. Prolonged detection times are characteristic for fluid feeding predators, such as hemipterans, spiders and carabid beetle larvae (Juen & Traugott 2005; Sheppard *et al.* 2005; Greenstone *et al.* 2007; Kuusk & Ekbom 2010). In contrast, *Lithobius* chews its prey and ingests solid particles, but supposedly uses also its poison to pre-digest prey (Lewis 1981). Unlike spiders, the alimentary canal of centipedes is rather straight, having no diverticula serving as food reservoir (Rosenberg 2009). Changes of metabolic rates due to starvation, however, may extend prey detection intervals: lithobiids regularly are starving during summer and winter (Pfleiderer-Gruber 1986), when drought and low temperatures are responsible for low prey activity, which possibly slows down their digestive activity. Similarly, in our experiment the centipedes were starved to empty their guts (for seven days). This suggests that the experimental set-up may not display conditions in the field when prey is abundant. Moreover, feeding the lithobiids *ad libitum* with non-target isopod prey ("chaser prey") could have provoked longer DNA retention times (Weber & Lundgren 2009).

In the second experiment we investigated the effect of prey quality on DNA detection success. Several studies have shown that post-feeding prey DNA detection intervals are prey-specific (Harwood *et al.* 2007; Gagnon *et al.* 2011; Kuusk & Ekbom 2010). Moreover, it has been demonstrated that food quality has a great impact on digestion and gut transit times (Karasov *et al.* 2011). Animals feeding on low quality food, such as phytoplankton or sclerotized arthropods, have extended digestion times in order to maximize nutrient uptake (Jaeger & Barnard 1981; Tirelli & Mayzaud 2005; Karasov *et al.* 2011; but see Mitra & Flynn, 2007), suggesting longer post feeding detection intervals of prey DNA than in high quality prey. In order to study the effect of prey DNA quantity on time-dependent prey DNA detection success in predators we therefore, for the first time, employed both diagnostic PCR and quantitative real-time PCR. We found that in protein-rich prey of Lumbricidae DNA detection intervals were significantly shorter than in Diptera and Collembola prey, apparently supporting our second hypothesis, and this was confirmed by GLM analysis of the pooled data. However, the qPCR results showed a different picture, with prey DNA quantity of each of the three prey taxa decreasing to a similar extend over time, indicating no prey identity effect. Two reasons might have been responsible for the discrepancy of amplification success in diagnostic and quantitative PCR. (1) Despite using the same mass of prey offered, the target DNA concentration differed strongly between the three prey types. The number of *Drosophila* template DNA was over 5,800 times higher than that for *Lumbricus* and 44 times higher than that for *Sinella*. This indicates a greater likelihood of successful detection of dipteran prey. (2) The sensitivity of the diagnostic PCR assays targeting Collembola and Lumbricidae prey was higher than the assay used to amplify Diptera prey DNA. Combining these two effects, an increasing amplification success in singleplex PCR in the order Collembola > Diptera > Lumbricidae is expected and this is consistent with the findings irrespective of prey quality.

Target gene and gene copy number are important factors in molecular gut content analysis and use of multi-copy genes (e.g., cytochrome *c* oxidase subunit I, 18S rDNA) has been recommended in order to increase detection probability (Symondson 2002; Gariepy *et al*. 2007). However, the number of these genes can differ largely between different prey taxa and even between different body parts of individual species (Alberts *et al*., 2002). Using two different genes for prey detection therefore complicates comparisons. Additional analysis of prey DNA detection using quantitative PCR allowed examining the efficiency of the

diagnostic PCR. GLM results revealed that detection of prey DNA depends on prey DNA quantity, indicating higher probability of positive detection at higher amounts of prey DNA. This might explain the lower proportion of centipedes tested positive for lumbricid prey immediately after feeding. However, prey DNA quantity did not significantly decrease with time in the three studied prey taxa. In fact, prey DNA quantity dropped within the first 32 h post-feeding, then levelling off close to detection threshold. However, the quantity of prey DNA was highly variable at specific time points post-feeding and characterized by high dropout rates of samples due to primer dimers and unspecific amplification, complicating the analysis. Rapid decrease in prey DNA template numbers and high variability in prey DNA concentrations have also been observed in other feeding studies employing qPCR, indicating that these findings are of general importance (Weber & Lundgren 2009; Durbin *et al.* 2011).

DNA has been shown to be a valuable marker in studying trophic interactions qualitatively and quantitatively (King *et al.* 2008; Pompanon *et al.* 2012). High sensitivity and specificity of PCR assays allows examination of the prey spectrum and prey preference of predators in unprecedented detail, however, the parameters affecting the detection success of prey DNA in a predator's gut are still poorly understood, necessitating further experiments. Such factors include predator body size and prey quality, which have been shown to drive predation (Eubanks & Denno 2000; Vucic-Pestic *et al.* 2010). In the present study both factors did not affect prey DNA detection success suggesting that other factors, such as DNA copy number and assay sensitivity, may be more important. Laboratory feeding studies, such as the present one, should guide us how to interpret field-derived data on prey DNA detection. Results of the present study stress the importance of standardising PCR assays. For example, the development and application of a new 18S rDNA based PCR assay to detect Lumbricidae prey, featuring similar detection sensitivity than the other assays for Diptera and Collembola, would facilitate comparisons between the three prey taxa. Further, the results showed that the combined application of diagnostic and quantitative PCR helps interpreting prey DNA detection rates, thereby allowing to identify factors which are actually driving predator-prey interactions.

Acknowledgments

The work has been funded by the DFG Priority Program 1374 “Infrastructure-Biodiversity-Exploratories”. We thank Benjamin Klein, Anina Vogt and David Ott for help with fieldwork. Special thanks to Alexandre Jousset for introducing us to the qPCR methodology and discussion of the results.

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Author contributions

B.E. and M.T. conceived and designed the experiments. B.E. performed all experiments and analyses, except the Diptera-*Lithobius* experiment, which formed part of E.M.U.'s bachelor thesis. B.E. wrote the manuscript with contributions of M.T. and S.S.

Chapter 4

Variations in prey choice of invertebrate soil predators with forest type as indicated by molecular gut content analysis

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Abstract

Predation is an important ecological factor driving animal population structures, ecosystem stability and biodiversity. Many environmental factors influence direction and intensity of predation, suggesting that anthropogenic change of habitats will affect prey choice. We investigated how the prey spectrum of common arthropod predators, i.e. *Lithobius* spp. (Chilopoda) and *Philonthus carbonarius* (Staphylinidae) varies with management of central European forests. Applying specific PCR assays to test for DNA of three abundant prey groups, i.e. Collembola, Diptera and Lumbricidae, in the predators' guts, we tracked trophic interactions. The results showed that *P. carbonarius* and two centipede species are generalist predators, the latter showing no differences in feeding behavior with forest management. The results indicate that variations in the prey spectrum of generalist predators with forest type are driven by changes in the depth of the litter layer, i.e. are due to changes in habitat structure. Trophic interactions varied between regions mainly due to changes in the consumption of Lumbricidae and Diptera. Further, prey consumption of centipedes significantly varied with prey density, predator body size and soil pH, and differed between the smaller *L. crassipes* and the larger *L. mutabilis.* The results complement food web analyses using fatty acids and stable isotopes by elucidating trophic interactions in soil in unprecedented detail.

Key-words: Soil food web, Predator-prey interactions, Molecular gut content analysis, Beech forest, Coniferous forest, Habitat structure, Body size, Centipedes, Staphylinid beetles

Introduction

Analyzing trophic interactions is essential to understand the dynamics and functioning of ecosystems. Food web connectivity and strength of interactions characterize stability and resilience of ecosystems, can help to identify keystone taxa, assess the impact of extinction and invasion events, and understand drivers of biodiversity (Brose 2005). The importance of the belowground system for ecosystem functioning is widely acknowledged (Bardgett and Wardle 2010), however, many interactions in the litter and soil layer are still unknown. Reasons for this include difficulties in visually observing feeding interactions, the small body size of many prey species, the frequency of omnivory and extraoral digestion hampering microscopic identification of gut contents (Scheu and Setälä 2002, Juen and Traugott 2007). Trophic interactions in soil food webs are of particular interest as most species feed on a wide range of prey organism and are thought to exert top-down-pressure on prey communities, thus shaping soil animal communities (Scheu and Setälä 2002). Prey choice and predation impact of these predators are driven by a variety of environmental and biological factors, suggesting that a specific predator affects its prey populations differently in different ecosystems. This also applies to differently managed forests as the structure of food webs and the relative importance of bottom-up and top-down forces vary with tree species and timber harvest practices (Scheu et al. 2003, Salmon et al. 2008). Generalist predators are supposed to adapt quickly to new environmental conditions allowing them to switch between different prey and to exploit new resources (Halaj and Wise 2002). However, information on how trophic interactions change with forest type and associated changes in habitat structure is scarce.

Changes in forest land use alter resource availability such as litter, therefore affecting abundance and population structure of decomposer animals and consequently their predators. Functional response models scaled by semi-natural laboratory experiments indicated that higher prey densities in soils result in higher predation impact (Vucic-Pestic et al. 2010a, Kalinkat et al. 2011). Moreover, habitat structure also influences the ecology of soil- and litter-dwelling invertebrates. Texture and thickness of the litter layer affects microclimate but also provides structural niches serving as microhabitats for a variety of soil invertebrates (Poser 1990, Jabin 2008). Habitat structure is among the most important drivers of predator-prey interactions (Langellotto and Denno 2004, Vucic-Pestic et al. 2010b), with microhabitats serving as refuge from predation. In laboratory experiments Kalinkat et al. (2012)

demonstrated that search time of centipede predators increases with habitat structure resulting in a decline in predation frequency.

In the present study we focus on factors affecting trophic interactions of three abundant predators in litter and soil of forests in central Europe, the rove beetle *Philonthus carbonarius* Gravenhorst, 1802 (Coleoptera, Staphylinidae), centipedes *Lithobius mutabilis* L. Koch, 1862 and *Lithobius crassipes* L. Koch, 1862 (Chilopoda, Lithobiidae). Each of these species lives as generalist predator feeding on a wide spectrum of invertebrate prey including collembolans and earthworms, however, displaying different hunting strategies. Staphylinid beetles are active foragers browsing the litter layer while lithobiid centipedes perform a sit-and-wait feeding strategy to capture prey (Poser 1988, Dennis and Sotherton 1994). The larger *L. mutabilis* colonizes the litter layer, but also occurs near tree trunks and in tree stumps (Fründ 1987, Poser 1990). In contrast, the smaller *L. crassipes* predominates in the F and H horizon of the litter layer as well as in dead wood.

In recent years molecular methods have been established to assess the trophic structure and interactions in soil systems (Post 2002, Ruess and Chamberlain 2010), of which PCR-based molecular gut content analysis provides the most sensitive and specific technique (Juen and Traugott 2007, King et al. 2008). This method is particularly advantageous when studying fluid feeding predators or in case predators consume soft-tissued prey organisms, such as earthworms, complicating conventional microscopic gut analysis. Using group- and species-specific PCR assays even small remnants of prey DNA can be detected allowing identification of prey taxa up to several days post feeding (Waldner and Traugott 2012).

Here, by applying molecular gut content analysis, we studied effects of forest type on the prey spectrum and prey consumption of staphylinid and lithobiid predators in forests of two regions in central and northeast Germany. We hypothesized that (1) Lithobiid and staphylinid predators display similar prey preferences across different forest types, (2) increasing thickness of the litter layer reduces the frequency of prey capture, (3) prey consumption increases with prey abundance, and (4) prey preference of predators varies with body-size.

Materials and methods

Study sites

The study was carried out in forest sites of the two regions, Schorfheide-Chorin (Brandenburg, Germany) and the Hainich in the western part of Thuringia, Germany. The study formed part of the interdisciplinary project "Biodiversity Exploratories" investigating effects of forest management and land use on biodiversity and ecosystem functioning. Altitude at the Schorfheide varies between 2–139 m asl; soils are sandy with the pH ranging between 3.30 and 6.65 (B. Klarner, unpublished data). Mean annual temperature is 6.5-8 °C and average precipitation is 520-580 mm. The climate at the Hainich is sub-oceanic with precipitation ranging between 630–800 mm, pH varies between 5.64 and 7.23 (B. Klarner, unpublished data) and the annual average temperature is 7.0°C. More details on the study regions are given in Fischer et al. (2010) and Ferlian and Scheu (in prep).

In each exploratory sixteen different forest sites were selected which represent four different forest types: (1) managed coniferous forests with Norway spruce (*Picea abies* H. Karst.) in the Hainich ("spruce") and Scots pine (*Pinus silvestris* L.) in the Schorfheide ("pine"), (2) 30 years old managed beech forests ("young beech") (3) 70 years old managed beech forests ("old beech") and (4) unmanaged natural beech forests ("unmanaged beech"). Beech forests were dominated by *Fagus sylvatica* L., interspersed with ash (*Fraxinus excelsior* L.) and maple (*Acer pseudoplatanus* L.).

Sampling and DNA extraction

Adult specimens of the centipedes *L. mutabilis*, *L. crassipes* and the staphylinid beetle *P. carbonarius* were collected by sieving litter through 18 mm mesh at each of the sites. In the Schorfheide we additionally collected *Lithobius curtipes* Koch, 1847, a close relative of *L. crassipes* with similar distribution and prey spectrum. The animals were transferred individually in cooled microcentrifuge tubes and stored at -21°C upon further processing. For gut content analysis we only used the mid body part of the lithobiids from the Hainich; the head capsule and the hind part were used for stable isotope and fatty acid analysis, respectively (see Ferlian and Scheu, in prep). Lithobiids from Schorfheide and staphylinid beetles were processed using the whole body.

All samples were extracted using a modified CTAB-based (cetyltrimethylammonium bromide) protocol (Juen and Traugott 2005) and subsequently purified using Geneclean Turbo Kit (MP Biomedicals, Solon, OH, USA). The extracts were tested using the universal invertebrate primer pair LCO1490/HCO2198 (Folmer et al. 1994) before screening for prey DNA (for PCR conditions see Eitzinger et al. 2013).

For detection of collembolan, dipteran and lumbricid prey in the predators we took a two-step approach: First, a multiplex PCR assay with primers 185F/14233R (Harper et al. 2005) and Col3F/Col5R (Kuusk and Agusti 2007) targeting 12S rDNA of earthworms and 18S rDNA of collembolans, respectively, was optimized following the manufacturer's instructions. After testing sensitivity and specificity of primer mixes on a series of prey DNA solutions in gradient PCR we used the following PCR protocol: Each 10 µl multiplex PCR contained 1 µl PCR water, 1 µl primer mix (final concentration of 4 µM for Col3F/Col5R and 2 µM for 185F/14233R), 5 µl multiplex PCR reaction mix (Qiagen, Hilden, Germany) and 3 µl of DNA extract. PCR cycling conditions were 95 °C for 15 min followed by 35 cycles of 95 °C for 30 s, 65 °C for 90 s, 72 °C for 45 s and a final elongation at 72 °C for 3 min. For detection of dipteran prey we used a singleplex PCR assay with dipteran primer DIP S16/DIP A17 and thermocycling conditions given in Eitzinger et al. (2013). All PCR products were analyzed using the capillary electrophoresis system QIAxcel and software ScreenGel (both Qiagen). Fragments of expected length and a relative fluorescent value (RFU) ≥ 0.08 were scored as positives. Samples showing negative results were tested twice.

Statistical analysis

For the statistical analysis of the data we measured centipede body length and converted it into predator body mass using a log-linear equation (Eitzinger et al. unpublished data). Consumption data was coded as binary 1/0 (prey DNA present or absent). Data on environmental factors were taken from Klarner et al. (in prep). We used a generalized linear model (GLM) with a binomial family in R 2.12.2 (R Development Core Team 2011) to analyze the relationships between predator-specific prey consumption and the explanatory variables exploratory region (factorial), forest type (factorial), predator body mass (continuous, $\log_{10}$ transformed), prey abundance (continuous, $\log_{10}$ transformed), soil pH

(continuous) and litter mass (continuous, $\log_{10}$ transformed). For model simplification non-significant explanatory variables were excluded using the step function (Crawley 2007). We selected the most parsimonious model using the Akaike Information Criterion (AIC; Burnham and Anderson 2004).

Results

Detection of prey DNA in field-caught predators

DNA of 355 individuals was successfully extracted and tested with universal primers showing no carry-over contamination. Unexpectedly, prey DNA detection frequency was higher in extractions only using the mid body (66.6%) than in whole-body-extracts (53.5%). However, dissections led to an unusual high fall out of purified DNA extracts due to low overall DNA content: fourteen samples (8.6%) of part-body-extracts had to be excluded from the analyses in contrast to only one of 208 whole-body-extracts. In order to achieve a higher number of DNA extracts allowing statistical analysis, we additionally included 32 non-purified part-body-extracts of lithobiid predators.

Prey detection frequency between the staphylinid beetle *P. carbonarius* (n = 37) and the two centipede species differed (Supplementary material Appendix 1, Table A1): 75.7% of the staphylinids consumed collembolan prey, 43.2% lumbricid prey and 13.5% dipteran prey; respective values for the two lithobiid species were 42.5%, 30.8% and 24.8%. As the overall number of staphylinid beetles tested positive was low, we refrained from formal statistical comparison.

Table 1. ANOVA of generalized linear model (GLM) on the effect of exploratory, predator species, prey type, predator body mass (mg), and prey abundance in Lithobius predators. Significant effects are indicated with (*).

	Df.	Deviance	Resid. Df	Resid. Dev	Pr(>Chi)
NULL			**953**	**1206.0**	
Exploratorium	**1**	**72.103**	**952**	**1133.9**	**< 0.0001 *** **
Prey type	**2**	**25.020**	**950**	**1108.9**	**< 0.0001 *** **
Predator body mass	**1**	**4.657**	**948**	**1086.2**	**0.0309 * **
Predator body mass²	**1**	**18.033**	**949**	**1090.8**	**< 0.0001 *** **
Exploratorium × Prey type	**2**	**35.528**	**946**	**1050.6**	**< 0.0001 *** **
Prey type × Predator body mass²	**2**	**10.013**	**944**	**1040.6**	**0.0067 ** **
Exploratorium × Predator body mass²	**1**	**4.577**	**943**	**1036.0**	**0.0324 * **
Prey type × Predator body mass	**2**	**9.412**	**941**	**1026.6**	**0.0090 ** **

In *L. mutabilis* (n= 192) *c*ollembolans were most often detected (43.8%) followed by lumbricids (36.5%) and dipterans (26.5%). Of the individuals tested positive 23.4% contained DNA of two different prey groups, 5.7% of all three groups. Of the 124 individuals of *L. crassipes* (n = 94) and *L. curtipes* (n = 32) 112 were tested positive. Detection frequency was highest for collembolans (45.5 %) followed by dipterans (26.8%) and lumbricids (25%). Of the individuals tested positive 15.9% had fed on two and 5.7% on three prey groups.

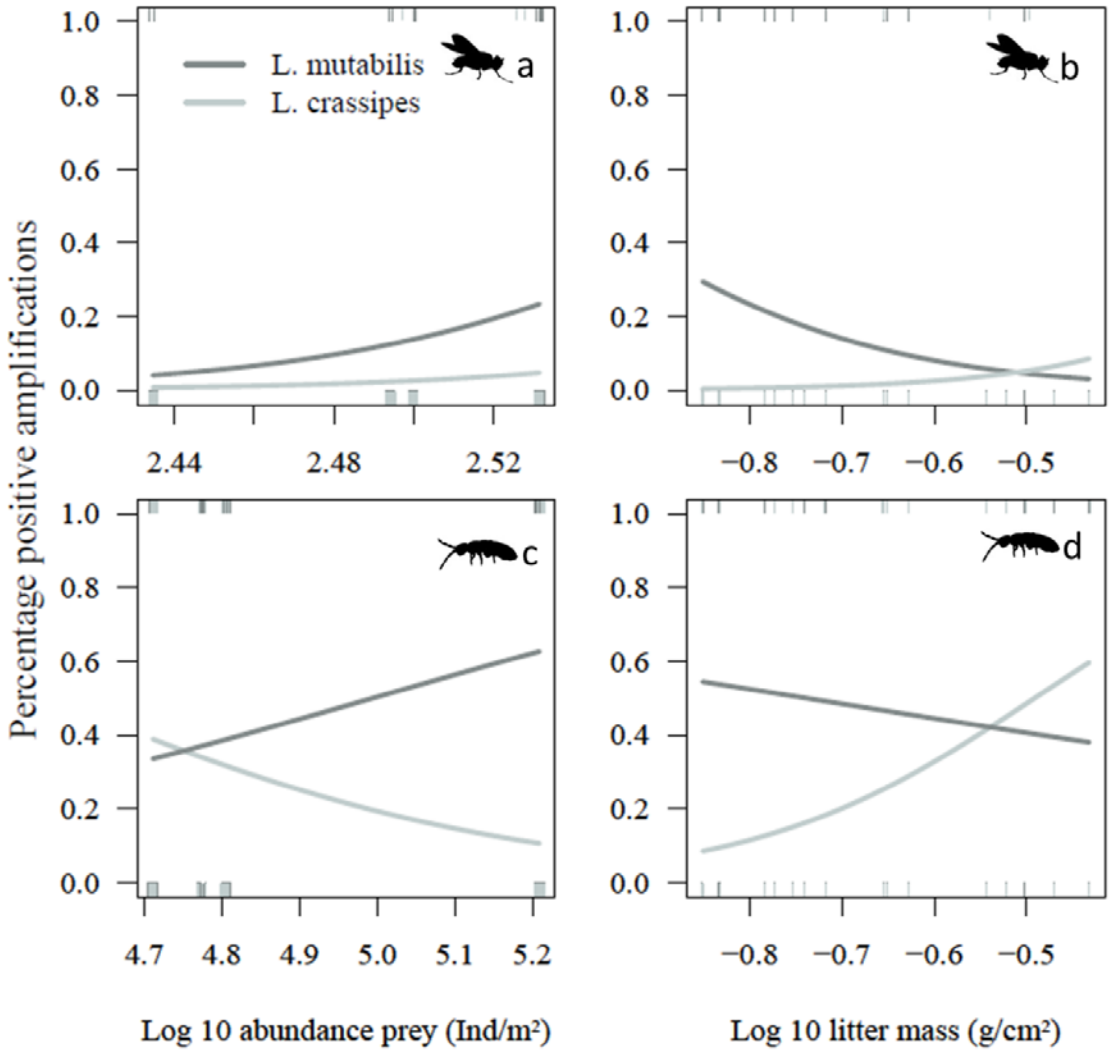

Figure 1. Changes in the frequency of detection of dipteran (a, b) and collembolan prey (c,d) in the Hainich with prey abundance (a, c) and amount of litter in the litter layer (b, d). Rugs along the x-axis indicate the measured data points colour coded as the centipede species.

Collembolans (40.9%) constituted the main prey of centipedes in the Hainich, while in the Schorfheide DNA of earthworms was most often detected (55.2%) considerably exceeding detection frequency in the Hainich (15.0%). Dipteran prey was detected in 46.4% of the Schorfheide samples but only in 10.0% of the tested individuals from the Hainich.

Overall prey detection frequency differed significantly between Hainich and Schorfheide ($p < 0.0001$; Table 1 and Supplementary material Appendix 1, Table A2). As forest type did not affect prey consumption in any other model it was excluded from the model.

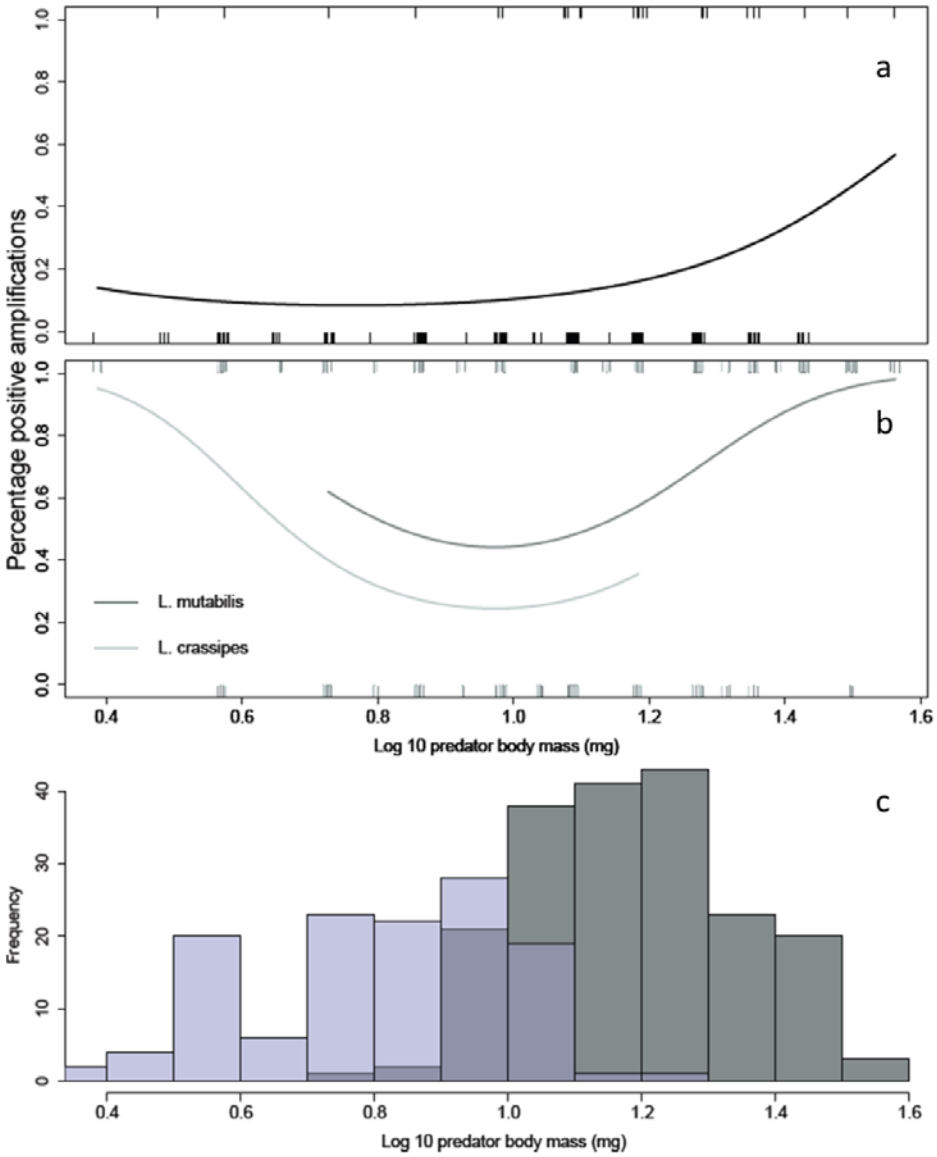

Figure 2. Detection frequency of lumbricid prey in the Schorfheide (a) and the Hainich (b) as affected by body mass of predators, and distribution of body mass of the measured centipedes (c). Rugs along the x-axis indicate the measured data points.

In the Hainich the variables predator body mass, soil pH and prey species significantly affected prey consumption. Feeding frequency in large as compared to small centipedes increased significantly with the density of dipteran prey ($p < 0.05$; Fig. 1a; Supplementary material Appendix 1, Table A3). Further, consumption of dipteran prey correlated negatively with litter mass ($p < 0.05$) in *L. mutabilis* but positively in *L. crassipes* (Fig. 1b).

In centipedes feeding frequency increased significantly with collembolan prey abundance in *L. mutabilis* ($p < 0.01$) but decreased in *L. crassipes* ($p < 0.05$; Fig. 1c; Supplementary material Appendix 1, Table A4). Additionally, higher litter mass corresponded with higher consumption frequency in *L. crassipes*, but lower frequency in *L. mutabilis* ($p < 0.05$, Fig. 1d). For *L. mutabilis*, feeding frequency decreased with increasing pH values ($p < 0.01$). Predator body mass strongly affected consumption of lumbricid prey ($p < 0.0001$; Fig. 2a; Supplementary material Appendix 1, Table A5); starting with predators of about 10 mg (equivalent to about 10 mm body length) detection of lumbricids increased exponentially.

In the Schorfheide only predator body mass and prey species significantly affected prey consumption. Body mass significantly affected consumption of lumbricids ($p < 0.001$; Supplementary material Appendix 1, Table A6) in both lithobiid species (Fig 2b). Similar to the Hainich, small (2.5-4 mg) and large centipedes (25-40 mg) were tested positive for lumbricid DNA whereas detection in medium-sized lithobiids (6-16 mg) was low.

Discussion

We hypothesized that feeding of soil predators is positively affected by low litter mass and high prey abundance while prey preference is driven by predator body size rather than forest type. Results of the present study suggest that the validity of these hypotheses depends on lithobiid centipede species.

Consumption frequency of collembolan and dipteran prey decreased significantly with the amount of litter in *L. mutabilis* while the opposite was true for *L. crassipes*. In addition, soil pH was associated with litter mass. Litter decomposition decreases with soil pH resulting in more pronounced litter layers in soils of low pH (Guckland *et al*. 2009, Trap *et al*. 2011). These results confirm findings from laboratory experiments by Kalinkat et al. (2012) that consumption frequency for collembolan prey in *L. mutabilis* decreases with increasing habitat structure. Similar results for spiders feeding on collembolans (Vucic-Pestic *et al*. 2010b) and tardigrades preying on nematodes (Hohberg and Traunspurger 2005) point to habitat structure as dominant factor driving predator–prey interaction strength in soil systems. Using fatty acids as trophic markers Ferlian and Scheu (in prep) also highlighted the importance of habitat structure for lithobiid - prey interactions with the small *L. crassipes* being more

effective in exploiting prey in deeper organic layers than the large *L. mutabilis*.

Thick organic layers increase the search time for prey resulting in predators spending more energy for catching prey and eventually to switch to other prey (Kalinkat et al. 2012). This suggests that prey dilution reduces overall predation, lessening top-down control of decomposer prey. In contrast, in aboveground systems Finke and Denno (2002) found predation to be higher in structured habitats. They assumed that in structured habitats intraguild predation is diminished, thereby increasing predation on mutual herbivore prey. This may apply to aboveground herbivore systems but not to soil food webs, where trophic links between predators are more frequent (Digel et al. in prep).

In contrast to *L. mutabilis* predation by *L. crassipes* was more pronounced in forests with thicker organic layers. Facilitated by its small body size, *L. crassipes* predominantly colonizes deeper litter layers (Fründ 1987, Jabin 2008). In contrast to *L. mutabilis*, *L. crassipes* therefore presumably benefited from thicker litter layers by its ability to hunt collembolan prey in this structured habitat. Further, low handling time at high humidity in deeper litter layers may have contributed to high prey detection frequency in *L. crassipes* (Kalinkat et al. 2012). Lithobiids are weakly sclerotized and suffer easily from water loss (Lewis 1981), and this applies in particular to the small *L. crassipes* (Fründ 1987, Jabin 2008). High litter accumulation therefore widens their foraging range and facilitates prey capture and consumption.

Conform to our expectation forest type did not significantly affect prey consumption. However, using fatty acid (FA) analysis Ferlian and Scheu (in prep) found the diet of centipedes to differ between spruce and beech forests. Despite we used in part the same individuals we could not confirm these results with molecular gut content analysis. However, the two methods target different aspects of predator – prey interactions with FA analysis providing information on the association of predators to different food web channels, such as the bacterial and fungal channel, whereas molecular gut content analysis providing information on links between predators and prey. In our study we used general prey primers neglecting e.g., that different collembolan species may feed on very different resources ranging from leaf litter to fungi to nematodes (Chahartaghi et al. 2005). The two methods therefore are complementary rather than redundant providing insight into different food web characteristics.

Based on functional response models of soil predators (Vucic-Pestic et al. 2010a, Rall et al. 2011) we expected the frequency of feeding of predators on prey to increase with increasing prey density. This could be demonstrated for *L. mutabilis* feeding on collembolan and dipteran prey, while consumption of *L. crassipes* did not change for dipteran prey or even decreased with increasing collembolan abundance.

Conform to our findings feeding frequency of predators vary with predator body size with smaller predators reaching maximum feeding at lower prey density than larger predators (Brose 2010). A higher abundance of collembolans prey might also form a kind of defense ("swarming") preventing predators from attacking (Vucic-Pestic 2010b), therefore explaining a negative correlation between predation rate and prey abundance.

Unexpectedly, the frequency of feeding on earthworms differed markedly between regions. Despite low lumbricid density, the studied predators more frequently fed on lumbricids in the Schorfheide. This discrepancy may be explained by predators in the Schorfheide feeding on small lumbricids, which typically are underrepresented in mustard and heat extracted samples (Eisenhauer et al. 2008).

Predator body mass of both lithobiid species was identified as the only factor driving feeding on lumbricids, thus confirming our fourth hypothesis. Only large *L. mutabilis* were tested positive for lumbricids in the Hainich, while in the Schorfheide also the small *L. crassipes* fed on lumbricids. Poser (1988) showed *L. crassipes* and *L. mutabilis* to be able to subdue and kill lumbricids if not longer than 1.5 and 3.0 cm, respectively, suggesting that mostly juvenile or small lumbricids are attacked. Epigeic lumbricids living in the litter layer typically are of that size and are present in high numbers in the litter layer of temperate forests (Scheu and Poser 1996). Earthworms constitute a protein-rich food source (Sun et al. 1997) which is digested more easily than collembolans or dipterans due to missing chitin cuticle (Karasov et al. 2011). Therefore, large predators may switch to lumbricid prey for saving costs for digestion. Furthermore, prey preferences increase significantly with the predator-prey body-mass ratio (Kalinkat et al. 2011). In the Schorfheide the frequency of feeding on dipteran prey by *L. mutabilis* exponentially increased with predator body mass, peaked at 25 mg and then decreased. Passive preference, as indicated by the allometric model (Kalinkat et al. 2011), may explain prey switching in predators exceeding 6-16 mg body mass from lumbricid towards dipteran prey.

The frequency of feeding on the prey taxa studied differed between *P. carbonarius* and lithobiids but, due to low predator numbers, the factors driving prey consumption could not be identified. However, the results prove *P. carbonarius* to function as generalist predator preferably feeding on collembolans. Preference for small and abundant prey is affirmed by field studies in agricultural systems (Nienstedt and Poehling 2004) indicating that genus *Philonthus* plays a crucial role in regulating aphid population. Staphylinids predominantly rely on their visual sense to capture prey, thereby able to kill fast moving prey such as collembolans. Unlike *Lithobius* spp., staphylinid beetles are ground cursorial predators actively foraging above- and belowground (Dennis and Sotherton 1994). There are no studies indicating that staphylinids enter burrows of earthworm, which make it plausible that only litter-dwelling lumbricids such as *Dendrobaena octaedra* are being fed on. Measuring up to 17 mm in length and equipped with strong mandibles, *P. carbonarius* may attack even large lumbricid individuals, however, scavenging on dead prey may also explain high consumption frequency. Low dipteran prey detection may be due to their preferred dwelling in lower horizons of litter (Hövemeyer 1992) which are not within the foraging range of larger staphylinids.

Results of this study indicate that consumption frequency of centipedes on collembolan, dipteran and lumbricid prey depend on a number of factors. Forest type does not control centipede feeding, as driving factors are not associated with forest management. Habitat structure, indicated as litter mass provides refuges for prey, impeding predation by the large but increasing predation facilities for the smaller centipede species. In contrast, the larger *L. mutabilis* benefits from higher prey abundance, while *L. crassipes* is confronted with prey defense mechanism. Overall, results suggest that direction of feeding differs markedly between the two lithobiid species as a result of their different body size, therefore confirming previous studies (Kalinkat 2011, Schneider et al. 2012). We conclude that when studying feeding interactions not only taxonomic affiliation, but also trans-species allometry to be considered.

Acknowledgments

We thank the managers of the three exploratories, Swen Renner, Sonja Gockel, Kerstin Wiesner, and Martin Gorke for their work in maintaining the plot and project infrastructure;

Simone Pfeiffer and Christiane Fischer giving support through the central office, Michael Owonibi for managing the central data base, and Markus Fischer, Eduard Linsenmair, Dominik Hessenmöller, Jens Nieschulze, Daniel Prati, Ingo Schöning, François Buscot, Ernst-Detlef Schulze, Wolfgang W. Weisser and the late Elisabeth Kalko for their role in setting up the Biodiversity Exploratories project. In addition, we are grateful for the field work permits of the state environmental office of Thuringia and Brandenburg according to § 72 BbgNatSchG. Special thanks to Mark Maraun, Christian Bluhm, David Ott, Emily Solly, Kerstin Heidemann and Amrei Binzer for support during field work, lab work, help with statistical analyses and the writing process.

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Chapter 5

Combining molecular gut content analysis and functional response models unravels how body size affects prey choice in soil predators

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Abstract

Predator-prey interactions in soil systems are driven by a variety of factors, of which body size presumably is among the most important. Functional response models allow to predict the strength of trophic links and assess motives for prey choice; however, due to their reductionist set-up these models may not display field conditions, possibly leading to skewed results. Therefore, we compared feeding patterns of two centipede predators using molecular gut content analysis and functional response models. Results showed that prey choice is driven by predator body size and prey identity while prey abundance had no effect. Results of functional response model significantly correlated with results from molecular gut content analysis for many prey, with the exception of intraguild prey. The results show that functional response models are a valuable instrument to assess trophic interactions in soil, however species-specific traits must be taken into account to assure correct predictions.

Keywords

Predator-prey interactions, PCR, forest soil, food web, centipedes, Collembola, Lumbricida, Diptera

Introduction

Soil animals play a fundamental role in delivering terrestrial ecosystem services. Their activity as decomposers, bioturbators, predators or root-feeders affects structure and functionality of communities below and above the ground (Bardgett & Wardle 2010). However, trophic interactions among soil animals are still largely unexplored and important processes, such as competition for resources or top-down control of prey populations, are little studied. It is assumed that the dense habitat structure and high abundance of potential prey organism promote ambush techniques and local feeding over active foraging for many predators such as centipedes and predatory coleopterans (Scheu & Setäla 2002). Following optimal foraging theory, this eventually leads many soil animals to feed on a wide range of prey organisms, i.e. to function as opportunistic or generalist predators (MacArthur & Pianka 1966).

Soil harbors an exceptional variety of organisms at high densities, leading to the designation

as “the poor man’s tropical rainforest” (Giller 1996). This also applies to the invertebrate predator community (Juen & Traugott 2007, Lundgren *et al*. 2009). For example, there are more than 40 species of mesostigmatid mites in litter and soil in forests of central Germany, reaching numbers as high as 10.000 individuals per m² (Čoja & Bruckner 2003; Klarner *et al*. 2013). Assuming that many of these predators compete for prey resources such as collembolans, we asked how food web stability can be maintained and the high diversity of prey and predator species can be preserved.

Body size of predators and prey may act as one of the major factors driving prey choice eventually reducing competition (Woodward *et al*., 2005). Predators are typically larger than their prey and forage on body sizes which they can handle at reasonable energy costs (Cohen *et al.*, 1993; Brose *et al.*, 2006). This eventually results in specific predator-prey body mass ratios, suggesting that small predators have narrow diets while large predators feed on a wider range of prey, occupying higher trophic levels (Woodward & Hildrew 2002; Riede *et al*. 2011). In laboratory feeding trials, body size dependent prey switching and feeding on intraguild prey were shown to relieve predation pressure on decomposer prey such as collembolans (Schneider *et al.* 2012). However, in the field strength of intraguild predation may be lowered at high extraguild prey densities (Halaj & Wise 2002; Eitzinger & Traugott 2011).

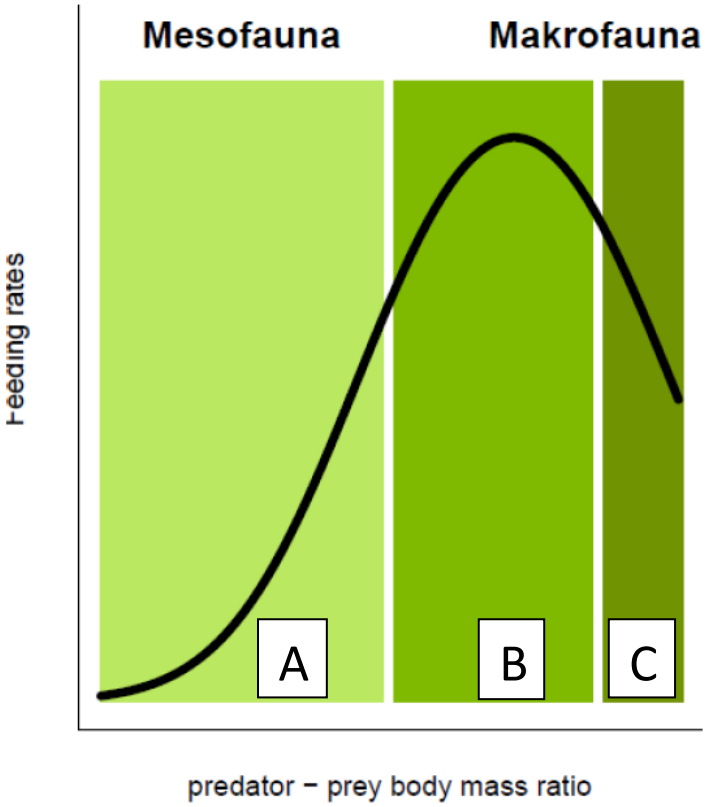

Fig 1. Hump-shaped relationship between feeding rates and predator-prey body mass ratio of macroinvertebrate predators in soil. A: low feeding rates due to long handling times; B: Optimal bodymass ratio leading to high feeding rates; C: low feeding rates due to low capture success.

Functional response models allow calculating intake rates of consumers based only on a small set of parameters including abundance and body sizes of prey and predator (Holling 1959). Comparing these prey-specific feeding rates allow predictions on prey choice and diet switch but also on impacts on prey communities (Kalinkat *et al*. 2011). Recent studies showed that allometric relationships between predator and prey are able to explain feeding interactions in soil systems, indicating a body-size based prey choice (Vucic-Pestic *et al*. 2010, Kalinkat *et al*. 2013b; Fig. 1). While this reductionist approach is desirable, as it allows handling large sets of data and comparing food webs of many habitats with a minimum of parameters, it may not reflect real processes in the field. Ignoring factors such as habitat structure defence mechanism or palatability of prey may bias results and lead to false conclusions.

In order to examine food webs comprehensively, it is therefore essential to analyse trophic links and interactions in the field. The opaque character of soil and the small size of most species do not allow for direct observations, calling for the use of indirect methods. Molecular gut content analysis is a state of-the-art technique to identify trophic links and assess the prey

spectrum of predators (King *et al*. 2008; Pompanon *et al*. 2012). Applying specific markers in PCR assays allows detection of prey DNA in the consumer's gut, even several days after the feeding event (Heidemann *et al*. 2011; Waldner *et al*. 2013), therefore allowing to unravel trophic links of consumers with high resolution.

We studied factors affecting centipede predation using molecular gut content analysis and compare results on prey DNA detection frequency with predictions from functional response models. We analysed gut content of field-collected centipede predators from unmanaged beech forest in central Germany. Eight species of lithobiid and three geophilomorph centipede taxa were investigated with group- and species-specific primers for DNA of 14 extra- and intraguild prey taxa common at the sampling sites. We hypothesized that (1) predator body size rather than predator identity affects prey choice and (2) feeding rate is positively correlated with prey abundance.

Materials and Methods

Sampling and DNA extraction

Invertebrate predators were collected on four plots (HEW 10, HEW 11, HEW 12, and HEW 36) of unmanaged beech forests (> 120 years old) within the national park Hainich near Mülverstedt (Thuringia, Germany). The plots span 100 × 100 m and form part of the Biodiversity Exploratories, an integrated biodiversity project (Fischer 2010). In order to avoid atypical feeding results due to dry and cold weather, we sampled by sieving of litter at four dates in autumn and spring respectively (8, 20 and 28 October and 3 November 2009; 15, 24 and 29 June and 8 July 2010). Predators were transferred individually to cooled 1.5 mL microcentrifuge tubes and placed immediately at -20 °C.

To record the species spectrum and abundance of prey organisms, two large (20 cm diameter, 10 cm deep) and two small (5 cm diameter, 10 cm deep) soil cores per plot were taken in spring 2008 and 2011. Animals were extracted using a high gradient extractor (Kempson *et al.* 1963), stored in 75% ethanol and identified to species level (except dipteran larvae). Additionally, lumbricids were collected by hand after application of mustard solution (*cf.* Eisenhauer *et al*. 2008).

A total of 532 field-caught *Lithobius* spp. and 65 geophilomorph centipedes were identified to species level using the key of Eason (1964) and Latzel (1880); sex, development stage and

body length were determined, and the predators subsequently subjected to a CTAB-based DNA-extraction protocol (Juen & Traugott 2005) with modifications given in Eitzinger *et al.* (2013). To test for DNA carry-over contamination a blank control was included within a batch of 47 specimens. DNA extracts were purified using Geneclean Kit (MP Biomedicals, Solon, OH, USA).

Successful DNA extraction was confirmed by PCR using universal invertebrate primers (Folmer *et al.* 1994) amplifying a ca. 700 bp fragment of the cytochrome *c* oxidase subunit I gene (*COI*). Each 10 µL PCR contained 5 µL PCR SuperHot Mastermix (2×), 1.25 mM $MgCl_2$ (both Geneaxxon, Ulm, Germany), 0.5 µL bovine serum albumin (BSA, 3%; Roth, Karlsruhe, Germany), 0.5 µM of each primer and 3 µL of DNA extract. PCR cycling conditions were 95 °C for 10 min followed by 35 cycles of 95 °C for 30 s, 48 °C for 30 s, 72 °C for 90 s and a final elongation of 10 min at 72 °C. PCR products were separated in 1% ethidium bromide-stained agarose gels and visualized under UV-light.

Screening predators for prey DNA

All DNA extracts were screened for five extraguild and three intraguild prey taxa in individual singleplex PCR assays using group-specific primers. PCR mixes and thermocycling conditions were the same as above only differing in applied primers, an elongation step at 72 °C for 45 s and the specific annealing temperature: Col3F/Col5R (Collembola; 60 °C), 185F/14233R (Lumbricida; 65 °C), DIP S16/DIP A17 (Diptera; 60 °C), ISO S6/ISO A3 (Isopoda; 60 °C), ORI S14/ORI A16 (oribatid mites; 68 °C), ARA S5/ARA A5 (spiders, 68 °C), GAM S7/GAM A8 (gamasid mites; 63 °C) and STA S6/STA A3 (staphylinid beetles; 65 °C). Geophilomorph centipedes were additionally tested for consumption of intraguild prey *Lithobius* spp. using LIT S13/LIT A8 (54 °C). All predator samples scoring positive for collembolans were subsequently tested for specific collembolans species using respective primers and annealing temperature: *Ceratophysella denticulata* (CERDEN S5/ CERDEN A3; 68 °C), *Folsomia quadrioculata* (FOLQUA S4/FOLQUA A1; 62 °C), *Lepidocyrtus lanuginosus* (LEPLAN S3/LEPLAN A1; 62 °C), *Protaphorura armata* (PROARM S3/PROARM A3; 68 °C) and *Pogonognathellus longicornis* (POGLON S4/POGLON A4; 62 °C). The general collembolan and earthworm primers used were developed by Kuusk & Agusti (2008) and Harper *et al*. (2005), respectively, all other primers used were developed by Eitzinger *et al*. (2013). Specificity of the PCR assays was warranted by testing against a set of up to 119 non-target organisms (Eitzinger *et al.* 2013). PCR

products were separated using the capillary electrophoresis system QIAxcel (Qiagen, Hilden, Germany); fragments of the expected size and a relative fluorescent value ≥ 0.1 RFU were scored as positive.

Statistical analysis

To analyse relationships between prey detection rates and predator identity, predator body mass, square of predator body mass, predator sex, predator development stage (immature or adult), prey identity, prey body mass and prey abundance, we calculated a generalized linear model (GLM) in R 2.12.2 (R Development Core Team 2011) using the function glm {stats} with subsequent step function. We did not include "sampling date" as factor as data on prey abundance were lacking for certain time points. Based on Akaike information criterion (AIC) we selected the most parsimonious model (Burnham and Anderson 2004). Prey DNA detection data was coded as binary 1/0 (prey DNA present or absent). Lithobiid body mass was calculated using equation (1):

$$log_{10} M = 2.32784 * log\ 10\ L - 1.24015 \quad \text{(eqn 1)}$$

where *M* is the fresh body mass (mg) and *L* the body length (mm) of lithobiid specimens. The equation is based on 560 lithobiid individuals used in laboratory studies by B. Eitzinger (unpubl. data). Geophilomorpha body mass was calculated using formula given in Gowin and Recher (1984) and Mercer (2001). Body mass (for predator and prey) and prey abundance were $\log_{10}$-transformed prior to statistical analyses. To compare prey detection rates between predator taxa at the $P < 0.05$ level, 95% tilting confidence intervals (CI; Hesterberg *et al.* 2003) were calculated by 9999 bootstrap resamples using s-plus 8.0 (Insightful Corporations, Seattle, WA, USA).

For calculation of functional responses of lithobiids, we implemented data on prey abundance, as well as prey and predator body mass in a Holling type II equation (Holling 1959). We used handling time, attack rate and capture exponent *q* from formulas given in Kalinkat *et al.* (2013b) including listed values for generalised allometric functional response. To allow comparisons between prey-specific DNA detection rates and modelled feeding rates we transformed the latter in plot-specific feeding ratios, displaying fraction of specific prey of

total. Additionally, we related both prey detection and feeding ratios to body size of predators. Comparison of body-size dependent data of prey DNA detection success and feeding rates was calculated using Pearson's correlation coefficient using R 2.12.2 (R Development Core Team 2011).

Results

Centipede community

Among the 597 centipedes collected during the sampling periods, nine species of lithobiid predators (*Lithobius aulacopus*, *Lithobius crassipes*, *Lithobius curtipes*, *Lithobius dentatus*, *Lithobius melanops*, *Lithobius muticus*, *Lithobius mutabilis*, *Lithobius nodulipes* and *Lithobius piceus*) and three species of geophilomorphs (*Geophilus* sp., *Schendyla nemorensis*, *Strigamia acuminata*) of both sexes and all developmental stages were identified. Body sizes ranged between 2-18 mm and 8-47 mm in lithobiid and in geophilomorph centipedes, respectively. Body masses ranged between 0.28 and 48.07 mg in lithobiids, and between 1.58 and 16.70 mg in geophilomorphs.

Prey DNA screening

A total of 532 *Lithobius* spp. and 65 geophilomorph centipedes collected at the eight sampling dates were tested for DNA of eight and nine extra- and intraguild prey groups, respectively. Per sampling date 41-91 *Lithobius* spp. and 4-12 geophilomorphs were investigated.

DNA of each of the prey organisms tested could be detected in at least one predator individual. Lithobiid predators were significantly more often tested positive for collembolans than for any other prey group (Fig 2A). Detection rates of dipterans and lumbricids were significantly higher than those of other extraguild prey, such as isopods and oribatid mites. Intraguild prey constituted only a minor fraction of lithobiid prey: detection frequencies of gamasid mites were followed by staphylinid beetles and spiders. In 69 predators two or three prey taxa were detected in one individual. The lithobiids which tested positive with the general collembolan primer pair (n=141) consumed significantly more *Folsomia quadrioculata* than any other of the four tested springtail species (Fig 2B).

Extraguild prey, such as collembolans and dipterans, were most often detected in geophilomorph centipedes followed by lumbricids, isopods and oribatid mites (Fig 2C). Detection rates for intraguild prey were highest in staphylinids, followed by spiders and

gamasid mites. None of the five springtail species could be detected in collembolan-positive geophilomorphs. In 14 geophilomorph individuals two or three prey taxa were detected.

Factors influencing prey consumption

Lithobiid feeding was significantly affected by prey type and predator body mass (Table 1 and 2), indicating prey preferences of predators for certain prey size. Changes in the probability of detection with predator body mass followed a humpback curve peaking at body masses of 6.3 mg and 4.9 mg for collembolans and lumbricids, respectively (Fig 3). In contrast, detection probability in dipterans increased exponentially, indicating that dipterans are increasingly fed by larger lithobiids while they are rejected by smaller specimens. Despite being generally low, prey detection probability also increased linearly for oribatid mites, gamasid mites, staphylinids and isopods, with the curve flattening at 25.1, 60.3, 61.7 and 69.2 mg body mass, respectively. Feeding on other intraguild prey, such as spiders, again followed a humpback curve peaking at 2.9 mg predator body mass. Geophilomorph feeding varied with prey type, predator body mass (including square of predator body mass) and prey abundance (Appendix S1). In contrast to lithobiids, however, detection rates followed a humpback curve for each of the prey taxa (Appendix S2).

In general, predator identity, development stage and sex did not significantly affect prey DNA detection rates. However, feeding on collembolans by lithobiid species varied with prey type, whereas neither body mass of prey and predator nor abundance significantly affected prey DNA detection.

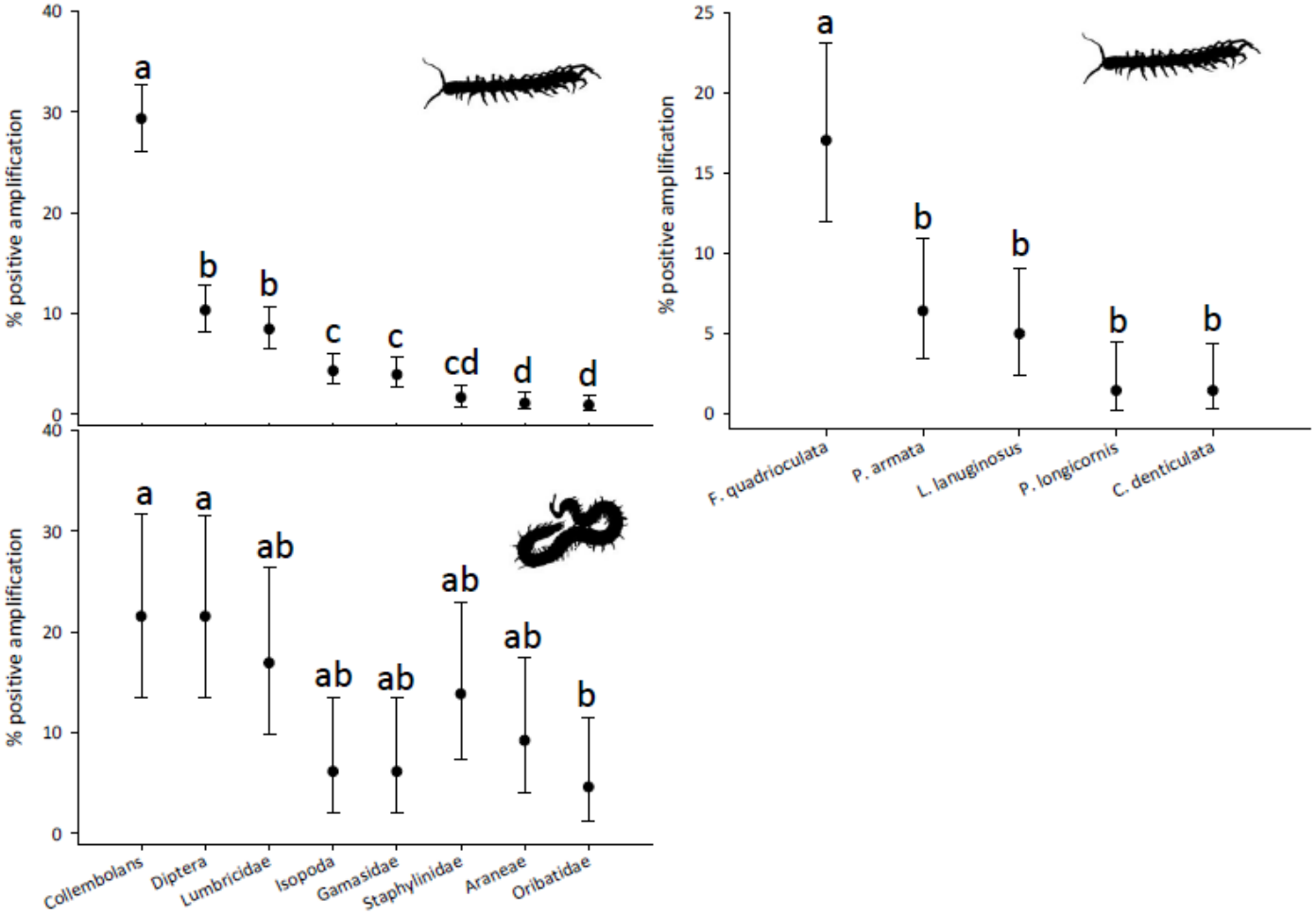

Fig 2. Prey detection rates of lithobiid (A; n= 532) and geophilomorph predators (C; n=65) collected in the Hainich forest in autumn 2009 and spring 2010. Lithobiids tested positive for collembolan prey (B; n=141) were tested further for collembolan prey species. Error bars indicated 95% confidence intervals and letters denote significant differences in DNA detection rates at $P < 0.05$.

Table 1. Generalized linear model (GLM) on the effect of predator body mass, square of predator body mass, prey type and the two-way interactions on the detection of prey DNA in *Lithobius* predators. Significant effects are highlighted in bold.

Variable	Df	Deviance	Resid. Df	Resid. Dev	P(>|Chi|)
NULL			4247	2270.2	
Log_{10} predator body mass	1	5.38	4246	2264.8	**0.02039**
Prey type	7	386.35	4239	1878.5	**<0.001**
Prey type× Log_{10} predator body mass²	8	19.05	4231	1859.5	**0.01461**

Comparison of functional response models with molecular gut content analysis

Feeding rates and prey DNA detection rates were significantly correlated for all lithobiid prey (Pearson correlation coefficient, $P < 0.001$). Collembolans, dipterans, isopods oribatid mites and staphylinid beetles displayed a positive relationship as feeding rates increased, while the other three prey species had a negative relationship (Fig. 4). In geophilomorph centipedes, only correlations with lumbricids and staphylinids were significantly positive ($P < 0.05$; Appendix S3), while the other prey did not show any correlation.

Feeding rates for all prey were low, except for collembolans, gamasid and oribatid mites, showing a roller-coaster-shaped relationship with two peaks (Fig. 5). Feeding of isopods and dipterans increased only slightly at high predator body masses.

Functional response models for lithobiid predators indicated high and low feeding rates for small and large prey, respectively, at each of the study sites. Calculated feeding rates declined from collembolans (average of 15.5 ind/h) to oribatid (8.5 ind/h) and gamasid mites (1.8 ind/h). In contrast, calculated feeding rates for staphylinids and lumbricids were very low (6.9×10^{-3} and 3.65×10^{-5} ind/h, respectively).

Based on functional response models geophilomorph centipedes showed generally lower feeding rates than lithobiids. The highest feeding rates were calculated for collembolan prey (24.3 ind/h), followed by oribatid mites (7.1 ind/h) and gamasid mites (1.6 ind/h). Feeding rates for dipterans (0.1 ind/h), isopods (0.02 ind/h), spiders (4.3×10^{-3} ind/h), staphylinid beetles (3.6×10^{-3} ind/h) and lumbricids (1.9×10^{-5} ind /h) were low. As for lithobiids, mesofauna formed the most important prey.

Table 2. Generalized linear model (GLM) on the effect of predator body mass (mg), square of predator body mass (mg), prey type, and the two-way interactions on the detection of prey DNA in *Lithobius* predators. SE represents the standard error of the estimated coefficient of the model. Significant effects are highlighted in bold.

Variable	**Estimate**	**SE**	**z-Value**	***P***
Intercept	-4.4795	0.7012	-6.388	**<0.001**
Log_{10} predator body mass	1.3197	0.7862	1.678	0.0933
Collembola prey	3.1776	0.6741	4.714	**<0.001**
Diptera prey	0.8872	0.7200	1.232	0.2179
Gamasidae prey	0.4830	0.7724	0.625	0.5317
Isopoda prey	0.5666	0.7637	0.742	0.4581
Lumbricida prey	1.7732	0.7049	2.516	**0.0119**
Oribatidae prey	-0.8956	1.0445	-0.857	0.3912
Staphylinidae prey	-0.3900	0.9016	-0.433	0.6653
Araneae prey × Log_{10} predator body mass²	-1.4409	0.9828	-1.466	0.1426
Collembola prey × Log_{10} predator body mass²	-0.8242	0.4972	-1.658	0.0973
Diptera prey × Log_{10} predator body mass²	0.2770	0.4843	0.572	0.5673
Gamasidae prey × Log_{10} predator body mass²	-0.3706	0.5833	-0.635	0.5252
Isopoda prey × Log_{10} predator body mass²	-0.3578	0.5723	-0.625	0.5319
Lumbricida prey × Log_{10} predator body mass²	-0.9581	0.5545	-1.728	0.0840
Oribatidae prey × Log_{10} predator body mass²	-0.4701	0.8921	-0.527	0.5982
Staphylinidae prey × Log_{10} predator body mass²	-0.3693	0.7220	-0.511	0.6090

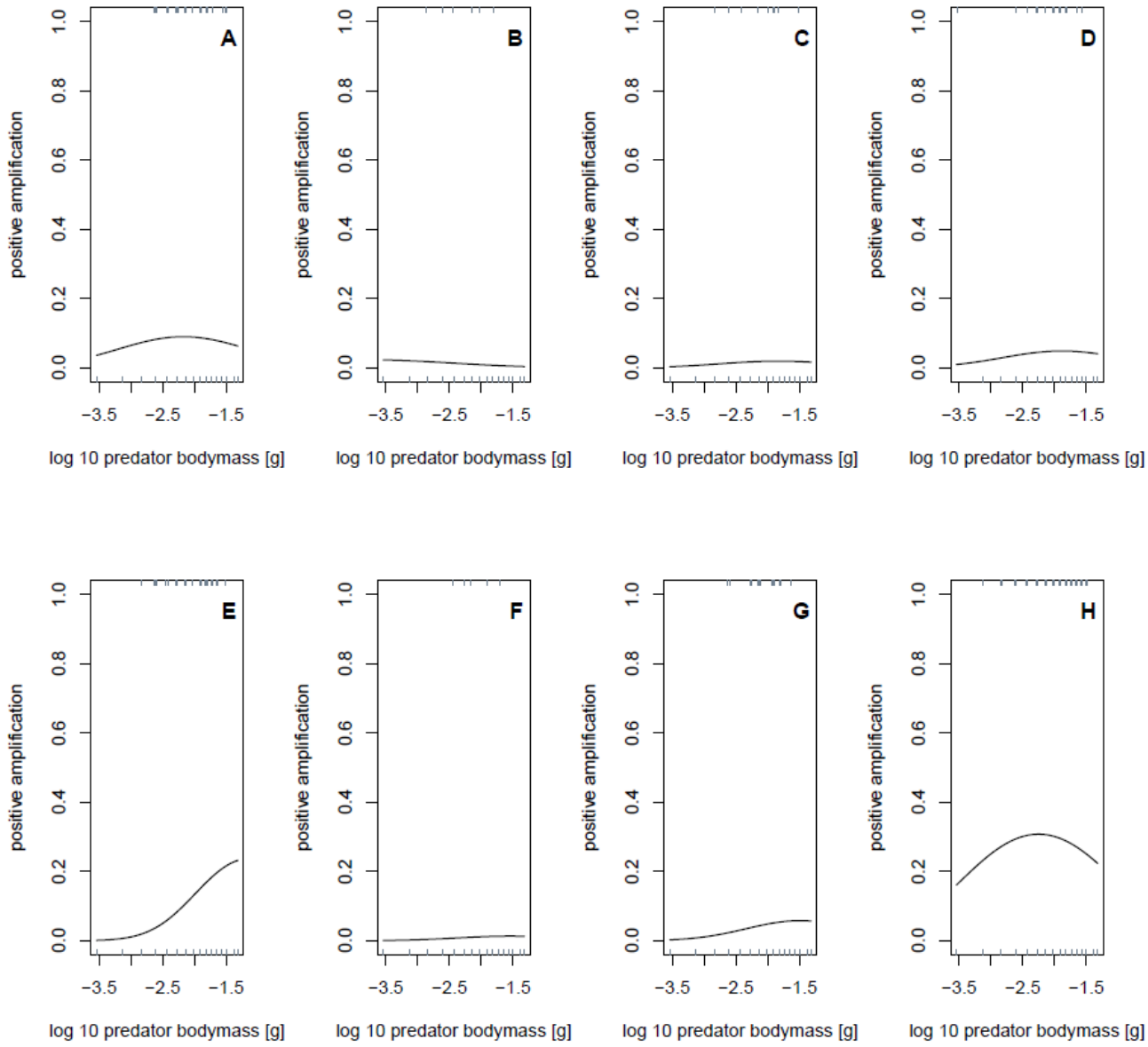

Fig 3. Body-size-dependent probability of positive detection of eight prey taxa in lithobiid predators (n= 532) collected in the Hainich forest in autumn 2009 and spring 2010. **A** Lumbricida, **B** Araneae, **C** Staphylinidae, **D** Isopoda, **E** Diptera, **F** Oribatidae, **G** Gamasidae, **H** Collembola. Rugs on top and bottom of each diagram display single data points with values 1 or 0.

Discussion

The present study, for the first time gives strong evidence that functional responses, despite their reductionist approach, are an appropriate method to assess predator-prey interactions

under field conditions. Positive correlation for most extraguild prey and staphylinid beetle prey suggest that explanatory power of functional responses is high, while results for intraguild prey and oribatid mites may be biased due to prey identity effects which are not considered in the models, such as defence mechanism. Here, results support previous studies emphasizing that body mass ratios are sufficient to calculate realistic functional responses (Vucic-Pestic *et al*. 2010, Kalinkat *et al*. 2013b), indicating that foraging behaviour is mainly driven by allometric rather than taxonomic constraints.

Predators of same size are confronted with similar challenges resulting in similar patterns (Peters 1983; Woodward *et al*. 2005). For example, metabolism increases with body size, requiring higher energy uptake by larger individuals which eventually leads to ingestion of more prey biomass, i.e. more small prey or larger prey individuals (Kalinkat, *et al*. 2011). Indeed, with predators becoming heavier more individuals were tested positive for prey DNA, indicating higher frequency of prey capture. This is also supported by our functional response model showing increased feeding rates. DNA detection frequency of spiders, lumbricids and collembolans, however, followed a hump-shaped curve, declining at low but also high body mass. Hump-shaped relationships between predator body mass and prey feeding are characterised by decreasing capture success above an optimum body mass ratio between predator and prey, resulting from an optimal ratio between costs of prey handling and benefits of energy uptake (Aljetlawi *et al.* 2004; Brose *et al*.2008; Vucic-Pestic *et al.* 2010). Using a combined taxonomic - allometric model, Rall *et al.* (2011) calculated an optimal body mass ratio of about 650 between *Lithobius forficatus* and the collembolan species *Heteromurus nitidus*. In our study a similar ratio also applied to the two collembolan species *L. lanuginosus* and *P. armata.* Medium-sized lithobiids, however, also fed intensively on earthworms which are much larger, apparently contradicting the calculated optimal predator – prey body mass ratio. Potentially, centipedes did not feed on large lumbricids but on juveniles of small epigeic species such as *Lumbricus castaneus*. The decline in feeding on lumbricids by large lithobiids may be due to difficulties of large lithobiids in following juvenile lumbricids in the dense lower litter layer where most lumbricids dwell (Phillipson *et al*. 1976). The strong increase in detecting dipteran prey with lithobiid body size indicates prey switching in larger lithobiid specimens to dipteran larvae as prey (Hohberg & Traunspurger 2005; Brose *et al.* 2008; Petchey *et al.* 2008). Dipteran larvae are abundant prey of high nutritional value (Oelbermann and Scheu 2002) and lithobiids prefer prey < 1 cm but also

feed on large tipulid larvae of 2.5 cm (Poser 1988).

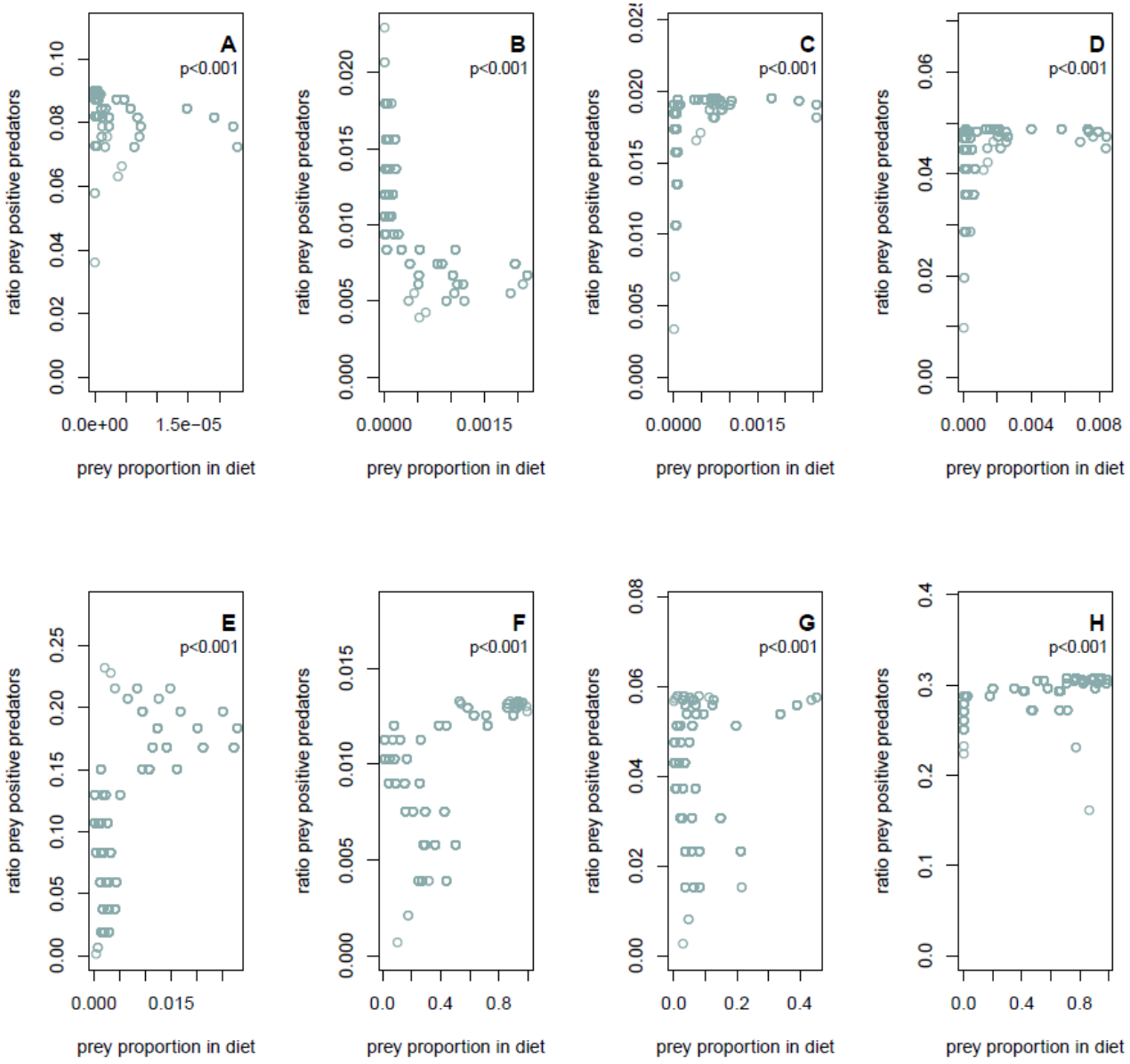

Fig 4. Pearson correlation coefficient test between functional response models (output: prey proportion in diet) and results of GLM with molecular gut content data (output: ratio of prey-positive tested predators) of centipede predator *Lithobius* sp. and eight different prey groups. **A** Lumbricida, **B** Araneae, **C** Staphylinidae, **D** Isopoda, **E** Diptera, **F** Oribatidae, **G** Gamasidae, **H** Collembola

Geophilomorph centipedes exhibited a hump-shaped feeding relationship with all prey types indicating no prey shift. Unlike lithobiids, geophilomorphs use specific techniques including hunting in collectives to subdue and kill prey larger than themselves particularly earthworms (Lewis 1981). Consequently, functional response models ignoring such traits presumably are

of limited use for predicting prey consumption by these large predators.

Contrary to our assumptions, prey abundance did not affect centipede feeding rates but rather was driven by prey type, indicating that prey-specific characteristics are important. While functional responses on oribatid and gamasid mite prey are high due to optimal body mass ratios and high abundances, feeding rates are consistently low. Due to their strong sclerotization and production of toxins mites are little fed by predators and have been suggested to live in enemy-free space (Peschel *et al*. 2006; Heethoff *et al*. 2011).

Collembolans are of high nutritional value (Bilde & Toft 2000, Agusti *et al*. 2003) and reach high density explaining the high feeding on collembolans by lithobiids. However, the functional response model also suggests that other factors, such as habitat structure, modify feeding on collembolans. Indeed, the results suggest that thick litter layers provide refuge for small prey, thereby reducing encounter rate and forcing predators to extend prey search time (Kalinkat *et al*. 2013a, Vucic-Pestic *et al*. 2010, Hohberg & Traunspurger 2005). In particular lower layers comprising small litter fragments such as the H horizon provides refuge from predation by large lithobiids (Günther *et al*. unpublished data).

Detection rate of intraguild prey, i.e. feeding on gamasid mites, spiders and staphylinid beetles, differed markedly between lithobiid and geophilomorph centipedes. Particularly small lithobiids did not feed on other predators while detection frequency of intraguild prey in lithobiids and particularly geophilomorphs increased with predator body size. This confirms earlier studies showing that the prey spectrum of predators increases with predator body size suggesting that large predators more efficiently exploit prey communities (Cohen *et al.* 1993; Woodward & Hildrew 2002). The results therefore contradict suggestions that at high density of extraguild prey intraguild predation is negligible (Halaj & Wise 2002; Eitzinger & Traugott 2011). Further, the results object findings that the role of intraguild predation is reduced in structured habitats providing refuge for intraguild prey (Finke & Denno 2002; Janssen *et al*. 2007).

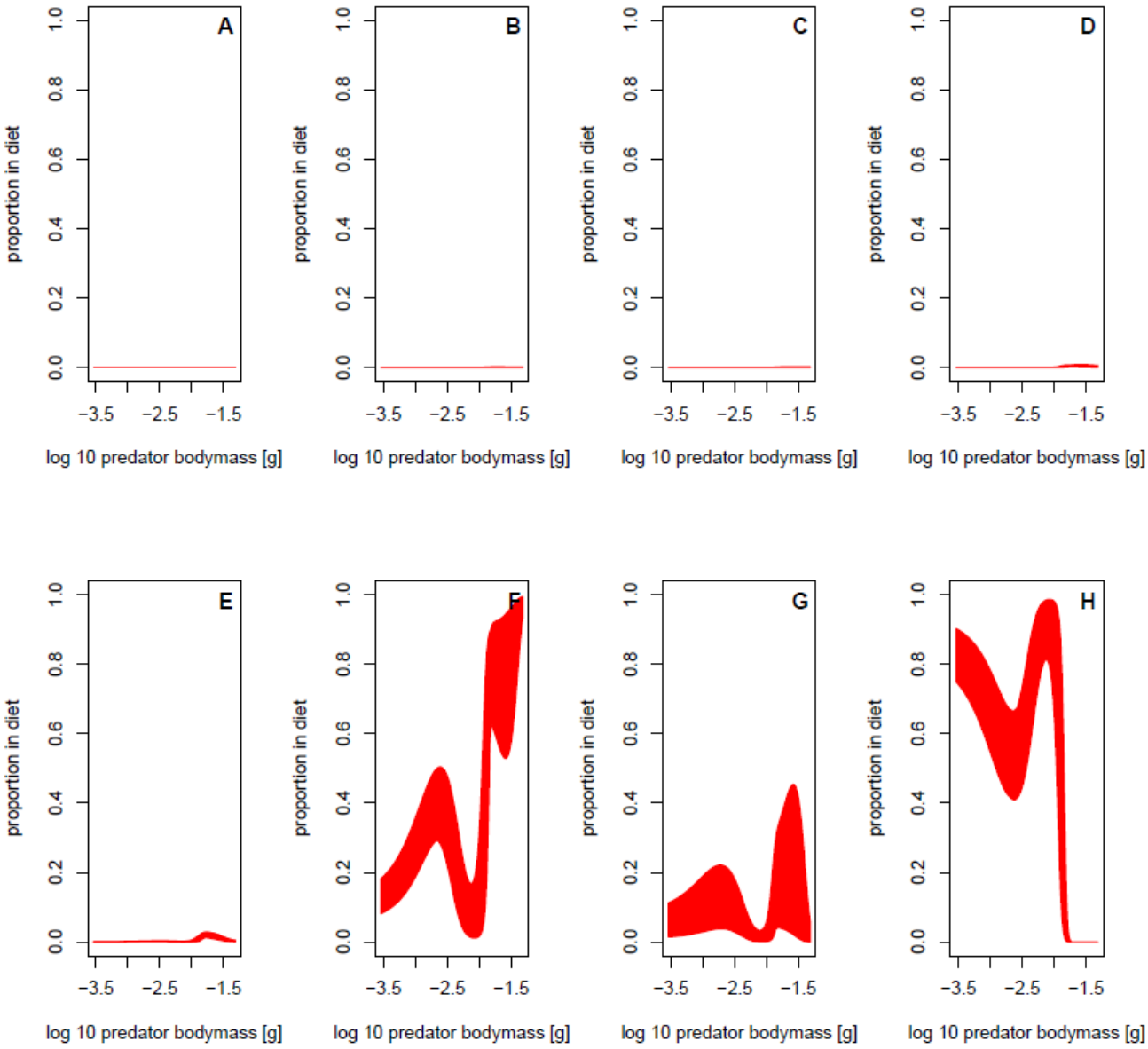

Fig. 5. Body-size-dependent proportion of eight prey taxa in centipede predators as based on abundance and body-size data of invertebrates collected in the Hainich forest in autumn 2009 and spring 2010. **A** Lumbricida, **B** Araneae, **C** Staphylinidae, **D** Isopoda, **E** Diptera, **F** Oribatidae, **G** Gamasidae, **H** Collembola. Upper and lower limit indicate highest and lowest diet proportion found in the four forest sites.

Caveats

The present study, for the first time, investigated the impact of predator body size and prey

abundance on predator consumption using two different methods, functional response models and molecular gut content analysis. Both methods proved to be useful to study trophic interactions, the first one to analyse whole food webs based on body-size ratios and abundances, the latter to examine predator prey interactions of individual predators on small scale (King *et al*. 2008). While these methods measure different parameters, feeding rate and prey DNA detection frequency, respectively, we demonstrated that results are comparable, allowing to study trophic interactions in a more comprehensive way than in previous studies. However, we would like to point out that some factors, immanent to the methods used may have affected our results. Detection success of prey DNA in a predator is influenced by a variety of factors of which the sensitivity of the PCR assay is among the most important. Applying PCR assays targeting different genes can lead to varying detection success resulting in over-or underestimation of feeding rates (Eitzinger *et al*. unpublished data). Moreover, molecular gut content analysis only yields data on absence or presence of prey, but not on the amount of prey ingested (King *et al*. 2008). Despite these pitfalls, results of gut content analysis and functional responses were widely consistent encouraging further studies combining these methods and helping to unravel the functioning of soil food webs and the factors affecting it.

Conclusions

Our results highlight the value of functional response models to assess predator-prey interactions under field conditions but also stress the usefulness of molecular gut content analysis to investigate prey choice of generalist predators at complex environmental settings such as those in soil. Comparing functional responses with gut content analyses and including predator-prey body size ratios we are able to explain a majority of feeding interactions in belowground systems. This supports previous assumptions emphasizing that allometric rather than taxonomic constraints are more powerful predictors for food web links (Rall *et al*. 2011). Further, in contrast to food webs in simply structured habitats, such as aquatic systems, prey abundance did not affect prey ingestion in soil pointing to the importance of prey identity effects as driving factors.

Acknowledgments

The work has been funded by the DFG Priority Program 1374 “Infrastructure-Biodiversity-Exploratories”. Field work permits were given by state environmental office of Thuringia according to § 72 BbgNatSchG. We thank the managers of the three exploratories, Swen Renner, Sonja Gockel, Kerstin Wiesner, and Martin Gorke for their work in maintaining the plot and project infrastructure; Simone Pfeiffer and Christiane Fischer giving support through the central office, Michael Owonibi for managing the central data base, and Markus Fischer, Eduard Linsenmair, Dominik Hessenmöller, Jens Nieschulze, Daniel Prati, Ingo Schöning, François Buscot, Ernst-Detlef Schulze, Wolfgang W. Weisser and the late Elisabeth Kalko for their role in setting up the Biodiversity Exploratories project. We particularly want to thank Olga Ferlian, Stephan Töppich and David Ott for great help with fieldwork, Bernhard Klarner for providing data on prey abundance and Christoph Digel and Amrei Binzer for help with statistics.

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Chapter 6

General discussion

I used molecular gut content analysis to unravel the trophic linkages of centipede predators and to identify factors driving prey choice and predation strength. I showed that centipedes are generalist predators as is also revealed in prey choice and feeding experiments (*e.g.* Lewis 1981, Pfleiderer-Gruber 1986, Poser 1988). The unspecific feeding ecology of centipedes helps explain the success and wide distribution of this ancient predator taxon. It is widely distributed in the World's temperate forests and frequently at high abundance (Lewis 1981). However, the details of the top-down trophic impact of prey choice and predation by different centipede species depend on a variety of environmental and biological factors, which are discussed in the following.

In Chapter 4, I demonstrate clearly that centipede prey choice is independent of forest management type. More important factors are soil pH, habitat structure, predator body size and prey density. The important influence of these factors on trophic interactions is also clear from fatty acid analysis (Ferlian *et al.* 2011) and stable isotope analysis (Klarner *et al.* in prep). Despite centipede generalist feeding behaviour, different species and size classes differ in their preference for specific prey organisms. The fatty acids in lithobiid body tissue reveal that this group make use of the fungal energy channel, one of the three pathways of nutrient fluxes in soil food webs (Moore & Hunt 1988, Scheu *et al.* 2005). As most collembolan species feed predominantly on fungi, collembola are likely to be the preferred prey of this group, which could be confirmed in the present study (Chapter 4 and 5).

I found clear evidence for the influence of habitat structure (Chapter 4). The thick litter layer of established coniferous and beech forests not only provides the basis for fungal growth, and consequently plentiful food for collembola but also negatively affects lithobiid feeding success. The small predator, *Lithobius crassipes*, more easily detected its prey in litter than did the larger *L. mutabilis*. Small centipedes benefit from the additional structure of deep litter (Fründ 1987, Jabin 2007) while large *L. mutabilis* are less able to penetrate the thick litter

layer. The results also suggest that habitat structure may affect food web stability by damping intraguild predation between centipedes of different sizes (Finke & Denno 2002, Janssen *et al.* 2007). Complex habitat structure not only reduces the encounter rate between large predators and their prey but also that between large and small predators. This facilitates the coexistence of predator species (Langellotto & Denno 2004). Such a mechanism would contribute to the great diversity of prey and predators in soil (Scheu 2002).

In contrast to the lithobiids, geophilomorph centipedes feed predominantly on earthworms as their bodies contain a high proportion of bacterial fatty acids (Ferlian *et al.* 2011) and bacteria are the predominant food of earthworms indicating feeding on earthworms. This is what I confirmed by revealing that geophilomorphs have no strong prey preferences compared to lithobiids (Chapter 5). This is understandable since, unlike lithobiids, which use a sit-and-wait ambush strategy to catch fast moving prey in the litter layer, the worm-shaped geophilomorphs are active hunters, pursuing and killing earthworms in small burrows in soil and litter layers (Lewis 1981). The geophilomorphs consequently encounter a greater proportion of low mobility prey species than do lithobiids. This difference is reflected in the differences between their gut contents.

Large lithobiids consume different collembolan and dipteran prey species in proportion to their specific densities. Prey density did not, however, affect overall predation (Chapter 4 and 5). *Lithobus crassipes* did not profit from higher collembolan abundance as it is more prone to prey defence mechanisms such as swarming (Vucic-Pestic *et al.* 2010). Functional response models (Chapter 5) predict increasing feeding rates with body size. However, they failed to differentiate between collembola, oribatid mite and gamasid mite prey. The low predation rates on mites are caused by prey-specific traits such as their heavily sclerotised bodies and their production of toxins. These defences protect mites from centipedes and thus relieve mites of much predation (Peschel *et al.* 2006, Heethoff *et al.* 2011).

The most important factor affecting centipede feeding is predator body size (Chapter 4 and 5). As large centipedes feed more often due to their large nutritional requirements, the probability of detecting prey DNA in large predators is higher than in small ones. In addition, large centipedes exhibit a wider prey spectrum than small ones. This difference is because large centipedes have lower overall handling times than small species (Woodward & Hildrew 2002; Riede *et al.* 2011). Predators of the same size are confronted with similar challenges resulting

in similar patterns (Peters 1983; Woodward *et al.* 2005). This means, therefore, that size, rather than taxonomy, affect feeding behaviour of same size predators, which was confirmed by the analysis of prey choice in nine lithobiid and three geophilomorph species (Chapter 5).

For most prey species there was a humpbacked relationship between prey size and predator size. This means that predator species eat fewer prey the more the prey body mass deviates from an optimum relative to the body mass of the predator species, as is common for soil predators (Brose *et al.* 2008). The predation success of small predators is low because they exploit a relatively small search area and have long handling times. Large predators have difficulties in catching small prey because these prey have high escape efficiencies (Vucic-Pestic *et al.* 2010). The trophic interaction strengths between small prey and large predators is therefore low and plays a minor role in centipede feeding ecologies (Brose *et al.* 2008). In contrast to other prey, DNA detection rates for dipteran larvae increases exponentially with body mass in lithobiid predators (Chapter 4 and 5). Due to their high nutrient requirements, large predators may be forced to switch to alternative prey that provide more energy with lower expenditure. Such switching is facilitated by the wide foraging area that large predators can exploit. Prey switching is thought to play a key role in food web stability. They may damp top-down pressure and so help to explain weak trophic interactions (Moore *et al.* 1988, Post *et al.* 2000).

The last chapter compares the predictability of feeding strength and prey choice from MGCA and functional response models. The more a predator species tested positive for a prey species the higher its feeding rate on that prey species, as I expected. However, there was a negative correlation for intraguild prey such as spiders and gamasid mites as well as for lumbricids. It is thus clear that prey-specific traits significantly affect feeding. They must therefore be fully taken into account if functional response results are to be correctly interpreted.

My study is the first to validate functional response models for predicting predation patterns. Furthermore, it also demonstrates that molecular gut content analysis is a reliable method for assessing feeding rates through prey DNA detection rates. This is especially important as PCR-based analyses examining gut content, regurgitates and faeces are prone to bias through variation in DNA detection success. In Chapters 1 and 2, I discuss these factors and focus on the effects of body size and prey quality on DNA detection success. These effects have not been adequately examined. I tested the hypotheses that DNA retention time in the predator

gut is negatively correlated with predator body size and prey quality. This relationship arises because large centipedes digest their prey more rapidly than small ones and high quality prey (defined as prey with a low carbon-nitrogen ratio) is more quickly processed than low quality prey (Lundgren & Weber 2010, Karasov *et al.* 2011). None of my hypotheses could be proved but the results indicated that prey DNA detection success is largely dependent on PCR assay sensitivity and DNA copy number in prey tissue. Highly sensitive PCR assays and high DNA copy number may lead to high prey DNA detection rates assuming a high consumption rate while the opposite is true for low sensitivity assays and low DNA copy number. Results of the present study stress the importance of standardising PCR assays in order to correctly interpret DNA prey detection results, particularly in generalist predators.

Conclusion & Outlook

I investigated prey choice and predation strength for centipede predators in forest soils and analysed the factors affecting them. I showed that centipedes are generalist predators, preferring soft-tissued prey such as collembola, dipteran larvae and earthworms, while intraguild prey and heavily sclerotised oribatid mites are rarely fed on. I identified habitat structure, prey density and, particularly, predator body size as the prime determinants of prey choice and strength of trophic interaction. The type of forest management type had no effect. I also showed that functional responses are significantly correlated with prey DNA detection rates. My study therefore provides important information on forest soil food webs and helps to elucidate the connectivity and strength of trophic links below ground. It will also contribute to important debates on how prey choice and prey switching are connected with predator diversity and soil food web stability.

Future work should therefore focus on the effects of multiple predators on mutual prey as well as on interactions among the predator community. Predator body size affects the strength of tropic interactions in feeding trials, which can lead to loss of species (Schneider *et al.* 2012). By applying multiplex PCR, which is able to screen a multitude of predator species for extra- and intraguild prey, we will be able to test these results under field conditions in various habitats.

In order to examine factors leading to prey switching, functional response experiments with predators of different body size, using multiple prey and varying habitat structure should be

conducted. This would enhance exisiting knowledge (Vucic-Pestic *et al.* 2010, Kalinkat *et al.* 2011, 2013) and provide a more realistic view of functional responses in soil systems.

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Acknowledgments

Ich danke...

….ganz besonders Stefan Scheu, für die äußerst interessante Arbeit, die hervorragende Betreuung und vor allem dem großen Vertrauen das in mich gesteckt wurde.
….Michael Traugott, der nicht nur immer ein zuverlässiger und geduldiger Ansprechpartner bei meinen häufigen Fragen war sondern auch manchmal den entscheidenden Anstoß gegeben hat weiterzumachen, wenns mal nicht ging.
….Mark Maraun, der immer ein offenes Ohr für meine Ideen und Anliegen hat und jegliche meiner Fragen zu Statistik zu beantworten wusste.
…. den Explorern Melanie, Georgia, Olga, Bernhard, David und Christian, für die schöne und lehrsame Zeit, die wir gemeinsam im Feld, hinterm Bino oder einfach beim Feierabendbier verbracht haben.
…der gesamten Molli-Gruppe, dafür dass es trotz engem Zeitplan, kaputten Cyclern oder fehlenden Eppis immer gute Laune im Labor und eine helfende Hand gab wenn sie nötig war.
….Kerstin, ohne die die Gut-Content –Gruppe nur halb so erfolgreich wäre. Ganz besonders danke für die Unterstützung zur Umsetzung des Molli-Kurses!
….Alex, der mich bei meinen Laborversuchen immer unterstützt hat aber mir auch zeigte, dass man durch feiern noch besser arbeiten kann.
…Björn Rall, der mich in die Welt von R, GLM und functional responses einführte.
…thanks to Kristy and Andrew Davis, for patiently proof-reading my manuscript.
… der gesamten AG Scheu für Hilfe und Freundschaft in den letzten Jahren.
…. Meinen Innsbrucker Freunden, die trotz großer Entfernung immer für mich da waren.
…und vor allem meinen Eltern, die mich immer mit großer Liebe unterstützt haben und mich nie zweifeln ließen an dem was ich tat.

Allen hiermit ein herzliches Dankeschön! Thank you very much!

List of publications

Published in peer-reviewed journals:

Eitzinger, B. & Traugott, M. (2011). Which prey sustains cold-adapted invertebrate generalist predators in arable land? Examining prey choices by molecular gut-content analysis. *Journal of Applied Ecology*, 48, 591–599.

Eitzinger, B., Micic, A., Körner, M., Traugott, M. & Scheu, S. (2013). Unveiling soil food web links: New PCR assays for detection of prey DNA in the gut of soil arthropod predators. *Soil Biology and Biochemistry*, 57, 943–945.

Submitted:

Eitzinger, B., Unger, E. M., Traugott, M., Scheu. Effects of prey quality and predator body size on prey DNA detection success in a centipede predator

Günther, B., Rall, B.C., Ferlian, O., Scheu, S. & **Eitzinger, B**. Variations in prey choice of invertebrate soil predators with forest type as indicated by molecular gut content analysis

Klarner, B., Ehnes, R., Erdmann, G., **Eitzinger, B**., Pollierer, M.M., Maraun, M. & Scheu, S. Trophic shift of soil animal species with forest type as indicated by stable isotope analysis

Ehnes, R.B., Pollierer, M.M., Erdmann, G., Klarner, B., **Eitzinger, B**., Digel, C., Ott, D., Maraun, M., Scheu, S. & Brose, U. Lack of energetic equivalence in forest soil invertebrates

Thesis declaration

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

Chapter 2 comprises a manuscript that has been published in a peer-reviewed journal; Chapter 3 and 4 comprise mansucripts that are currently submitted to peer reviewed journals. In all manuscripts except that presented in Chapter 4 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. Chapter 2 contains work done by A. Micic in her diploma thesis, as well as M. Körners bachelor thesis. I also

supervised the master thesis of B. Günther which is presented in Chapter 4. E. M. Ungers bachelor thesis formed part of Chapter 3.

To the study presented in Chapter 4 I contributed to the design, data analysis and writing of the manuscript. 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.

Bernhard Eitzinger Göttingen, July 2013

APPENDIX

Appendix Chapter 2

Supplementary Material S1. DNA sequencing protocol

Mitochondrial Cytochrome C oxidase subunit I (COI) gene (710 bp)

Collembolan DNA extracts were subjected to PCR using universal invertebrate primers LCO1490 and HCO2198 (Folmer *et al*. 1994). Each 25 µL PCR contained 12.5 µL PCR Mastermix (2×), 2 mM $MgCl_2$ (both Geneaxxon, Ulm, Germany), 1 µL bovine serum albumin (BSA, 3%; Roth, Karlsruhe, Germany), 0.8 µM of each primer, 6.5 µL PCR-water and 3 µL of DNA extract. Thermocycling included 95 °C for 10 min, 35 cycles of 95 °C for 30 s, 48 °C for 30 s, 72 °C for 90 s and a final elongation at 72 °C for 10 min. PCR products were purified with QIAquick Purification Kit (Qiagen, Hilden, Germany) and sequenced in both directions using primers LCO1490 and HCO2198 at the Institute of Microbiology, University of Goettingen, Germany.

Nuclear 18S ribosomal DNA (1800 bp)

DNA extracts of dipterans, gamasid mites, spiders and staphylinid beetles were amplified with primers 18Sforward and 18Sreverse and subsequently sequenced using 18Sforward, 18Sreverse, 18S554f, 18S1282r, 18S1150f and 18S614r (Turbeville *et al*. 1991). Sequencing protocol was same as for COI (see above) but with 57 °C annealing temperature.

References

Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R.,1994. DNA primers for the amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3, 294–299.

Turbeville, J. M., Pfeifer, D.M., Field, K.G., Raff, R.A., 1991. The Phylogenetic Status of Arthropods, as inferred from 18S rRNA Sequences. Molecular Biology and Evolution 8, 669-686.

Table S1: Details of sequences downloaded from GenBank used for design of general primers targeting dipterans, gamasid and oribatid mites, spiders, staphylinid beetles and woodlice.

Class	Order	Family	Species	GenBank Accession number
Arachnida	Araneae	Amaurobiidae	*Amaurobius similis* (Blackwall, 1861)	DQ628699
			Amaurobius similis (Blackwall, 1861)	DQ628736
			Coelotes terrestris (Wider, 1834)	AJ007986
			Coelotes terrestris (Wider, 1834)	DQ628761
			Coelotes terrestris (Wider, 1834)	DQ628762
		Clubionidae	*Clubiona pseudogermanica* Schenkel, 1936	AY633869
		Hahniidae	*Hahnia clathrata* Simon, 1898	FJ948881
			Hahnia clathrata Simon, 1898	FJ948923
		Linyphiidae	*Diplocephalus cristatus* (Blackwall, 1833)	GU338490
			Microneta viaria (Blackwall, 1841)	FJ838708
			Microneta viaria (Blackwall, 1841)	GU338502
			Walckenaeria clavicornis (Emerton, 1882)	GU338483
			Walckenaeria keikoae H. Saito, 1988	GU338484
		Theridiidae	*Robertus neglectus* (O. P.-Cambridge, 1871)	AY230922
		Tetragnathidae	*Pachygnatha degeeri* Sundevall, 1830	EU003363
		Thomisidae	*Xysticus croceus* Fox, 1937	AY671972
Arachnida	Mesostigmat	Parasitidae	*Pergamasus cf. canestrinii* (Berlese, 1884)	AY620934
			Pergamasus sp. Berlese, 1904	FJ911803
		Veigaiidae	*Veigaia* sp. Oudemans, 1905	AY620941
			Veigaia sp. Oudemans 1905	FJ911804
Arachnida	Oribatida	Achipteriidae	*Achipteria coleoptrata* (Linnaeus, 1758)	EF091418
		Camisiidae	*Platynothrus peltifer* (C. L. Koch, 1839)	EF091422
			Platynothrus peltifer (C. L. Koch, 1839)	GQ864291
		Chamobatidae	*Chamobates voigtsi* (Oudemans, 1902)	EU432189
		Galumnidae	*Galumna lanceata* (Oudemans, 1900)	EU432197
		Gehypochthoni	*Gehypochthonius urticinus* (Berlese, 1910)	EU433994
		Hypochthoniid	*Hypochthonius rufulus* (C. L. Koch, 1836)	EF091427
			Hypochthonius rufulus (C. L. Koch, 1836)	EF093784
			Hypochthonius rufulus (C. L. Koch, 1836)	EF093785
		Nothridae	*Nothrus silvestris bistilus* Jacot, 1937	EF081305
			Nothrus silvestris Nicolet, 1855	AF022039
			Nothrus silvestris Nicolet, 1855	EF091425
			Nothrus silvicus Jacot, 1937	EF204473
			Nothrus sp. Koch, 1836	GQ864292

(continued from page 100)

			Nothrus truncatus Banks, 1895	EF081306
		Oribatulidae	*Oribatula tibialis* (Nicolet, 1855)	EU433990
		Phenopelopida	*Eupelops acromios* (Hermann, 1804)	EU432192
			Eupelops hirtus (Berlese, 1916)	EF093782
			Eupelops hirtus (Berlese, 1916)	EF093783
			Eupelops plicatus (C. L. Koch, 1835)	EF091419
		Phtiacaridae	*Atropacarus striculus* (Koch, 1835)	EF091416
			Steganacarus applicatus (Sellnick, 1920)	GQ864301
Insecta	Coleoptera	Staphylinidae	*Acrotona* sp. Thomson, 1859	GQ981091
		Staphylinidae	*Acrotona* sp. Thomson, 1859	GQ981093
		Staphylinidae	*Aleochara lata* Gravenhorst, 1802	EF213791
		Staphylinidae	*Aleochara moerens* Gyllenhal, 1827	GQ981070
		Staphylinidae	*Atheta graminicola* (Gravenhorst, 1806)	GQ981134
		Staphylinidae	*Atheta myrmecobia* Kraatz, 1856	GQ981136
		Staphylinidae	*Atheta myrmecobia* Kraatz, 1856	GQ981137
		Staphylinidae	*Geostiba circellaris* (Gravenhorst, 1806)	GQ981160
		Staphylinidae	*Gyrohypnus* sp. Leach, 1819	AY745637
		Staphylinidae	*Habrocerus capillaricornis* (Gravenhorst, 1806)	AY745613
		Staphylinidae	*Lathrobium brunnipes* (Fabricius, 1793)	AY745634
		Staphylinidae	*Leptusa kitazawai* (Sawada, 1970)	FJ749926
		Staphylinidae	*Liogluta microptera* Thomson, 1867	GQ981142
		Staphylinidae	*Liogluta nigropolita* Bernhauer, 1907	GQ981144
		Staphylinidae	*Oxypoda praecox* Erichson, 1839	GQ981089
		Staphylinidae	*Quedius mesomelinus* (Marsham, 1802)	AJ810738
		Staphylinidae	*Tachyporus* sp. Gravenhorst, 1802	DQ337148
		Staphylinidae	*Xantholinus linearis* (Olivier, 1795)	AY745633
Insecta	Diptera	Chironomidae	*Telmatogeton macswaini* Wirth, 1949	GU356739
		Muscidae	*Musca domestica* Linnaeus, 1758	GQ465780
		Stratiomyidae	*Hermetia illucens* (Linnaeus, 1758)	GQ465779
		Tipulidae	*Tipula* sp. Linnaeus, 1758	X89496
Malacostrac	Isopoda	Armadillidiidae	*Armadillidium vulgare* (Latreille, 1804)	AJ267293
		Ligiidae	*Ligidium hypnorum* (Cuvier, 1792)	AJ287056
		Oniscidae	*Oniscus asellus* Linnaeus, 1758	AY692348
		Oniscidae	*Oniscus asellus* Linnaeus, 1758	AF255699
		Oniscidae	*Oniscus asellus* Linnaeus, 1758	AJ287057
		Philosciidae	*Philoscia muscorum* (Scopoli, 1763)	AJ287058
		Porcellionidae	*Porcellio scaber* Latreille, 1804	AJ287062

Table S2: Cross reactivity set of 120 target and nontarget species tested with newly designed primers.

"+" denotes positive, "-" negative amplification using listed annealing temperatures

Species marked with "NA" were not tested against specific primer

No	phylum	class	order	family	*species*	Araneae group ARA S5/ ARA A5 (68 °C)	Diptera group DIP S16/ DIP A17 (60 °C)	Gamasina group GAM S7/ GAM A8 (63 °C)	Isopoda group ISO S6/ ISO A3 (60 °C)
1	Annelida	Oligochaeta	Opisthopora	Lumbricidae	*Aporrectodea caliginosa* (Savigny, 1826)	-	-	-	-
2					*Dendrobaena octaedra* (Savigny, 1826)	-	-	-	-
3					*Lumbricus terrestris* Linnaeus, 1758	-	-	-	-
4					*Octolasion cyaneum* (Savigny, 1826)	-	-	-	-
5			Tubificida	Enchytraeidae	*unidentified specimen*	-	-	-	-
6	Arthropoda	Arachnida	Araneae	Agelenidae	*Coelotes inermis* (L. Koch, 1855)	+	-	-	-
7					*Coelotes terrestris* (Wider, 1834)	+	-	-	-
8					*Histopona torpida* (C. L. Koch, 1837)	+	-	-	-
9				Clubionidae	*Clubiona terrestris* Westring, 1851	+	-	-	-
10				Dysderidae	*Harpactea lepida* (C. L. Koch, 1838)	+	-	-	-
11				Linyphiidae	*Diplocephalus picinus* (Blackwall, 1841)	+	-	-	-
12					*Micrargus apertus* (O. P.-Cambridge, 1871)	+	-	-	-
13					*Micrargus herbigradus* (Blackwall, 1854)	+	-	-	-
14					*Microneta viaria* (Blackwall, 1841)	+	-	-	-
15					*Tapinocyba insecta* (L. Koch, 1869)	+	-	-	-
16					*Tenuiphantes tenebricola* (Wider, 1834)	+	-	-	-
17					*Walckenaeria cucullata* (C. L. Koch, 1836)	+	-	-	-
18					*Walckenaeria cuspidata* Blackwall, 1833	+	-	-	-
19					*Walckenaeria obtusa* Blackwall, 1836	+	-	-	-
20				Salticidae	*Neon reticulatus* (Blackwall, 1853)	+	-	-	-
21				Theridiidae	*Robertus lividus* (Blackwall, 1836)	+	-	-	-
22			Mesostigmata	Parasitidae	*Parasitus lunulatus* (J. Müller, 1859)	-	-	+	-
23					*Pergamasus septentrionalis* (Oudemans, 1902)	-	-	+	-
24				Polyaspididae	*Trachytes aegrota* (C.L. Koch, 1841)	-	-	-	-
25					*Uroseius cylindricus* (Berlese, 1916)	-	-	-	-
26				Urodinychidae	*Urodiaspis tecta* (Kramer, 1876)	-	-	-	-
27				Uropodina	*Uropoda cassidea* (Hermann, 1804)	-	-	-	-
28				Veigaiidae	*Veigaia nemorensis* (C.L. Koch 1839)	-	-	+	-
29				Zerconidae	*Zercon gurensis* Mihelcic, 1962	-	-	+	-

30				*Zercon vagabundus* Karg, 1971	-	-	+	-
31		Opiliones	Trogulidae	*Anelasmocephalus cambridgei* (Westwood, 1847)	-	-	-	-
32				*Trogulus nepaeformis* (Scopoli, 1763)	-	-	-	-
33		Oribatida	Achipteriidae	*Achipteria coleoptrata* (Linnaeus, 1758)	-	-	-	-
34			Belbidae	*unidentified specimen*	-	-	-	-
35			Camisiidae	*Platynothrus peltifer* (C. L. Koch, 1839)	-	-	-	-
36			Carabodidae	*Carabodes sp.* C. L. Koch, 1835	-	-	-	-
37			Cepheidae	*Tritegeus bisulcatus* Grandjean, 1953	-	-	-	-
38			Galumnidae	*Galumna sp.* Von Heyden, 1826	-	-	-	-
39			Liacaridae	*Liacarus sp. Michael, 1898*	-	-	-	-
40			Oribatellidae	*Oribatella calcarata (C. L. Koch, 1836)*	-	-	-	-
41			Oribatellidae	*Oribatella quadricornuta (Michael, 1884)*	-	-	-	-
42			Oribatidae	*Hypochtonius rufulus C. L. Koch, 1836*	-	-	-	-
43			Phthiacaridae	*Steganacerus magnus (Nicolet, 1855)*	-	-	-	-
44		Pseudoscorpiones	Neobisiidae	*Neobisium carcinoides (Hermann, 1804)*	-	-	-	-
45	Chilopoda	Geophilomorpha	Geophilidae	*Geophilus flavus (De Geer, 1778)*	-	-	-	-
46	Chilopoda	Geophilomorpha	Linotaeiniidae	*Strigamia acuminata Leach (1815)*	-	-	-	-
47	Chilopoda	Lithobiomorpha	Lithobiidae	*Lithobius crassipes L. Koch, 1862*	-	-	-	-
48	Chilopoda	Lithobiomorpha	Lithobiidae	*Lithobius lapidicola Meinert, 1872*	-	-	-	-
49	Chilopoda	Lithobiomorpha	Lithobiidae	*Lithobius muticus C.L. Koch, 1847*	-	-	-	-
50	Chilopoda	Lithobiomorpha	Lithobiidae	*Lithobius nodulipes Latzel, 1880*	-	-	-	-
51	Chilopoda	Lithobiomorpha	Lithobiidae	*Lithobius mutabilis L. Koch, 1862*	-	-	-	-
52	Diplopoda	Chordeumatida	Mastigophorophyllidae	*Haploporatia eremita (Verhoeff, 1909)*	-	-	-	-
53	Diplopoda	Glomerida	Glomeridae	*Glomeris marginata (Villers, 1789)*	-	-	-	-
54	Diplopoda	Glomerida	Glomeridae	*Glomeris sp. Latreille, 1803*	-	-	-	-
55	Diplopoda	Polydesmida	Polydesmidae	*Polydesmus complanatus (Linnaeus, 1761)*	-	-	-	-
56	Entognatha	Collembola	Entomobryidae	*Sinella curviseta (Brook, 1882)*	NA	NA	NA	NA
57	Entognatha	Collembola	Isotomidae	*Isotomurus palustris (Muller, 1776)*	NA	NA	NA	NA
58	Entognatha	Collembola	Isotomidae	*Proisotoma minuta (Tullberg, 1871)*	NA	NA	NA	NA
59	Entognatha	Collembola	Neanuridae	*Neanura muscorum (Templeton, 1835)*	NA	NA	NA	NA
60	Entognatha	Collembola	Onychiuridae	*Supraphorura furcifera (Borner, 1901)*	NA	NA	NA	NA
61	Entognatha	Collembola	Tomoceridae	*Tomocerus vulgaris (Tullberg, 1871)*	NA	NA	NA	NA
62	Entognatha	Collembola	Entomobryidae	*Heteromurus nitidus (Templeton, 1835)*	-	-	-	-
63	Entognatha	Collembola	Entomobryidae	*Lepidocyrtus lanuginosus (Gmelin, 1788)*	-	-	-	-
64	Entognatha	Collembola	Hypogastruridae	*Ceratophysella denticulata (Bagnall, 1941)*	-	-	-	-
65	Entognatha	Collembola	Hypogastruridae	*Hypogastrura burkilli (Bagnall, 1940)*	NA	NA	NA	NA
66	Entognatha	Collembola	Isotomidae	*Folsomia candida Willem, 1902*	NA	NA	NA	NA

67	Entognatha	Collembola	Isotomidae	*Folsomia quadrioculata (Tullberg, 1871)*	-	-	-	-
68	Entognatha	Collembola	Isotomidae	*Isotoma viridis Bourlet, 1839*	-	-	-	-
69	Entognatha	Collembola	Isotomidae	*Parisotoma notabilis (Schaeffer, 1896)*	NA	NA	NA	NA
70	Entognatha	Collembola	Onychiuridae	*Protaphorura armata (Tullberg, 1869)*	-	-	-	-
71	Entognatha	Collembola	Tomoceridae	*Pogonognathellus longicornis (Müller, 1776)*	-	-	-	-
72	Entognatha	Diplura	Campodeidae	*Campodea sp. Westwood, 1842*	-	-	-	-
73	Insecta	Coleoptera	Carabidae	*Abax parallelepipedus (Piller & Mitterpacher, 1783)*	-	-	-	-
74	Insecta	Coleoptera	Carabidae	*Carabus irregularis Fabricius, 1792*	-	-	-	-
75	Insecta	Coleoptera	Carabidae	*Nebria brevicollis (Fabricius, 1792)*	-	-	-	-
76	Insecta	Coleoptera	Carabidae	*Pterostichus melanarius (Illiger, 1798)*	-	-	-	-
77	Insecta	Coleoptera	Carabidae	*Pterostichus oblongopunctatus (Fabricius, 1787)*	-	-	-	-
78	Insecta	Coleoptera	Carabidae	*Pterostichus burmeisteri Heer, 1838*	-	-	-	-
79	Insecta	Coleoptera	Carabidae	*Trechus nigrinus Putzeys, 1847*	-	-	-	-
80	Insecta	Coleoptera	Elateridae	*Athous haemorrhoidalis (Fabricius, 1801)*	-	-	-	-
81	Insecta	Coleoptera	Elateridae	*Athous jejunus Kiesenwetter, 1858*	-	-	-	-
82	Insecta	Coleoptera	Elateridae	*Athous subfuscus (O. F. Muller, 1764)*	-	-	-	-
83	Insecta	Coleoptera	Elateridae	*Dalopius marginatus (Linnaeus, 1758)*	-	-	-	-
84	Insecta	Coleoptera	Lampyridae	*Lamprohiza splendidula (Linnaeus, 1767)*	-	-	-	-
85	Insecta	Coleoptera	Leiodidae	*Nargus anisotomoides (Spence, 1815)*	-	-	-	-
86	Insecta	Coleoptera	Staphylinidae	*Domene scabricollis (Erichson 1840)*	-	-	-	-
87	Insecta	Coleoptera	Staphylinidae	*Eusphalerum sp. Kraatz, 1857*	-	-	-	-
88	Insecta	Coleoptera	Staphylinidae	*Habrocerus capillaricornis (Gravenhorst, 1806)*	-	-	-	-
89	Insecta	Coleoptera	Staphylinidae	*Lathrobium fulvipenne (Gravenhorst, 1806)*	-	-	-	-
90	Insecta	Coleoptera	Staphylinidae	*Othius punctulatus (Goeze, 1777)*	-	-	-	-
91	Insecta	Coleoptera	Staphylinidae	*Philonthus carbonarius (Gravenhorst, 1802)*	-	-	-	-
92	Insecta	Coleoptera	Staphylinidae	*Philonthus laevicollis (Lacordaire, 1835)*	-	-	-	-
93	Insecta	Coleoptera	Staphylinidae	*Rugilus rufipes Germar, 1836*	-	-	-	-
94	Insecta	Coleoptera	Staphylinidae	*Xantholinus laevigatus Jacobsen, 1849*	-	-	-	-
95	Insecta	Coleoptera	Staphylinidae	*Xantholinus tricolor (Fabricius, 1787)*	-	-	-	-
96	Insecta	Dermaptera	Forficulidae	*Chelidurella sp. Verhoeff, 1902*	-	-	-	-
97	Insecta	Diptera	Cecidomyidae	*unidentified specimen*	-	+	-	-
98	Insecta	Diptera	Ceratopogonidae	*unidentified specimen species 1*	-	+	-	-
99	Insecta	Diptera	Ceratopogonidae	*unidentified specimen species 2*	-	+	-	-
100	Insecta	Diptera	Chironomidae	*unidentified specimen*	-	+	-	-
101	Insecta	Diptera	Fanniidae	*Fannia sp. Robineau-Desvoidy, 1830*	-	+	-	-
102	Insecta	Diptera	Rhagionidae	*unidentified specimen*	-	+	-	-
103	Insecta	Diptera	Tipulidae	*unidentified specimen*	-	+	-	-

104		Malacostraca	Isopoda	Ligiidae	*Ligidium cf. hypnorum (Cuvier, 1792)*	-	-	-	+
105		Malacostraca	Isopoda	Porcellionidae	*Porcellio sp. Latreille, 1804*	-	-	-	+
106		Malacostraca	Isopoda	Trichoniscidae	*Trichoniscus pusillus Brandt, 1833*	-	-	-	+
107		Symphyla			*unidentified specimen*	-	-	-	-
108	Mollusca	Gastropoda	Pulmonata	Agriolimacidae	*Deroceras sp. Rafinesque, 1820*	-	-	-	-
109	Mollusca	Gastropoda	Pulmonata	Arionidae	*Arion cf. silvaticus Lohmander, 1937*	-	-	-	-
110	Mollusca	Gastropoda	Pulmonata	Boettgerillidae	*Boettgerilla pallens Simroth, 1912*	-	-	-	-
111	Mollusca	Gastropoda	Pulmonata	Euconulidae	*Euconulus fulvus (O.F. Muller, 1774)*	-	-	-	-
112	Mollusca	Gastropoda	Pulmonata	Patulidae	*Discus rotundatus (O.F. Muller, 1774)*	-	-	-	-
113	Nematoda		Plectida	Plectidae	*Plectus minimus Cobb, 1893*	-	-	-	-
114	Nematoda		Plectida	Plectidae	*Plectus velox Bastian, 1865*	-	-	-	-
115	Nematoda		Rhabditida	Cephalobidae	*Acrobeloides buetschlii (de Man, 1884)*	-	-	-	-
116	Nematoda		Rhabditida	Panagrolaimidae	*Panagrellus sp.Thorne, 1938*	-	-	-	-
117	Nematoda		Rhabditida	Rhabditidae	*Caenorhabditis elegans (Maupas, 1900)*	-	-	-	-
118	Nematoda		Strongylida	Heterorhabditidae	*Heterorhabditis megidis Poinar, Jackson & Klein 1988*	-	-	-	-
119	Nematoda		Tylenchida	Pratylenchidae	*Pratylenchus zeae Graham, 1951*	-	-	-	-
120	Nematomorpha				*unidentified specimen*	-	-	-	-

N0	Lithobius group	Oribatida group	Staphylinidae group	Ceratophysella denticulata	*Folsomia quadrioculata*	Lepidocyrtus lanuginosus	Pogonognathellus longicornis	Protaphorura armata
	LIT S13/ LIT A8 (54 °C)	ORI S14/ ORI A16 (68 °C)	STA S6/ STA A3 (65 °C)	CERDEN S5/ CERDEN A3 (68 °C)	*FOLQUA S4/ FOLQUA A1 (62 °C)*	LEPLAN S3/ LEPLAN A1 (62 °C)	POGLON S4/ POGLON A4 (62 °C)	PROARM S3/ PROARM A3 (68 °C)
1	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-	-

8	-	-	-	-	-		-	-	-
9	-	-	-	-	-		-	-	-
10	-	-	-	-	-		-	-	-
11	-	-	-	-	-		-	-	-
12	-	-	-	-	-		-	-	-
13	-	-	-	-	-		-	-	-
14	-	-	-	-	-		-	-	-
15	-	-	-	-	-		-	-	-
16	-	-	-	-	-		-	-	-
17	-	-	-	-	-		-	-	-
18	-	-	-	-	-		-	-	-
19	-	-	-	-	-		-	-	-
20	-	-	-	-	-		-	-	-
21	-	-	-	-	-		-	-	-
22	-	-	-	-	-		-	-	-
23	-	-	-	-	-		-	-	-
24	-	-	-	-	-		-	-	-
25	-	-	-	-	-		-	-	-
26	-	-	-	-	-		-	-	-
27	-	-	-	-	-		-	-	-
28	-	-	-	-	-		-	-	-
29	-	-	-	-	-		-	-	-
30	-	-	-	-	-		-	-	-
31	-	-	-	-	-		-	-	-
32	-	-	-	-	-		-	-	-
33	-	+	-	-	-		-	-	-
34	-	+	-	-	-		-	-	-
35	-	+	-	-	-		-	-	-
36	-	+	-	-	-		-	-	-
37	-	+	-	-	-		-	-	-
38	-	+	-	-	-		-	-	-
39	-	+	-	-	-		-	-	-
40	-	+	-	-	-		-	-	-
41	-	+	-	-	-		-	-	-
42	-	+	-	-	-		-	-	-
43	-	+	-	-	-		-	-	-
44	-	-	-	-	-		-	-	-

45	-	-	-	-	-	-	-	-
46	-	-	-	-	-	-	-	-
47	+	-	-	-	-	-	-	-
48	+	-	-	-	-	-	-	-
49	+	-	-	-	-	-	-	-
50	+	-	-	-	-	-	-	-
51	+	-	-	-	-	-	-	-
52	-	-	-	-	-	-	-	-
53	-	-	-	-	-	-	-	-
54	-	-	-	-	-	-	-	-
55	-	-	-	-	-	-	-	-
56	NA	NA	NA	-	-	-	-	-
57	NA	NA	NA	-	-	-	-	-
58	NA	NA	NA	-	-	-	-	-
59	NA	NA	NA	-	-	-	-	-
60	NA	NA	NA	-	-	-	-	-
61	NA	NA	NA	-	-	-	-	-
62	-	-	-	-	-	-	-	-
63	-	-	-	-	-	+	-	-
64	-	-	-	+	-	-	-	-
65	NA	NA	NA	-	-	-	-	+
66	NA	NA	NA	-	-	-	-	-
67	-	-	-	-	+	-	-	-
68	-	-	-	-	-	-	-	-
69	NA	NA	NA	-	-	-	-	-
70	-	-	-	-	-	-	-	+
71	-	-	-	-	-	-	+	-
72	-	-	-	-	-	-	-	-
73	-	-	-	-	-	-	-	-
74	-	-	-	-	-	-	-	-
75	-	-	-	-	-	-	-	-
76	-	-	-	-	-	-	-	-
77	-	-	-	-	-	-	-	-
78	-	-	-	-	-	-	-	-
79	-	-	-	-	-	-	-	-
80	-	-	-	-	-	-	-	-
81	-	-	-	-	-	-	-	-

82	-	-	-	-	-	-	-	-
83	-	-	-	-	-	-	-	-
84	-	-	-	-	-	-	-	-
85	-	-	-	-	-	-	-	-
86	-	-	+	-	-	-	-	-
87	-	-	+	-	-	-	-	-
88	-	-	+	-	-	-	-	-
89	-	-	+	-	-	-	-	-
90	-	-	+	-	-	-	-	-
91	-	-	+	-	-	-	-	-
92	-	-	+	-	-	-	-	-
93	-	-	+	-	-	-	-	-
94	-	-	+	-	-	-	-	-
95	-	-	+	-	-	-	-	-
96	-	-	-	-	-	-	-	-
97	-	-	-	-	-	-	-	-
98	-	-	-	-	-	-	-	-
99	-	-	-	-	-	-	-	-
100	-	-	-	-	-	-	-	-
101	-	-	-	-	-	-	-	-
102	-	-	-	-	-	-	-	-
103	-	-	-	-	-	-	-	-
104	-	-	-	-	-	-	-	-
105	-	-	-	-	-	-	-	-
106	-	-	-	-	-	-	-	-
107	-	-	-	-	-	-	-	-
108	-	-	-	-	-	-	-	-
109	-	-	-	-	-	-	-	-
110	-	-	-	-	-	-	-	-
111	-	-	-	-	-	-	-	-
112	-	-	-	-	-	-	-	-
113	-	-	-	-	-	-	-	-
114	-	-	-	-	-	-	-	-
115	-	-	-	-	-	-	-	-
116	-	-	-	-	-	-	-	-
117	-	-	-	-	-	-	-	-
118	-	-	-	-	-	-	-	-

119	-	-	-	-	-		-	-	-
120	-	-	-	-	-		-	-	-

Appendix Chapter 3

Appendix: Predator biomass and prey DNA detection data

Sample code	Prey	Time post feeding [h]	Size class	Body size [mm]	Predator species	Predator sex	Predator body mass before feeding [mg]	Predator body mass after feeding [mg]	Prey DNA detection (yes/no)	Prey DNA quantity [ng/µL]	DNA copies/µL	Remarks
Col_265	Collembola	0	large	12	L. sp.	Female	23.6	22.9	yes	NA	NA	
Col_343	Collembola	0	large	12	L. mutabilis	Female	26.5	26.4	yes	NA	NA	
Col_425	Collembola	0	large	13	L. dentatus	Male	30.8	30.6	yes	NA	NA	
Col_460	Collembola	0	large	14	L. mutabilis	Female	28.6	29	yes	NA	NA	
Col_491	Collembola	0	large	14	L. dentatus	Female	28.2	28.5	yes	NA	NA	
Col_497	Collembola	0	large	14	L. mutabilis	Female	25	25.1	yes	NA	NA	
Col_523	Collembola	0	large	12	L. dentatus	Female	24.7	25	yes	NA	NA	
Col_600	Collembola	0	large	15	L. muticus	Female	29.7	29.7	yes	NA	NA	
Col_617	Collembola	0	large	12	L. nodulipes	Male	24.6	24.5	yes	NA	NA	
Col_622	Collembola	0	large	11	L. mutabilis	Female	20.4	20.3	yes	NA	NA	
Col_1122	Collembola	16	large	12	L. dentatus	Female	30	31.3	no	NA	NA	
Col_1138	Collembola	16	large	12	L. mutabilis	Male	28.5	28.6	yes	NA	NA	
Col_1145	Collembola	16	large	12	L. dentatus	Male	21.6	22.3	NA	NA	NA	not used for any PCR
Col_1151	Collembola	16	large	13	L. dentatus	Female	24.4	24.1	yes	NA	NA	
Col_1170	Collembola	16	large	12	L. mutabilis	Female	21.4	21.7	yes	NA	NA	
Col_1173	Collembola	16	large	14	L. dentatus	Female	31.8	32.5	yes	NA	NA	
Col_1178	Collembola	16	large	NA	NA	NA	19.7	20.2	yes	NA	NA	
Col_1184	Collembola	16	large	11	L. dentatus	Male	26.9	27.6	no	NA	NA	
Col_1390	Collembola	16	large	12	L. mutabilis	Male	26.7	27.5	yes	NA	NA	

Col_1409	Collembola	16	large	12	L. mutabilis	Male	26.1	28.1	no	NA	NA	
Col_982	Collembola	24	large	12	L. mutabilis	Male	19.7	18.6	no	NA	NA	
Col_989	Collembola	24	large	11	L. mutabilis	Female	19.8	17.6	yes	NA	NA	
Col_1005	Collembola	24	large	11	L. mutabilis	Male	19.7	18.4	yes	NA	NA	
Col_1032	Collembola	24	large	11	L. sp.	Male	19.6	17.3	yes	NA	NA	
Col_1054	Collembola	24	large	14	L. mutabilis	Male	32.6	30.5	yes	NA	NA	
Col_1231	Collembola	24	large	14	L. dentatus	Female	20.8	21.5	NA	NA	NA	not used for any PCR
Col_1247	Collembola	24	large	15	L. mutabilis	Male	27.5	28.6	no	NA	NA	
Col_1253	Collembola	24	large	12	L. mutabilis	Female	18.9	19.4	no	NA	NA	
Col_1299	Collembola	24	large	14	L. sp.	Female	21.8	22.3	yes	NA	NA	
Col_1314	Collembola	24	large	12	L. dentatus	Female	19.8	20.5	yes	NA	NA	
Col_446	Collembola	32	large	14	L. dentatus	Male	33.7	33.5	yes	NA	NA	
Col_611	Collembola	32	large	15	L. mutabilis	Female	32.4	31.9	yes	NA	NA	
Col_659	Collembola	32	large	14	L. dentatus	Female	29.8	30.4	yes	NA	NA	
Col_794	Collembola	32	large	12	L. mutabilis	Male	26.1	30.5	yes	NA	NA	
Col_922	Collembola	32	large	11	L. mutabilis	Female	19.5	19.5	no	NA	NA	
Col_936	Collembola	32	large	12	L. dentatus	Male	33.5	29.9	yes	NA	NA	
Col_971	Collembola	32	large	10	L. mutabilis	Male	19	18.3	yes	NA	NA	
Col_985	Collembola	32	large	11	L. mutabilis	Female	19.3	18.5	no	NA	NA	
Col_1096	Collembola	32	large	10	L. mutabilis	Male	19.1	18.1	no	NA	NA	
Col_1136	Collembola	32	large	13	L. dentatus	Male	32.7	34.3	yes	NA	NA	
Col_1205	Collembola	40	large	12	L. mutabilis	Male	20.7	21.4	no	NA	NA	
Col_1213	Collembola	40	large	13	L. mutabilis	Female	30.7	31.1	yes	NA	NA	
Col_1214	Collembola	40	large	14	L. dentatus	Female	22.8	23.2	no	NA	NA	
Col_1216	Collembola	40	large	12	L. mutabilis	Male	23.5	24.1	yes	NA	NA	
Col_1223	Collembola	40	large	12	L. mutabilis	Female	20.3	20.5	yes	NA	NA	
Col_1230	Collembola	40	large	12	L. mutabilis	Male	20.6	21.5	yes	NA	NA	
Col_1237	Collembola	40	large	12	L. mutabilis	Female	21.6	22.1	yes	NA	NA	

Col_1266	Collembola	40	large	12	L. mutabilis	Female	24	25.2	yes	NA	NA	
Col_1295	Collembola	40	large	12	L. mutabilis	Male	19.4	20.1	no	NA	NA	
Col_1312	Collembola	40	large	13	L. piceus	Male	29.9	30.4	no	NA	NA	
Col_453	Collembola	48	large	11	L. mutabilis	Female	21.8	21.9	no	NA	NA	
Col_900	Collembola	48	large	12	L. mutabilis	Male	18	18.3	yes	NA	NA	
Col_1044	Collembola	48	large	12	L. mutabilis	Female	26	26.7	yes	NA	NA	
Col_1057	Collembola	48	large	11	L. mutabilis	Male	20.3	20.2	yes	NA	NA	
Col_1107	Collembola	48	large	13	L. piceus	Male	31.5	31.7	no	NA	NA	
Col_1208	Collembola	48	large	13	L. mutabilis	Male	26.8	27.5	no	NA	NA	
Col_1215	Collembola	48	large	18	L. mutabilis	Male	28.1	28.2	no	NA	NA	
Col_1221	Collembola	48	large	13	L. mutabilis	Male	25.4	25.7	no	NA	NA	
Col_1232	Collembola	48	large	11	L. nodulipes	Female	22.6	23.3	yes	NA	NA	
Col_1243	Collembola	48	large	13	L. mutabilis	Female	18.2	18.6	no	NA	NA	
Col_1269	Collembola	48	large	13	L. mutabilis	Male	27.7	27.7	no	NA	NA	
Col_1273	Collembola	48	large	12	L. dentatus	Male	25.7	26	yes	NA	NA	
Col_1280	Collembola	48	large	17	L. mutabilis	Female	31.7	31.5	NA	NA	NA	not used for any PCR
Col_1291	Collembola	48	large	13	L. mutabilis	Male	30.3	31	NA	NA	NA	not used for any PCR
Col_1301	Collembola	48	large	11	L. mutabilis	Male	20.5	21	no	NA	NA	
Col_1009	Collembola	56	large	13	L. dentatus	Female	42.1	41.3	NA	NA	NA	not used for any PCR
Col_1015	Collembola	56	large	11	L. mutabilis	Male	21.6	21.5	no	NA	NA	
Col_1020	Collembola	56	large	12	L. mutabilis	Male	20.7	20.7	yes	NA	NA	
Col_1029	Collembola	56	large	12	L. mutabilis	Female	25.2	24.9	no	NA	NA	
Col_1035	Collembola	56	large	12	L. piceus	Male	22.1	23	no	NA	NA	
Col_1043	Collembola	56	large	10	L. mutabilis	Male	20.3	21.4	no	NA	NA	
Col_1047	Collembola	56	large	12	L. nodulipes	Female	25.8	26.4	no	NA	NA	
Col_1080	Collembola	56	large	12	L. mutabilis	Female	23.4	23.5	yes	NA	NA	
Col_1090	Collembola	56	large	13	L. mutabilis	Male	27.1	26.6	no	NA	NA	
Col_1098	Collembola	56	large	13	L. mutabilis	Female	22.3	22.3	yes	NA	NA	

Col_1106	Collembola	56	large	10	L. nodulipes	Female	22	21.5	yes	NA	NA	
Col_729	Collembola	72	large	13	L. sp.	Male	28.8	29.2	NA	NA	NA	not used for any PCR
Col_825	Collembola	72	large	12	L. mutabilis	Male	24	23.9	no	NA	NA	
Col_829	Collembola	72	large	13	L. mutabilis	Male	26.4	25.9	yes	NA	NA	
Col_1016	Collembola	72	large	13	L. mutabilis	Female	31.3	30.5	yes	NA	NA	
Col_1024	Collembola	72	large	12	L. piceus	Male	29.6	30.3	yes	NA	NA	
Col_1062	Collembola	72	large	12	L. mutabilis	Female	24	24.5	yes	NA	NA	
Col_1100	Collembola	72	large	12	L. mutabilis	Female	23.7	23.3	yes	NA	NA	
Col_1270	Collembola	72	large	14	L. piceus	Male	30	30.9	NA	NA	NA	not used for any PCR
Col_1305	Collembola	72	large	12	L. mutabilis	Male	28.8	28.9	no	NA	NA	
Col_x2	Collembola	72	large	13	L. dentatus	Male	25.9	25.1	no	NA	NA	
Col_1258	Collembola	88	large	12	L. mutabilis	Male	23	23.5	no	NA	NA	
Col_1354	Collembola	88	large	12	L. mutabilis	Male	22.3	22.8	yes	NA	NA	
Col_1376	Collembola	88	large	13	L. mutabilis	Male	22	22.8	yes	NA	NA	
Col_1382	Collembola	88	large	12	L. muticus	Male	28.5	29	yes	NA	NA	
Col_1386	Collembola	88	large	11	L. mutabilis	Female	25.8	26.1	yes	NA	NA	
Col_1410	Collembola	88	large	13	L. mutabilis	Female	27.8	28.1	yes	NA	NA	
Col_1415	Collembola	88	large	11	L. mutabilis	Female	20.6	21	no	NA	NA	
Col_1420	Collembola	88	large	11	L. mutabilis	Male	23.4	23.9	yes	NA	NA	
Col_1424	Collembola	88	large	13	L. mutabilis	Female	24.5	25.1	yes	NA	NA	
Col_1428	Collembola	88	large	12	L. mutabilis	Male	24.4	24.8	yes	NA	NA	
Col_243	Collembola	104	large	14	L. piceus	Female	30.2	29.4	no	NA	NA	
Col_252	Collembola	104	large	12	L. mutabilis	Female	23.8	23.1	yes	NA	NA	
Col_349	Collembola	104	large	14	L. piceus	Male	29.3	28.8	no	NA	NA	
Col_417	Collembola	104	large	15	L. dentatus	Female	39.1	38.7	yes	NA	NA	
Col_609	Collembola	104	large	13	L. piceus	Female	26.7	27.3	no	NA	NA	
Col_616	Collembola	104	large	13	L. dentatus	Female	31.7	32.1	yes	NA	NA	
Col_834	Collembola	104	large	14	L. dentatus	Female	30	30.3	NA	NA	NA	not used for any PCR

Col_845	Collembola	104	large	14	L. dentatus	Male	35.7	35.3	NA	NA	NA	not used for any PCR
Col_876	Collembola	104	large	12	L. mutabilis	Female	26.7	27.1	no	NA	NA	
Col_902	Collembola	104	large	11	L. dentatus	Male	27.2	27.1	no	NA	NA	
Col_904	Collembola	104	large	13	L. mutabilis	Female	29.9	29.7	yes	NA	NA	
Col_345	Collembola	120	large	12	L. piceus	Male	25.4	25.6	no	NA	NA	
Col_410	Collembola	120	large	11	L. piceus	Female	22.5	22.8	NA	NA	NA	not used for any PCR
Col_458	Collembola	120	large	12	L. mutabilis	Female	22.8	22.9	no	NA	NA	
Col_485	Collembola	120	large	12	L. mutabilis	Female	26.3	25.9	yes	NA	NA	
Col_509	Collembola	120	large	12	L. mutabilis	Female	23.4	22.9	no	NA	NA	
Col_513	Collembola	120	large	11	L. mutabilis	Female	22	21.8	yes	NA	NA	
Col_540	Collembola	120	large	12	L. dentatus	Female	30.2	30	NA	NA	NA	not used for any PCR
Col_604	Collembola	120	large	12	L. mutabilis	Female	27.4	26.6	yes	NA	NA	
Col_623	Collembola	120	large	12	L. dentatus	Female	22.4	21	NA	NA	NA	not used for any PCR
Col_934	Collembola	120	large	12	L. dentatus	Male	23.5	23.4	NA	NA	NA	not used for any PCR
Col_303	Collembola	144	large	12	L. mutabilis	Female	27	26.8	yes	NA	NA	
Col_613	Collembola	144	large	12	L. mutabilis	Male	29.1	27.9	no	NA	NA	
Col_619	Collembola	144	large	10	L. dentatus	Female	23.4	23.9	yes	NA	NA	
Col_621	Collembola	144	large	10	L. mutabilis	Female	19.8	19.8	no	NA	NA	
Col_919	Collembola	144	large	10	L. mutabilis	Male	23.6	23.5	NA	8.76E-06	4.27E+04	not used for singleplex PCR
Col_932	Collembola	144	large	12	L. mutabilis	Female	26.8	27.5	yes	NA	NA	
Col_973	Collembola	144	large	13	L. sp.	Male	23.7	24.3	no	NA	NA	
Col_978	Collembola	144	large	12	L. mutabilis	Male	27.2	27.4	yes	NA	NA	
Col_996	Collembola	144	large	12	L. mutabilis	Female	20.5	21.8	no	NA	NA	
Col_1008	Collembola	144	large	11	L. muticus	Female	21.2	22	no	NA	NA	
Col_916	Collembola	168	large	11	L. mutabilis	Female	20.3	21.4	no	NA	NA	
Col_935	Collembola	168	large	12	L. mutabilis	Female	23.9	24.5	no	NA	NA	
Col_946	Collembola	168	large	11	L. mutabilis	Female	22	22.6	no	NA	NA	
Col_1003	Collembola	168	large	12	L. mutabilis	Female	22.6	23.1	yes	NA	NA	

Col_1219	Collembola	168	large	13	L. mutabilis	Male	24	24.7	no	NA	NA		
Col_1224	Collembola	168	large	11	L. mutabilis	Male	24.8	25.6	no	NA	NA		
Col_1229	Collembola	168	large	13	L. mutabilis	Male	29.3	30.2	no	NA	NA		
Col_1235	Collembola	168	large	11	L. mutabilis	Male	21.3	22	no	NA	NA		
Col_1238	Collembola	168	large	12	L. mutabilis	Male	19.7	20.5	no	NA	NA		
Col_1290	Collembola	168	large	11	L. dentatus	Male	23.3	23.2	NA	NA	NA	not used for any PCR	
Col_1426	Collembola	NA	large	NA	NA	NA	22.6	23.2	NA	NA	NA		
Col_1363	Collembola	NA	large	NA	NA	NA	27.9	28.9	NA	NA	NA		
Col_1423	Collembola	NA	large	NA	NA	NA	20.6	20.5	NA	NA	NA		
Col_995	Collembola	NA	large	NA	NA	NA	21	21.8	NA	NA	NA		
Col_289	Collembola	0	medium	8	L. crassipes	Male	5.4	5.5	yes	3.52E-07	1.26E+03		
Col_481	Collembola	0	medium	7	L. crassipes	Male	5.9	6.1	yes	3.84E-10	1.38E+00		
Col_503	Collembola	0	medium	10	L. crassipes	Female	9.5	9.7	yes	7.40E-04	2.65E+06		
Col_514	Collembola	0	medium	10	L. nodulipes	Male	9.7	10.6	yes	3.48E-07	1.25E+03		
Col_527	Collembola	0	medium	9	L. sp.	NA	8.6	8.9	yes	8.99E-05	3.22E+05		
Col_653	Collembola	0	medium	10	L. mutabilis	Male	12.9	13.2	yes	No Ct	No Ct	ambiguous qPCR results	
Col_714	Collembola	0	medium	7	L. sp.	Male	5.7	6	yes	NA	NA		
Col_720	Collembola	0	medium	10	L. sp.	Male	12.4	12.7	yes	7.67E-07	2.75E+03		
Col_734	Collembola	0	medium	9	L. sp.	NA	9.4	9.3	yes	4.97E-04	1.78E+06		
Col_799	Collembola	0	medium	5	L. sp.	Male	5.5	5.6	yes	3.12E-05	1.12E+05		
Col_928	Collembola	0	medium	7	L. sp.	Male	6.2	6.7	yes	4.62E-03	1.65E+07		
Col_medxx	Collembola	0	medium	10	L. sp.	Female	12.8	12.9	yes	NA	NA		
Col_841	Collembola	16	medium	8	L. sp.	Male	6.6	6.6	yes	7.27E-07	2.60E+03		
Col_1018	Collembola	16	medium	8	L. mutabilis	Female	6.8	7.3	yes	2.87E-08	1.03E+02		

Col_1019	Collembola	16	medium	10	L. mutabilis	Female	11.6	11.9	yes	3.54E-06	1.27E+04	
Col_1025	Collembola	16	medium	9	L. mutabilis	Female	8.4	8.8	yes	8.20E-09	2.94E+01	ambiguous qPCR results
Col_1049	Collembola	16	medium	8	L. mutabilis	Female	7.8	8.8	yes	1.68E-08	6.01E+01	
Col_1052	Collembola	16	medium	10	L. mutabilis	Female	10.6	10.5	yes	NA	NA	
Col_1067	Collembola	16	medium	9	L. mutabilis	Male	10.2	10.4	yes	NA	NA	
Col_1070	Collembola	16	medium	9	L. mutabilis	Male	9	9.3	yes	3.38E-07	1.21E+03	
Col_1078	Collembola	16	medium	10	L. nodulipes	Female	11.2	11.5	yes	7.70E-06	2.76E+04	
Col_1083	Collembola	16	medium	6	L. crassipes	Male	5.7	5.8	yes	3.54E-08	1.27E+02	
Col_1093	Collembola	16	medium	10	L. mutabilis	Female	9	9.2	yes	NA	NA	
Col_1094	Collembola	16	medium	9	L. mutabilis	Male	7.6	7.9	yes	7.61E-09	2.73E+01	
Col_1112	Collembola	16	medium	10	L. dentatus	Female	13.3	13.1	NA	1.20E-07	4.31E+02	not used for singleplex PCR
Col_1159	Collembola	16	medium	8	L. dentatus	Male	7.3	7.4	yes	1.54E-09	5.51E+00	
Col_1164	Collembola	16	medium	6	L. sp.	Male	6.5	7.1	yes	1.38E-06	4.95E+03	
Col_1180	Collembola	16	medium	8	L. mutabilis	Male	10	10.1	yes	4.07E-09	1.46E+01	
Col_1183	Collembola	16	medium	10	L. piceus	Female	14.5	15.4	yes	5.12E-07	1.83E+03	
Col_1189	Collembola	16	medium	10	L. dentatus	Female	15.1	16	yes	4.09E-07	1.47E+03	
Col_1193	Collembola	16	medium	10	L. sp.	Male	13.9	14.5	yes	4.23E-06	1.52E+04	
Col_235	Collembola	24	medium	7	L. crassipes	Female	5.8	5.7	yes	3.25E-07	1.17E+03	
Col_260	Collembola	24	medium	7	L. crassipes	Male	6.2	6.5	yes	1.24E-07	4.44E+02	
Col_263	Collembola	24	medium	7	L. crassipes	Male	5.2	5.2	no	9.71E-06	3.48E+04	
Col_353	Collembola	24	medium	7	L. crassipes	Male	5.1	5.1	yes	4.85E-08	1.74E+02	
Col_359	Collembola	24	medium	7	L. sp.	Male	7.3	7.5	yes	1.48E-08	5.30E+01	

Col_502	Collembola	24	medium	10	L. mutabilis	Male	14.8	14.8	yes	NA	NA	
Col_668	Collembola	24	medium	10	L. mutabilis	Male	12.6	12.8	yes	NA	NA	
Col_702	Collembola	24	medium	8	L. sp.	Male	11.6	11.7	NA	NA	NA	not used for any PCR
Col_709	Collembola	24	medium	8	L. sp.	NA	7.2	7.5	yes	No Ct	No Ct	ambiguous qPCR results
Col_732	Collembola	24	medium	11	L. dentatus	Male	11.9	12.4	yes	5.95E-09	2.13E+01	
Col_742	Collembola	24	medium	8	L. sp.	Male	7.7	8.3	yes	5.09E-08	1.82E+02	
Col_431	Collembola	32	medium	9	L. mutabilis	Male	10.9	11.1	yes	NA	NA	
Col_482	Collembola	32	medium	10	L. mutabilis	Female	15	14.8	yes	3.33E-07	1.19E+03	
Col_486	Collembola	32	medium	8	L. crassipes	Female	6.1	5.9	no	5.19E-08	1.86E+02	
Col_1102	Collembola	32	medium	7	L. mutabilis	Male	6.9	5.5	yes	NA	NA	
Col_1124	Collembola	32	medium	9	L. mutabilis	Male	8.9	10	NA	NA	NA	not used for any PCR
Col_1143	Collembola	32	medium	7	L. sp.	Male	7.4	7.4	yes	2.85E-08	1.02E+02	
Col_1155	Collembola	32	medium	9	L. sp.	Male	10.7	11.6	yes	NA	NA	
Col_1162	Collembola	32	medium	9	L. curtipes	Male	10.4	10.4	yes	NA	NA	
Col_1181	Collembola	32	medium	8	L. mutabilis	Male	6.9	7.4	yes	6.70E-05	2.40E+05	
Col_x1	Collembola	32	medium	10	L. dentatus	Male	14.6	14.6	yes	NA	NA	
Col_1012	Collembola	40	medium	10	L. mutabilis	Male	9.6	10	no	NA	NA	
Col_1036	Collembola	40	medium	7	L. sp.	Male	7.4	8.6	yes	NA	NA	
Col_1066	Collembola	40	medium	8	L. nodulipes	Male	10.1	10.7	yes	1.30E-07	4.65E+02	
Col_1148	Collembola	40	medium	9	L. crassipes	Male	9.3	9.9	yes	1.25E-06	4.48E+03	
Col_1150	Collembola	40	medium	8	L. crassipes	Male	5.6	5.9	yes	2.15E-07	7.71E+02	
Col_1152	Collembola	40	medium	7	L. mutabilis	Male	7.1	8	yes	1.82E-08	6.51E+01	
Col_1171	Collembola	40	medium	8	L. sp.	Male	9.3	9.8	yes	4.98E-08	1.78E+02	
Col_1172	Collembola	40	medium	9	L. crassipes	Male	7	7.3	yes	4.46E-07	1.60E+03	

Col_1187	Collembola	40	medium	8	L. dentatus	Male	8.6	9.8	yes	4.33E-09	1.55E+01	
Col_1199	Collembola	40	medium	11	L. crassipes	Female	8.7	9.2	yes	2.80E-07	1.00E+03	
Col_1028	Collembola	44	medium	11	L. mutabilis	Male	11.4	12	NA	NA	NA	not used for any PCR
Col_1031	Collembola	44	medium	9	L. crassipes	Male	9	9.3	NA	NA	NA	not used for any PCR
Col_1041	Collembola	44	medium	10	L. sp.	Male	12.4	12.9	NA	NA	NA	not used for any PCR
Col_1048	Collembola	44	medium	10	L. dentatus	Female	11.6	11.4	NA	NA	NA	not used for any PCR
Col_1061	Collembola	44	medium	9	L. mutabilis	Male	8.3	9.5	NA	NA	NA	not used for any PCR
Col_1065	Collembola	44	medium	10	L. sp.	Male	10.1	10.2	NA	NA	NA	not used for any PCR
Col_1075	Collembola	44	medium	11	L. mutabilis	Male	9.2	9.4	NA	NA	NA	not used for any PCR
Col_1077	Collembola	44	medium	8	L. mutabilis	Female	7.1	7.4	NA	NA	NA	not used for any PCR
Col_1099	Collembola	44	medium	9	L. sp.	Male	8.9	9.1	NA	NA	NA	not used for any PCR
Col_1109	Collembola	44	medium	9	L. mutabilis	Female	11.5	11.2	NA	NA	NA	not used for any PCR
Col_316	Collembola	48	medium	8	L. sp.	Male	5.4	5.3	yes	1.51E-07	5.42E+02	
Col_441	Collembola	48	medium	8	L. crassipes	Male	5.2	5.2	yes	1.81E-05	6.47E+04	
Col_1014	Collembola	48	medium	9	L. mutabilis	Female	9.2	9.6	no	NA	NA	
Col_1034	Collembola	48	medium	9	L. nodulipes	Male	14.6	15	yes	4.01E-07	1.44E+03	
Col_1051	Collembola	48	medium	8	L. mutabilis	Female	7.2	7.6	yes	NA	NA	
Col_1074	Collembola	48	medium	8	L. mutabilis	Male	8.9	9.1	yes	NA	NA	
Col_1085	Collembola	48	medium	7	L. sp.	Male	6.5	6.7	no	NA	NA	
Col_1089	Collembola	48	medium	7	L. sp.	Male	7.8	8.1	yes	3.81E-08	1.36E+02	
Col_1105	Collembola	48	medium	9	L. dentatus	Female	9.9	10.2	yes	2.03E-07	7.29E+02	
Col_1111	Collembola	48	medium	9	L. mutabilis	Female	8.6	9.6	no	6.12E-09	2.19E+01	
Col_247	Collembola	56	medium	8	L. crassipes	Male	6.2	6.4	no	1.35E-06	4.85E+03	
Col_329	Collembola	56	medium	7	L. crassipes	Male	5.4	5.6	yes	5.38E-08	1.93E+02	
Col_373	Collembola	56	medium	6	L. sp.	Male	5.1	5.3	no	NA	NA	

Col_933	Collembola	56	medium	9	L. nodulipes	Male	9.8	10	NA	NA	NA	not used for any PCR
Col_958	Collembola	56	medium	8	L. mutabilis	Male	8.2	8.8	yes	NA	NA	
Col_959	Collembola	56	medium	10	L. muticus	Male	12.5	13.1	NA	NA	NA	not used for any PCR
Col_961	Collembola	56	medium	10	L. sp.	Male	12.6	13.7	NA	NA	NA	not used for any PCR
Col_968	Collembola	56	medium	10	L. mutabilis	Male	11.6	11.8	no	NA	NA	
Col_979	Collembola	56	medium	7	L. crassipes	Male	5.1	5.6	yes	8.94E-08	3.20E+02	
Col_1010	Collembola	56	medium	10	L. muticus	Male	12.7	12.5	yes	2.66E-07	9.54E+02	
Col_526	Collembola	72	medium	7	L. sp.	Female	5.1	5.6	yes	4.72E-04	1.69E+06	
Col_1017	Collembola	72	medium	9	L. dentatus	Male	13	13.2	no	2.96E-09	1.06E+01	
Col_1022	Collembola	72	medium	7	L. mutabilis	Female	7.4	7.7	yes	7.62E-06	2.73E+04	
Col_1023	Collembola	72	medium	9	L. sp.	Female	7.9	9.2	yes	1.46E-05	5.23E+04	
Col_1037	Collembola	72	medium	10	L. mutabilis	Female	12	12.3	yes	NA	NA	
Col_1055	Collembola	72	medium	7	L. sp.	Male	6.2	6.6	NA	NA	NA	not used for any PCR
Col_1056	Collembola	72	medium	9	L. mutabilis	Female	6.1	5.8	no	1.91E-07	6.85E+02	ambiguous qPCR results
Col_1060	Collembola	72	medium	8	L. crassipes	Male	5.7	5.9	no	NA	NA	
Col_1091	Collembola	72	medium	10	L. sp.	Male	8.4	9.3	yes	1.48E-06	5.31E+03	
Col_1110	Collembola	72	medium	10	L. nodulipes	Female	13.3	13.8	no	4.70E-08	1.69E+02	
Col_330	Collembola	88	medium	7	L. sp.	Male	5.2	5.3	yes	4.33E-07	1.55E+03	
Col_488	Collembola	88	medium	7	L. sp.	Male	5.4	5.7	yes	No Ct	No Ct	ambiguous qPCR results
Col_809	Collembola	88	medium	6	L. sp.	Male	5.1	5.3	yes	NA	NA	
Col_1013	Collembola	88	medium	9	L. mutabilis	Female	9	9.7	no	5.11E-10	1.83E+00	ambiguous qPCR results
Col_1046	Collembola	88	medium	9	L. sp.	Male	7.8	8.4	no	NA	NA	
Col_1071	Collembola	88	medium	7	L. mutabilis	Male	5.3	5.6	no	NA	NA	
Col_1095	Collembola	88	medium	8	L. sp.	Female	8.2	8.7	no	7.19E-08	2.58E+02	

Col_1103	Collembola	88	medium	8	L. crassipes	Male	5.4	5.6	yes	4.23E-09	1.52E+01	
Col_1114	Collembola	88	medium	9	L. dentatus	Female	10.8	11.5	no	2.60E-08	9.33E+01	ambiguous qPCR results
Col_1115	Collembola	88	medium	10	L. sp.	Male	14.2	14.5	no	2.40E-06	8.61E+03	
Col_323	Collembola	104	medium	7	L. crassipes	Female	6.8	6.8	yes	5.35E-07	1.92E+03	
Col_684	Collembola	104	medium	10	L. dentatus	Male	12.8	13.1	yes	NA	NA	
Col_713	Collembola	104	medium	9	L. sp.	Male	8.4	9.1	NA	NA	NA	not used for any PCR
Col_726	Collembola	104	medium	7	L. sp.	Male	5.6	5.6	no	2.26E-09	8.09E+00	
Col_747	Collembola	104	medium	10	L. sp.	NA	5.3	5.6	yes	NA	NA	
Col_761	Collembola	104	medium	10	L. sp.	Male	13.3	13.2	NA	NA	NA	not used for any PCR
Col_918	Collembola	104	medium	8	L. crassipes	Male	7.5	7.8	yes	NA	NA	
Col_938	Collembola	104	medium	7	L. crassipes	Male	13.9	13.5	no	NA	NA	
Col_1000	Collembola	104	medium	7	L. mutabilis	Male	6.8	6.9	yes	NA	NA	
Col_1002	Collembola	104	medium	NA	NA	NA	8.3	8.7	yes	NA	NA	
Col_296	Collembola	120	medium	10	L. sp.	Male	5.5	5.2	no	5.33E-07	1.91E+03	
Col_929	Collembola	120	medium	10	L. aulacopus	Male	11.5	12.1	no	2.30E-06	8.24E+03	ambiguous qPCR results
Col_937	Collembola	120	medium	9	L. mutabilis	Male	7.9	8.3	no	NA	NA	
Col_939	Collembola	120	medium	7	L. sp.	NA	6.2	6.2	NA	1.24E-04	6.02E+05	not used for singleplex PCR
Col_947	Collembola	120	medium	7	L. sp.	Male	5.9	6.3	yes	NA	NA	
Col_951	Collembola	120	medium	10	L. sp.	Male	9.1	9.7	yes	NA	NA	
Col_972	Collembola	120	medium	8	L. sp.	Male	5.9	6.5	yes	NA	NA	
Col_993	Collembola	120	medium	8	L. sp.	Male	10.1	10.1	no	5.54E-08	1.98E+02	
Col_1004	Collembola	120	medium	7	L. muticus	Male	7.9	8.4	yes	NA	NA	
Col_1006	Collembola	120	medium	7	L. mutabilis	Male	6.2	6.7	yes	NA	NA	
Col_918x	Collembola	144	medium	11	L. mutabilis	Male	10.1	11.1	no	NA	NA	
Col_920	Collembola	144	medium	7	L. sp.	Male	7.2	8.2	yes	1.52E-09	5.45E+00	

Col_930	Collembola	144	medium	10	L. crassipes	Female	8.7	8.8	yes	1.14E-07	4.07E+02	
Col_954	Collembola	144	medium	9	L. sp.	Female	7.8	8.3	NA	5.94E-06	2.13E+04	not used for singleplex PCR
Col_956	Collembola	144	medium	9	L. nodulipes	Male	10.5	10.8	NA	4.33E-07	1.55E+03	not used for singleplex PCR
Col_967	Collembola	144	medium	8	L. mutabilis	Male	6.9	6.9	no	NA	NA	
Col_974	Collembola	144	medium	10	L. mutabilis	Male	12.3	14	yes	3.11E-09	1.11E+01	
Col_976	Collembola	144	medium	7	L. sp.	NA	8	8.3	yes	4.32E-09	1.55E+01	
Col_977	Collembola	144	medium	9	L. nodulipes	Male	12.8	13.8	NA	1.65E-07	5.93E+02	not used for singleplex PCR
Col_986	Collembola	144	medium	10	L. mutabilis	Female	14.3	17	yes	NA	NA	
Col_412	Collembola	168	medium	10	L. crassipes	Female	5.5	5.6	yes	NA	NA	
Col_917	Collembola	168	medium	8	L. crassipes	Male	7.7	8.3	yes	NA	NA	
Col_960	Collembola	168	medium	8	L. mutabilis	Male	9.1	9.2	yes	NA	NA	
Col_963	Collembola	168	medium	7	L. crassipes	Male	6.4	6.5	NA	NA	NA	not used for any PCR
Col_966	Collembola	168	medium	9	L. sp.	Female	7.7	8.2	yes	4.31E-07	1.54E+03	
Col_980	Collembola	168	medium	10	L. mutabilis	Female	8.2	8.3	yes	1.78E-07	6.38E+02	
Col_988	Collembola	168	medium	8	L. sp.	Male	8.8	9.5	NA	NA	NA	not used for any PCR
Col_994	Collembola	168	medium	8	L. mutabilis	Male	7.2	8	no	2.56E-09	9.16E+00	
Col_1007	Collembola	168	medium	9	L. mutabilis	Female	13	14.3	no	NA	NA	
Col_1082	Collembola	168	medium	10	L. mutabilis	Male	13.3	13.8	yes	NA	NA	
Col_375	Collembola	0	small	6	L. sp.	Male	3.9	3.7	yes	NA	NA	
Col_438	Collembola	0	small	5	L. crassipes	Female	3	3.2	yes	NA	NA	
Col_449	Collembola	0	small	6	L. sp.	NA	3.7	4.1	yes	NA	NA	
Col_459	Collembola	0	small	6	L. crassipes	Male	4.6	4.6	yes	NA	NA	
Col_727	Collembola	0	small	5	L. sp.	NA	2.9	2.9	yes	NA	NA	
Col_831	Collembola	0	small	5	L. sp.	Male	3.8	3.7	yes	NA	NA	
Col_853	Collembola	0	small	5	L. sp.	NA	3.4	3.2	yes	NA	NA	

Col_867	Collembola	0	small		6	L. sp.	Male		4.7	5.1	yes	NA	NA	
Col_877	Collembola	0	small		5	L. crassipes	Male		2.1	2.4	yes	NA	NA	
Col_908	Collembola	0	small		5	L. sp.	Male		2	2.1	yes	NA	NA	
Col_515	Collembola	16	small		5	L. sp.	NA		3.6	3.6	yes	NA	NA	
Col_715	Collembola	16	small		6	L. crassipes	Male		3.8	4.1	yes	NA	NA	
Col_795	Collembola	16	small		5	L. sp.	Male		3.5	3.5	yes	NA	NA	
Col_840	Collembola	16	small		5	L. sp.	Male		3.8	4.2	yes	NA	NA	
Col_880	Collembola	16	small		7	L. sp.	Male		4.5	4.7	yes	NA	NA	
Col_884	Collembola	16	small		6	L. sp.	Male		3.5	3.4	yes	NA	NA	
Col_897	Collembola	16	small		6	L. crassipes	Male		3.8	3.8	yes	NA	NA	
Col_905	Collembola	16	small		5	L. sp.	NA		3.8	4.2	yes	NA	NA	
Col_909	Collembola	16	small		5	L. sp.	NA		3.2	3.2	yes	NA	NA	
Col_912x	Collembola	16	small		6	L. crassipes	Male		1.7	2	yes	NA	NA	
Col_258	Collembola	24	small		6	L. crassipes	Male		4.3	4.7	yes	NA	NA	
Col_269	Collembola	24	small		5	L. crassipes	Female		1.8	1.8	yes	NA	NA	
Col_278	Collembola	24	small		8	L. sp.	Male		4.9	5	yes	NA	NA	
Col_308	Collembola	24	small		7	L. crassipes	Female		3.9	3.9	yes	NA	NA	
Col_332	Collembola	24	small		7	L. crassipes	Female		3.8	3.8	yes	NA	NA	
Col_439	Collembola	24	small		7	L. crassipes	Female		4.7	4.8	yes	NA	NA	
Col_724	Collembola	24	small	NA		NA	NA		1.6	1.8	yes	NA	NA	
Col_731	Collembola	24	small		5	L. mutabilis	Male		4.7	4.9	no	NA	NA	
Col_744	Collembola	24	small		6	L. crassipes	Male		4.3	4.3	yes	NA	NA	
Col_749	Collembola	24	small		12	L. mutabilis	Female		3.4	3.7	yes	NA	NA	
Col_630	Collembola	32	small		6	L. crassipes	Female		3.3	3.5	yes	NA	NA	
Col_631	Collembola	32	small		7	L. crassipes	Female		3.6	3.9	yes	NA	NA	
Col_644	Collembola	32	small		7	L. crassipes	Male		4	4.3	yes	NA	NA	
Col_650	Collembola	32	small		5	L. crassipes	Female	NA		4	yes	NA	NA	not used for body mass measurement
Col_655	Collembola	32	small		5	L. crassipes	Female		2.4	2.6	yes	NA	NA	

Col_671	Collembola	32	small	6	L. crassipes	Female	4	4.4	yes	NA	NA	
Col_676	Collembola	32	small	5	L. crassipes	Female	2.3	2.8	yes	NA	NA	
Col_677	Collembola	32	small	5	L. crassipes	Male	2.1	2.5	yes	NA	NA	
Col_690	Collembola	32	small	6	L. sp.	Female	3.9	4.3	no	NA	NA	
Col_691	Collembola	32	small	6	L. crassipes	Female	3.9	4.2	yes	NA	NA	
Col_305	Collembola	40	small	8	L. crassipes	Female	4.6	4.7	yes	NA	NA	
Col_400	Collembola	40	small	5	L. sp.	NA	2.5	2.4	no	NA	NA	
Col_415	Collembola	40	small	6	L. sp.	NA	4.3	4.5	no	NA	NA	
Col_440	Collembola	40	small	6	L. crassipes	Female	4.4	4.4	yes	NA	NA	
Col_454	Collembola	40	small	5	L. crassipes	Female	1.4	1.5	yes	NA	NA	
Col_487	Collembola	40	small	4	L. sp.	NA	1.1	1.1	yes	NA	NA	
Col_826	Collembola	40	small	4	L. sp.	NA	1.5	1.5	NA	NA	NA	not used for any PCR
Col_855	Collembola	40	small	6	L. sp.	NA	2.5	2.6	yes	NA	NA	
Col_886	Collembola	40	small	5	L. crassipes	Male	1.9	2	yes	NA	NA	
Col_890	Collembola	40	small	6	L. crassipes	Male	2.2	2.4	no	NA	NA	
Col_254	Collembola	44	small	7	L. crassipes	Male	6.7	6.7	yes	NA	NA	not used for any PCR
Col_261	Collembola	44	small	7	L. mutabilis	Female	5.2	5.7	yes	NA	NA	not used for any PCR
Col_266	Collembola	44	small	6	L. crassipes	Male	4.3	4.6	no	NA	NA	not used for any PCR
Col_272	Collembola	44	small	5	L. crassipes	Male	2.3	2.6	no	NA	NA	not used for any PCR
Col_295	Collembola	44	small	6	L. sp.	Male	5.9	6	yes	NA	NA	not used for any PCR
Col_368	Collembola	44	small	6	L. crassipes	Female	4.3	4.1	yes	NA	NA	not used for any PCR
Col_483	Collembola	44	small	7	L. sp.	Male	4.4	4.5	yes	NA	NA	not used for any PCR
Col_495	Collembola	44	small	8	L. crassipes	Female	4.2	4.5	no	NA	NA	not used for any PCR
Col_718	Collembola	44	small	6	L. sp.	Male	4.9	5.4	yes	NA	NA	not used for any PCR
Col_757	Collembola	44	small	6	L. crassipes	Female	5	5	no	NA	NA	not used for any PCR
Col_371	Collembola	48	small	6	L. sp.	Male	3.8	4	yes	NA	NA	
Col_376	Collembola	48	small	7	L. crassipes	Male	4.1	4.4	yes	NA	NA	
Col_393	Collembola	48	small	5	L. sp.	NA	2.2	2.3	yes	NA	NA	

Col_442	Collembola	48	small	7	L. crassipes	Female		4		4.2	no	NA	NA	
Col_480	Collembola	48	small	5	L. crassipes	Male		2.2		2.4	no	NA	NA	
Col_501	Collembola	48	small	5	L. crassipes	Male		3.1		2.7	no	NA	NA	
Col_652	Collembola	48	small	5	L. crassipes	Female		2		2.2	yes	NA	NA	
Col_711	Collembola	48	small	5	L. crassipes	Male		2.7		3	yes	NA	NA	
Col_808	Collembola	48	small	5	L. crassipes	Male		3.9		4.4	yes	NA	NA	
Col_842	Collembola	48	small	6	L. crassipes	Female		3.1		3.3	yes	NA	NA	
Col_242	Collembola	56	small	8	L. crassipes	Male		4.8		5.2	yes	NA	NA	
Col_288	Collembola	56	small	6	L. sp.	Male		3.8		4.2	yes	NA	NA	
Col_304	Collembola	56	small	6	L. crassipes	Female		4.5		4.8	yes	NA	NA	
Col_377	Collembola	56	small	7	L. crassipes	Male		3.6		4	no	NA	NA	
Col_402	Collembola	56	small	4	L. sp.	NA	NA		NA		no	NA	NA	not used for body mass measurement
Col_558	Collembola	56	small	7	L. crassipes	Male		3.4		3.3	no	NA	NA	
Col_562	Collembola	56	small	7	L. crassipes	Male		3.4		3.7	no	NA	NA	
Col_948	Collembola	56	small	8	L. crassipes	Female		3.3		3.5	no	NA	NA	
Col_965	Collembola	56	small	8	L. sp.	Male		4.2		4.3	yes	NA	NA	
Col_987	Collembola	56	small	5	L. mutabilis	Female		4.2		4.4	yes	NA	NA	
Col_998	Collembola	56	small	5	L. crassipes	Male		2.4		3	no	NA	NA	
Col_380	Collembola	72	small	6	L. sp.	Male		4.3		4.2	yes	NA	NA	
Col_395	Collembola	72	small	5	L. sp.	NA		3		3	yes	NA	NA	
Col_398	Collembola	72	small	5	L. sp.	Male		2.2		2.3	yes	NA	NA	
Col_404	Collembola	72	small	5	L. sp.	Male		2.7		2.8	yes	NA	NA	
Col_405	Collembola	72	small	6	L. sp.	Male		4.9		4.7	no	NA	NA	
Col_414	Collembola	72	small	5	L. crassipes	Male		1.9		2	no	NA	NA	
Col_434	Collembola	72	small	5	L. sp.	Male		2.1		2.2	yes	NA	NA	
Col_456	Collembola	72	small	6	L. crassipes	Male		2.9		2.7	yes	NA	NA	
Col_730	Collembola	72	small	6	L. crassipes	Male		3.3		3.6	no	NA	NA	
Col_879	Collembola	72	small	7	L. sp.	Male		3.9		4.2	yes	NA	NA	

Col_285	Collembola	88	small	6	L. crassipes	Female	3.8	3.8	yes	NA	NA	
Col_338	Collembola	88	small	6	L. sp.	Male	3.8	4.2	yes	NA	NA	
Col_348	Collembola	88	small	6	L. crassipes	Female	4.2	4	yes	NA	NA	
Col_379	Collembola	88	small	6	L. sp.	Male	4.4	4.6	NA	NA	NA	not used for any PCR
Col_528	Collembola	88	small	5	L. crassipes	Male	3.2	3.1	no	NA	NA	
Col_818	Collembola	88	small	8	L. sp.	NA	5	5	yes	NA	NA	
Col_846	Collembola	88	small	5	L. crassipes	Male	1.3	1.4	yes	NA	NA	
Col_1084	Collembola	88	small	7	L. crassipes	Male	4.4	4.3	no	NA	NA	
Col_1092	Collembola	88	small	7	L. crassipes	Male	3.8	4.1	no	NA	NA	
Col_1108	Collembola	88	small	6	L. sp.	Male	3.7	3.9	no	NA	NA	
Col_518	Collembola	104	small	4	L. sp.	NA	1.5	2.1	yes	NA	NA	
Col_542	Collembola	104	small	5	L. crassipes	Male	1.4	1.8	no	NA	NA	
Col_928	Collembola	104	small	5	L. sp.	NA	3.7	3.5	yes	4.62E-03	1.65E+07	
Col_942	Collembola	104	small	5	L. sp.	NA	1.9	2.7	no	NA	NA	
Col_944	Collembola	104	small	5	L. sp.	NA	2.8	3.4	yes	NA	NA	
Col_950	Collembola	104	small	6	L. crassipes	Male	3.3	3.5	NA	NA	NA	not used for any PCR
Col_969	Collembola	104	small	6	L. sp.	Male	4.6	5.1	yes	NA	NA	
Col_981	Collembola	104	small	7	L. crassipes	Male	4.8	4.9	yes	NA	NA	
Col_1073	Collembola	104	small	7	L. sp.	NA	3.1	3.8	yes	NA	NA	
Col_1079	Collembola	104	small	5	L. sp.	Male	3.3	3.2	yes	NA	NA	
Col_1146	Collembola	104	small	7	L. crassipes	Male	2.7	3.3	yes	NA	NA	
Col_1176	Collembola	104	small	6	L. crassipes	Male	3.5	3.4	yes	NA	NA	
Col_294	Collembola	120	small	7	L. crassipes	Female	4.7	4.6	yes	NA	NA	
Col_314	Collembola	120	small	9	L. crassipes	NA	3.8	4	no	NA	NA	
Col_333	Collembola	120	small	6	L. sp.	NA	2	2.1	yes	NA	NA	
Col_465	Collembola	120	small	6	L. crassipes	Male	3.1	3.2	yes	NA	NA	
Col_500	Collembola	120	small	6	L. sp.	NA	4.9	5.3	yes	NA	NA	
Col_519	Collembola	120	small	4	L. sp.	NA	2.1	2.2	yes	NA	NA	

Col_529	Collembola	120	small	5	L. mutabilis	NA	3	3.2	NA	NA	NA	not used for any PCR
Col_796	Collembola	120	small	6	L. sp.	Male	4.5	5	no	NA	NA	
Col_820	Collembola	120	small	6	L. crassipes	Male	4.7	4.7	no	NA	NA	
Col_997	Collembola	120	small	7	L. mutabilis	Male	4.7	5.5	no	NA	NA	
Col_256	Collembola	144	small	6	L. crassipes	Male	4.7	4.6	yes	NA	NA	
Col_279	Collembola	144	small	6	L. crassipes	Female	4.2	4.3	no	NA	NA	
Col_418	Collembola	144	small	7	L. mutabilis	Male	4.7	4.6	yes	NA	NA	
Col_423	Collembola	144	small	7	L. mutabilis	Male	3.2	3.4	no	NA	NA	
Col_708	Collembola	144	small	6	L. crassipes	Male	3.6	3.8	no	NA	NA	
Col_738	Collembola	144	small	6	L. crassipes	Male	3.5	4.2	NA	NA	NA	not used for any PCR
Col_758	Collembola	144	small	6	L. mutabilis	Male	3.8	4.1	yes	NA	NA	
Col_915	Collembola	144	small	6	L. crassipes	Female	3.4	3.5	yes	NA	NA	
Col_953	Collembola	144	small	7	L. crassipes	Male	4.8	4.5	yes	NA	NA	
Col_990	Collembola	144	small	7	L. mutabilis	Male	3.8	3.8	yes	NA	NA	
Col_310	Collembola	168	small	5	L. crassipes	Male	3.4	3.5	yes	NA	NA	
Col_321	Collembola	168	small	7	L. crassipes	Male	3.5	3.4	yes	NA	NA	
Col_344	Collembola	168	small	5	L. crassipes	Male	1.5	1.7	no	NA	NA	
Col_722	Collembola	168	small	6	L. crassipes	Female	3.3	3.7	no	NA	NA	
Col_921	Collembola	168	small	5	L. sp.	NA	2.8	3.4	NA	NA	NA	not used for any PCR
Col_927	Collembola	168	small	5	L. crassipes	Male	3.9	4.1	NA	NA	NA	not used for any PCR
Col_941	Collembola	168	small	6	L. crassipes	Male	3.3	3.4	no	NA	NA	
Col_943	Collembola	168	small	5	L. sp.	NA	4.9	4.3	NA	NA	NA	not used for any PCR
Col_975	Collembola	168	small	7	L. sp.	NA	4.7	5.1	no	NA	NA	
Col_984	Collembola	168	small	5	L. crassipes	Male	2.8	2.9	no	NA	NA	
Col_1087	Collembola	168	small	6	L. sp.	Male	3.3	3.4	yes	NA	NA	
Col_T168	Collembola	168	small	NA	NA	NA	NA	NA	no	NA	NA	not used for body mass measurement
Lum_1118	Lumbricidae	0	medium	8	L. aulacopus	Female	10	10.1	yes	No Ct	No Ct	ambiguous qPCR results
Lum_1142	Lumbricidae	0	medium	10	L. mutabilis	Male	13.8	14.2	yes	No Ct	No Ct	ambiguous qPCR results

Lum_1196	Lumbricidae	0	medium	7	L. mutabilis	Male	4.5	5.1	yes	5.46E-07	2.28E+03	
Lum_1204	Lumbricidae	0	medium	11	L. mutabilis	Male	14.8	15.3	yes	4.05E-07	1.69E+03	
Lum_1222	Lumbricidae	0	medium	10	L. sp.	Female	14.7	15.1	no	1.17E-08	4.87E+01	
Lum_1267	Lumbricidae	0	medium	9	L. mutabilis	Male	6	5.9	yes	1.28E-05	5.35E+04	ambiguous qPCR results
Lum_1289	Lumbricidae	0	medium	10	L. mutabilis	Female	9.1	10.9	yes	2.92E-06	1.22E+04	
Lum_1292	Lumbricidae	0	medium	11	L. mutabilis	Male	15.4	15.3	no	4.05E-06	1.69E+04	ambiguous qPCR results
Lum_1294	Lumbricidae	0	medium	10	L. mutabilis	Male	11.9	11.7	yes	1.82E-06	7.63E+03	
Lum_1303	Lumbricidae	0	medium	10	L. mutabilis	Male	9.6	10.1	yes	5.22E-08	2.18E+02	
Lum_1021	Lumbricidae	16	medium	9	L. mutabilis	Female	8.3	9	yes	No Ct	No Ct	ambiguous qPCR results
Lum_1179	Lumbricidae	16	medium	11	L. mutabilis	Female	13.9	14.6	yes	NA	NA	
Lum_1185	Lumbricidae	16	medium	9	L. mutabilis	Male	5.6	6.2	yes	5.76E-07	2.41E+03	ambiguous qPCR results
Lum_1190	Lumbricidae	16	medium	10	L. mutabilis	Male	9.4	10	yes	1.77E-08	7.38E+01	
Lum_1202	Lumbricidae	16	medium	12	L. mutabilis	Male	9.8	10.6	no	1.59E-08	6.64E+01	
Lum_1206	Lumbricidae	16	medium	10	L. mutabilis	Male	14.7	14.8	no	1.97E-09	8.22E+00	
Lum_1244	Lumbricidae	16	medium	11	L. dentatus	Male	15.1	15.3	no	1.15E-09	4.83E+00	
Lum_1255	Lumbricidae	16	medium	10	L. aulacopus	Male	9.1	9.6	no	4.28E-09	1.79E+01	
Lum_1256	Lumbricidae	16	medium	11	L. aulacopus	Male	10.2	10.4	yes	2.36E-08	9.88E+01	
Lum_1302x	Lumbricidae	16	medium	9	L. aulacopus	Male	7.6	7.2	no	4.45E-08	1.86E+02	
Lum_1097	Lumbricidae	24	medium	10	L. nodulipes	Male	11.2	10.9	yes	2.65E-07	1.11E+03	
Lum_1154	Lumbricidae	24	medium	12	L. aulacopus	Male	9.9	10.5	yes	5.52E-09	2.31E+01	
Lum_1156	Lumbricidae	24	medium	11	L. sp.	Male	10.5	10.7	yes	7.88E-10	3.30E+00	
Lum_1157	Lumbricidae	24	medium	12	L. aulacopus	Male	12.8	13.1	yes	1.98E-07	8.26E+02	

Lum_1209	Lumbricidae	24	medium	12	L. aulacopus	Female	13.6	13.9	no	9.11E-10	3.81E+00	
Lum_1233	Lumbricidae	24	medium	10	L. aulacopus	Male	7	7	NA	2.10E-08	8.80E+01	not used for singleplex PCR
Lum_1265	Lumbricidae	24	medium	11	L. aulacopus	Male	10.1	10.8	yes	2.84E-08	1.19E+02	
Lum_1293	Lumbricidae	24	medium	8	L. aulacopus	Male	6.5	6.8	no	3.62E-08	1.51E+02	
Lum_1304	Lumbricidae	24	medium	10	L. sp.	Male	5.7	5.8	no	1.02E-06	4.25E+03	
Lum_1308	Lumbricidae	24	medium	8	L. mutabilis	Female	7.2	7.6	no	3.89E-08	1.63E+02	ambiguous qPCR results
Lum_1116	Lumbricidae	32	medium	12	L. mutabilis	Male	6.9	7.3	yes	7.12E-08	2.98E+02	
Lum_1117	Lumbricidae	32	medium	8	L. mutabilis	Male	6.4	6.5	yes	6.34E-08	2.65E+02	
Lum_1119	Lumbricidae	32	medium	10	L. crassipes	Male	8.2	8.4	yes	2.39E-05	1.00E+05	
Lum_1120	Lumbricidae	32	medium	8	L. aulacopus	Male	7.7	7.9	yes	1.01E-08	4.22E+01	
Lum_1125	Lumbricidae	32	medium	11	L. dentatus	Female	13.2	13.8	yes	No Ct	No Ct	ambiguous qPCR results
Lum_1149	Lumbricidae	32	medium	8	L. mutabilis	Female	7.1	7.3	yes	8.62E-06	3.60E+04	ambiguous qPCR results
Lum_1165	Lumbricidae	32	medium	8	L. mutabilis	Male	6.6	7.2	yes	6.45E-08	2.70E+02	
Lum_1195	Lumbricidae	32	medium	10	L. crassipes	Male	8.4	8.8	no	1.97E-06	8.23E+03	
Lum_1226	Lumbricidae	32	medium	11	L. mutabilis	Male	11.4	11.9	no	1.20E-09	5.03E+00	
Lum_1245	Lumbricidae	32	medium	9	L. mutabilis	Male	9.4	10.4	no	5.26E-09	2.20E+01	
Lum_945	Lumbricidae	40	medium	11	L. mutabilis	Male	13.9	14	no	NA	NA	
Lum_1127	Lumbricidae	40	medium	11	L. piceus	Male	15	15.7	no	5.13E-07	2.15E+03	ambiguous qPCR results
Lum_1160	Lumbricidae	40	medium	10	L. aulacopus	Male	8.4	8.6	yes	2.15E-08	8.98E+01	
Lum_1210	Lumbricidae	40	medium	10	L. aulacopus	Female	7.6	8.4	no	1.33E-09	5.57E+00	
Lum_1268	Lumbricidae	40	medium	10	L. aulacopus	Male	7.9	8.2	no	1.72E-07	7.19E+02	ambiguous qPCR results
Lum_1271	Lumbricidae	40	medium	10	L. sp.	Male	9.6	9.9	no	6.33E-08	2.65E+02	ambiguous qPCR results

Lum_1272	Lumbricidae	40	medium	10	L. sp.	Female	7.8	7.6	no	1.89E-06	7.89E+03	ambiguous qPCR results
Lum_1286	Lumbricidae	40	medium	10	L. sp.	Female	10.4	10.5	no	8.27E-09	3.46E+01	ambiguous qPCR results
Lum_1306	Lumbricidae	40	medium	11	L. aulacopus	Male	12.6	12.8	NA	5.03E-11	2.10E-01	not used for singleplex PCR
Lum_1307	Lumbricidae	40	medium	10	L. mutabilis	Male	8.2	8.7	no	1.81E-07	7.57E+02	ambiguous qPCR results
Lum_1128	Lumbricidae	48	medium	10	L. aulacopus	Male	6.8	7.5	no	NA	NA	
Lum_1129	Lumbricidae	48	medium	12	L. mutabilis	Male	15.6	15.8	no	3.40E-09	1.42E+01	ambiguous qPCR results
Lum_1147	Lumbricidae	48	medium	10	L. mutabilis	Female	8.4	8.6	no	3.43E-05	1.43E+05	ambiguous qPCR results
Lum_1169	Lumbricidae	48	medium	10	L. piceus	Female	11.3	11.8	no	No Ct	No Ct	ambiguous qPCR results
Lum_1241	Lumbricidae	48	medium	9	L. mutabilis	Male	6.1	6.2	no	3.81E-09	1.59E+01	ambiguous qPCR results
Lum_1263	Lumbricidae	48	medium	8	L. aulacopus	Male	7.9	8.3	no	3.48E-09	1.46E+01	ambiguous qPCR results
Lum_1283	Lumbricidae	48	medium	8	L. mutabilis	Male	5.7	5.7	no	6.51E-08	2.72E+02	ambiguous qPCR results
Lum_1285	Lumbricidae	48	medium	11	L. dentatus	Male	13.9	13.9	NA	3.43E-09	1.43E+01	not used for singleplex PCR
Lum_1296	Lumbricidae	48	medium	10	L. mutabilis	Male	13.6	11.8	no	2.40E-08	1.00E+02	
Lum_1298	Lumbricidae	48	medium	10	L. mutabilis	Female	11.6	11.8	no	2.91E-08	1.22E+02	
Lum_1220	Lumbricidae	56	medium	11	L. mutabilis	Male	14.1	14.6	NA	1.19E-07	4.98E+02	not used for singleplex PCR
Lum_1227	Lumbricidae	56	medium	9	L. mutabilis	Female	7.6	8.1	no	8.92E-11	3.73E-01	ambiguous qPCR results
Lum_1240	Lumbricidae	56	medium	10	L. mutabilis	Male	7.7	8.2	no	1.33E-08	5.56E+01	ambiguous qPCR results
Lum_1250	Lumbricidae	56	medium	10	L. mutabilis	Male	8.9	10.1	no	4.37E-10	1.83E+00	ambiguous qPCR results
Lum_1251	Lumbricidae	56	medium	9	L. mutabilis	Male	6.6	7.3	no	6.88E-10	2.88E+00	
Lum_1257	Lumbricidae	56	medium	10	L. aulacopus	Male	9.7	10.3	NA	No Ct	No Ct	
Lum_1259	Lumbricidae	56	medium	10	L. mutabilis	Female	8.8	9.7	no	NA	NA	
Lum_1262	Lumbricidae	56	medium	8	L. sp.	Male	6.1	6.2	no	1.16E-06	4.83E+03	ambiguous qPCR results

Lum_1284	Lumbricidae	56	medium	11	L. aulacopus	Male	11.9	12.8	NA			not used for any PCR
Lum_1287	Lumbricidae	56	medium	10	L. mutabilis	Male	7.8	8.4	no	2.76E-08	1.16E+02	ambiguous qPCR results
Lum_1131	Lumbricidae	72	medium	10	L. mutabilis	Male	9	9.2	no	No Ct	No Ct	ambiguous qPCR results
Lum_1139	Lumbricidae	72	medium	11	L. mutabilis	Female	10.9	11.3	no	No Ct	No Ct	ambiguous qPCR results
Lum_1203	Lumbricidae	72	medium	10	L. mutabilis	Male	11.9	12	no	2.13E-09	8.92E+00	ambiguous qPCR results
Lum_1207	Lumbricidae	72	medium	10	L. mutabilis	Female	9.4	10.8	no	1.16E-09	4.86E+00	
Lum_1212	Lumbricidae	72	medium	11	L. sp.	Male	10.9	12.6	no	NA	NA	
Lum_1225	Lumbricidae	72	medium	10	L. sp.	Female	14.5	14.7	no	NA	NA	
Lum_1228	Lumbricidae	72	medium	9	L. mutabilis	Male	6.6	6.7	no	7.46E-09	3.12E+01	ambiguous qPCR results
Lum_1234	Lumbricidae	72	medium	12	L. dentatus	Male	10.6	11.5	NA	4.26E-10	1.78E+00	not used for singleplex PCR
Lum_1276	Lumbricidae	72	medium	8	L. sp.	Male	4.7	5.2	no	1.92E-09	8.03E+00	
Lum_1282	Lumbricidae	72	medium	10	L. sp.	Male	8.3	8.2	no	3.39E-09	1.42E+01	ambiguous qPCR results
Lum_1191	Lumbricidae	88	medium	10	L. mutabilis	Male	9.9	9.5	no	9.46E-06	3.96E+04	
Lum_1318	Lumbricidae	88	medium	10	L. mutabilis	Female	9.8	10.2	no	NA	NA	
Lum_1320	Lumbricidae	88	medium	10	L. sp.	Female	9.8	11.4	no	1.92E-10	8.03E-01	ambiguous qPCR results
Lum_1323	Lumbricidae	88	medium	10	L. aulacopus	Male	8.9	9.3	no	1.14E-06	4.78E+03	ambiguous qPCR results
Lum_1324	Lumbricidae	88	medium	8	L. sp.	Male	7.4	7.1	no	1.25E-08	5.24E+01	
Lum_1331	Lumbricidae	88	medium	NA	NA	NA	5.7	6.1	no	NA	NA	
Lum_1332	Lumbricidae	88	medium	12	L. nodulipes	Female	14.4	14.9	no	5.20E-06	2.17E+04	ambiguous qPCR results
Lum_1338	Lumbricidae	88	medium	8	L. mutabilis	Female	8.2	9	no	1.97E-09	8.22E+00	
Lum_1349	Lumbricidae	88	medium	10	L. mutabilis	Male	13.7	13.7	no	1.77E-09	7.39E+00	ambiguous qPCR results
Lum_1351	Lumbricidae	88	medium	10	L. mutabilis	Male	10.3	11.3	no	NA	NA	
Lum_1391	Lumbricidae	88	medium	8	L. mutabilis	Male	NA	NA	NA	1.53E-09	6.39E+00	not used for any PCR

Lum_1175	Lumbricidae	104	medium	8	L. crassipes	Female	6.5	6.5	no	No Ct	No Ct	ambiguous qPCR results; not used for body mass measurements
Lum_1217	Lumbricidae	104	medium	NA	NA	NA	NA	NA	no	NA	NA	not used for body mass measurements
Lum_1218	Lumbricidae	104	medium	12	L. aulacopus	Male	12.2	12.9	NA	NA	NA	not used for any PCR
Lum_1248	Lumbricidae	104	medium	10	L. dentatus	Male	13.2	13.6	NA	2.47E-10	1.03E+00	not used for singleplex PCR
Lum_1249	Lumbricidae	104	medium	10	L. mutabilis	Male	9.8	11.3	no	2.12E-09	8.85E+00	ambiguous qPCR results
Lum_1260	Lumbricidae	104	medium	10	L. mutabilis	Male	9.8	10.5	no	1.49E-09	6.25E+00	ambiguous qPCR results
Lum_1261	Lumbricidae	104	medium	8	L. aulacopus	Male	6.4	7.8	no	1.47E-08	6.16E+01	ambiguous qPCR results
Lum_1274	Lumbricidae	104	medium	9	L. sp.	Male	7	7.3	no	NA	NA	
Lum_1279	Lumbricidae	104	medium	11	L. piceus	Female	11.7	13.1	no	7.34E-07	3.07E+03	ambiguous qPCR results
Lum_1313	Lumbricidae	104	medium	10	L. mutabilis	Female	12.4	12.7	no	1.64E-06	6.86E+03	ambiguous qPCR results
Lum_1130	Lumbricidae	120	medium	10	L. dentatus	Female	11.4	11.5	NA	NA	NA	not used for any PCR
Lum_1194	Lumbricidae	120	medium	8	L. crassipes	Male	4.8	5.4	no	No Ct	No Ct	ambiguous qPCR results
Lum_1211	Lumbricidae	120	medium	11	L. dentatus	Female	13.6	14.3	no	1.80E-08	7.52E+01	
Lum_1239	Lumbricidae	120	medium	10	L. mutabilis	Male	12	12.2	no	3.71E-09	1.55E+01	ambiguous qPCR results
Lum_1315	Lumbricidae	120	medium	7	L. crassipes	Female	5	4.8	no	1.20E-07	5.03E+02	ambiguous qPCR results
Lum_1317	Lumbricidae	120	medium	10	L. sp.	Male	9	9.3	no	8.34E-07	3.49E+03	ambiguous qPCR results
Lum_1339	Lumbricidae	120	medium	9	L. sp.	Male	7.8	7.2	no	2.63E-07	1.10E+03	ambiguous qPCR results
Lum_1340	Lumbricidae	120	medium	10	L. sp.	Male	14.3	13.3	NA	NA	NA	not used for any PCR
Lum_1341	Lumbricidae	120	medium	12	L. sp.	Male	12.7	14.5	no	2.97E-07	1.24E+03	ambiguous qPCR results
Lum_1342	Lumbricidae	120	medium	9	L. mutabilis	Female	7.5	7.7	no	6.90E-10	2.89E+00	ambiguous qPCR results
Lum_1319	Lumbricidae	144	medium	7	L. sp.	Male	6.5	7.1	no	2.26E-07	9.44E+02	ambiguous qPCR results
Lum_1327	Lumbricidae	144	medium	7	L. sp.	Male	5.7	6.2	no	2.18E-06	9.12E+03	ambiguous qPCR results
Lum_1328	Lumbricidae	144	medium	7	L. crassipes	Female	4.4	4.8	no	2.82E-07	1.18E+03	ambiguous qPCR results

Lum_1334	Lumbricidae	144	medium	12	L. dentatus	Female	12.2	12.8	no	9.86E-07	4.12E+03	ambiguous qPCR results
Lum_1336	Lumbricidae	144	medium	12	L. dentatus	Female	9.6	10.5	no	3.10E-09	1.30E+01	ambiguous qPCR results
Lum_1337	Lumbricidae	144	medium	10	L. sp.	Female	13.3	14.2	no	1.77E-06	7.40E+03	ambiguous qPCR results
Lum_1343	Lumbricidae	144	medium	10	L. dentatus	Female	12.2	12.8	NA	4.64E-08	1.94E+02	not used for singleplex PCR
Lum_1347	Lumbricidae	144	medium	8	L. mutabilis	Male	7	7	no	4.42E-08	1.85E+02	ambiguous qPCR results
Lum_1348	Lumbricidae	144	medium	10	L. sp.	Male	10.1	10.5	no	3.15E-07	1.32E+03	ambiguous qPCR results
Lum_1352	Lumbricidae	144	medium	10	L. mutabilis	Female	13.3	14	no	7.27E-08	3.04E+02	ambiguous qPCR results
Lum_1316	Lumbricidae	168	medium	10	L. dentatus	Male	8.7	8.7	no	5.52E-10	2.31E+00	ambiguous qPCR results
Lum_1326	Lumbricidae	168	medium	10	L. mutabilis	Female	9.1	9.4	no	1.19E-09	4.97E+00	ambiguous qPCR results
Lum_1329	Lumbricidae	168	medium	13	L. dentatus	Male	12.6	12.9	no	3.93E-10	1.64E+00	not used for any PCR
Lum_1330	Lumbricidae	168	medium	10	L. sp.	Male	12	12.2	NA	NA	NA	not used for any PCR
Lum_1333	Lumbricidae	168	medium	7	L. mutabilis	Male	6	6.2	no	3.85E-08	1.61E+02	ambiguous qPCR results
Lum_1335	Lumbricidae	168	medium	10	L. sp.	Male	12.3	13.9	no	4.15E-07	1.74E+03	ambiguous qPCR results
Lum_1344	Lumbricidae	168	medium	10	L. mutabilis	Female	13.7	14	no	NA	NA	
Lum_1346	Lumbricidae	168	medium	10	L. sp.	Male	11.3	11.6	no	2.13E-07	8.92E+02	ambiguous qPCR results
Lum_1350	Lumbricidae	168	medium	8	L. mutabilis	Male	7.4	7.8	no	1.40E-09	5.86E+00	ambiguous qPCR results
Lum_1353	Lumbricidae	168	medium	10	L. sp.	Male	13.9	14.3	no	7.48E-08	3.13E+02	ambiguous qPCR results
Lum_1123	Lumbricidae	NA	medium	NA	NA	NA	9.4	10	NA	NA	NA	not used for any PCR
Lum_1252	Lumbricidae	NA	medium	NA	NA	NA	15.6	15.8	NA	NA	NA	not used for any PCR
Lum_1325	Lumbricidae	NA	medium	NA	NA	NA	4.7	4.7	NA	NA	NA	not used for any PCR
Dipt_1000	Diptera	0	medium	8	L. sp.	Male	9.65	10.62	yes	2.31E-04	1.13E+06	
Dipt_1001	Diptera	0	medium	8	L. sp.	Male	9.65	10.62	yes	2.25E-03	1.10E+07	
Dipt_1002	Diptera	0	medium	8.5	L. sp.	Male	9.65	10.62	yes	4.10E-	2.00E+06	

										04		
Dipt_1003	Diptera	0	medium	9	L. sp.	Male	9.65	10.62	yes	4.18E-05	2.04E+05	
Dipt_1004	Diptera	0	medium	9.5	L. sp.	Male	9.65	10.62	yes	1.84E-03	8.99E+06	
Dipt_1005	Diptera	0	medium	9.5	L. sp.	Male	9.65	10.62	yes	2.79E-04	1.36E+06	
Dipt_1006	Diptera	0	medium	10.5	L. sp.	Male	9.65	10.62	yes	7.38E-04	3.60E+06	
Dipt_1007	Diptera	0	medium	10.5	L. sp.	Male	9.65	10.62	yes	6.11E-05	2.98E+05	
Dipt_1008	Diptera	0	medium	11.5	L. sp.	Female	9.65	10.62	yes	3.48E-04	1.70E+06	
Dipt_1009	Diptera	0	medium	NA	NA	NA	NA	NA	yes	NA	NA	
Dipt_250	Diptera	16	medium	10	L. muticus	Male	7.2	7.5	no	NA	NA	
Dipt_435	Diptera	16	medium	11.5	L. mutabilis	Male	17.5	17.7	no	NA	NA	
Dipt_484	Diptera	16	medium	10	L. mutabilis	Female	6.9	7	yes	1.60E-05	7.78E+04	
Dipt_490	Diptera	16	medium	7	L. crassipes	Female	3.6	4.5	yes	NA	NA	
Dipt_536	Diptera	16	medium	11	L. sp.	Male	12.5	13.1	yes	No Ct	No Ct	ambiguous qPCR results
Dipt_544	Diptera	16	medium	9	L. crassipes	Male	7.4	6.6	yes	NA	NA	
Dipt_550	Diptera	16	medium	9	L. nodulipes	Female	11.9	12.1	yes	NA	NA	
Dipt_871	Diptera	16	medium	8	L. crassipes	Male	6.5	7.2	yes	NA	NA	
Dipt_915	Diptera	16	medium	8	L. crassipes	Female	6.3	7.1	yes	NA	NA	
Dipt_919	Diptera	16	medium	10	L. muticus	Male	9.6	9.9	yes	8.76E-06	4.27E+04	
Dipt_406	Diptera	24	medium	9	L. sp.	Male	8.5	9.4	yes	NA	NA	
Dipt_466	Diptera	24	medium	8.5	L. crassipes	Female	7.1	7.9	yes	NA	NA	
Dipt_535	Diptera	24	medium	8.5	L. crassipes	Male	7.1	7.3	yes	NA	NA	
Dipt_547	Diptera	24	medium	12	L. mutabilis	Female	16.1	16	no	NA	NA	
Dipt_628	Diptera	24	medium	8.5	L. crassipes	Male	6.9	7.5	yes	1.12E-07	5.43E+02	
Dipt_775	Diptera	24	medium	7	L. crassipes	Male	3.7	4	no	NA	NA	
Dipt_806	Diptera	24	medium	11	L. muticus	Male	13	13.9	no	NA	NA	

Dipt_819	Diptera	24	medium	10.5	L. mutabilis	Male	14	15.5	yes	1.22E-05	5.95E+04	
Dipt_913	Diptera	24	medium	7.5	L. crassipes	Female	4.6	5.5	yes	NA	NA	
Dipt_939	Diptera	24	medium	11	L. sp.	Male	10.6	12	yes	1.24E-04	6.02E+05	
Dipt_399	Diptera	32	medium	8.5	L. crassipes	Female	9.8	11.1	yes	NA	NA	
Dipt_517	Diptera	32	medium	10	L. muticus	Male	8.1	8.4	yes	NA	NA	
Dipt_830	Diptera	32	medium	8	L. crassipes	Male	6	6.9	no	NA	NA	
Dipt_838	Diptera	32	medium	10.5	L. muticus	Female	11.9	11.3	no	NA	NA	
Dipt_872	Diptera	32	medium	10	L. sp.	Male	11.7	11.9	yes	NA	NA	
Dipt_914	Diptera	32	medium	9	L. aulacopus	Male	6.7	7.2	yes	NA	NA	
Dipt_916	Diptera	32	medium	8	L. crassipes	Female	5.1	5.2	no	NA	NA	
Dipt_926	Diptera	32	medium	10.5	L. mutabilis	Male	13.9	14.5	yes	7.56E-08	3.68E+02	
Dipt_930	Diptera	32	medium	10.5	L. mutabilis	Male	12.5	13.3	yes	3.34E-08	1.63E+02	
Dipt_931	Diptera	32	medium	11	L. mutabilis	Male	14.6	14.6	yes	1.62E-01	7.90E+08	
Dipt_525	Diptera	40	medium	10	L. sp.	Male	6.5	7.8	yes	No Ct	No Ct	ambiguous qPCR results
Dipt_541	Diptera	40	medium	10.5	L. muticus	Female	10.7	11.4	yes	NA	NA	
Dipt_811	Diptera	40	medium	9.5	L. sp.	Male	10.1	10.3	yes	NA	NA	
Dipt_917	Diptera	40	medium	10.5	L. nodulipes	Male	11.9	12.1	no	NA	NA	
Dipt_920	Diptera	40	medium	10	L. sp.	Male	9.8	9.8	yes	6.22E-08	3.03E+02	
Dipt_921	Diptera	40	medium	9	L. crassipes	Male	5.4	5.5	yes	NA	NA	
Dipt_922	Diptera	40	medium	7.5	L. crassipes	Male	5.1	5.6	yes	NA	NA	
Dipt_925	Diptera	40	medium	12	L. mutabilis	Male	12.1	12.8	yes	NA	NA	
Dipt_929	Diptera	40	medium	9.5	L. sp.	Male	7.3	8.4	yes	NA	NA	
Dipt_940	Diptera	40	medium	8.5	L. crassipes	Female	6	6.1	yes	NA	NA	
Dipt_276	Diptera	48	medium	9	L. crassipes	Female	16.9	17.5	yes	No Ct	No Ct	ambiguous qPCR results
Dipt_331	Diptera	48	medium	11	L. muticus	Male	12.4	12.8	no	No Ct	No Ct	ambiguous qPCR results
Dipt_336	Diptera	48	medium	9.5	L. aulacopus	Male	10.9	12	no	NA	NA	

Dipt_350	Diptera	48	medium	11	L. mutabilis	Male	14.1	14.4	no	NA	NA
Dipt_352	Diptera	48	medium	9	L. nodulipes	Male	7.3	9.2	no	NA	NA
Dipt_451	Diptera	48	medium	9.5	L. nodulipes	Male	10.5	10.6	no	NA	NA
Dipt_464	Diptera	48	medium	9	L. aulacopus	Male	8.8	8.9	no	NA	NA
Dipt_493	Diptera	48	medium	8.2	L. crassipes	Male	7	6.8	no	NA	NA
Dipt_776	Diptera	48	medium	10	L. aulacopus	Male	11	12.1	yes	NA	NA
Dipt_792	Diptera	48	medium	9	L. crassipes	Female	8.8	9.2	no	NA	NA
Dipt_255	Diptera	56	medium	10	L. mutabilis	Male	14.4	14.8	no	NA	NA
Dipt_346	Diptera	56	medium	11	L. aulacopus	Male	14.9	14.9	no	NA	NA
Dipt_358	Diptera	56	medium	11	L. dentatus	Male	15.7	16.1	no	NA	NA
Dipt_391	Diptera	56	medium	10.5	L. mutabilis	Male	11.3	11.2	yes	NA	NA
Dipt_422	Diptera	56	medium	10.5	L. sp.	Male	10.7	12.9	no	NA	NA
Dipt_521	Diptera	56	medium	11	L. mutabilis	Male	16.6	16.7	no	NA	NA
Dipt_555	Diptera	56	medium	10.5	L. muticus	Male	16.1	15.7	yes	NA	NA
Dipt_817	Diptera	56	medium	9	L. crassipes	Male	10.3	10.9	no	NA	NA
Dipt_823	Diptera	56	medium	9.5	L. sp.	Male	10.7	11.4	yes	1.80E-05	8.78E+04
Dipt_859	Diptera	56	medium	11	L. sp.	?	16.3	17	no	NA	NA
Dipt_281	Diptera	72	medium	8.5	L. sp.	Male	11	11.4	no	NA	NA
Dipt_788	Diptera	72	medium	10.5	L. muticus	Female	10.3	10.8	no	NA	NA
Dipt_789	Diptera	72	medium	8.5	L. aulacopus	Male	6.3	7.5	no	1.48E-08	7.20E+01
Dipt_821	Diptera	72	medium	11.5	L. mutabilis	Female	11.5	12.4	no	NA	NA
Dipt_832	Diptera	72	medium	10	L. mutabilis	Female	14.8	15	no	NA	NA
Dipt_863	Diptera	72	medium	9	L. crassipes	Male	10.4	11.6	no	NA	NA
Dipt_885	Diptera	72	medium	10	L. sp.	Female	10	10.3	no	NA	NA
Dipt_888	Diptera	72	medium	10	L. aulacopus	Male	8.9	10.3	no	NA	NA
Dipt_895	Diptera	72	medium	10	L. mutabilis	Female	8.8	9.9	no	NA	NA
Dipt_923	Diptera	72	medium	9	L. crassipes	Female	6	6.6	no	NA	NA

Dipt_767	Diptera	88	medium	9	L. sp.	Male	10.8	11.5	yes	No Ct	No Ct	ambiguous qPCR results
Dipt_781	Diptera	88	medium	9.5	L. mutabilis	Female	14	14.8	no	NA	NA	
Dipt_793	Diptera	88	medium	10	L. mutabilis	Male	11.8	12.2	no	No Ct	No Ct	ambiguous qPCR results
Dipt_800	Diptera	88	medium	9.5	L. aulacopus	Female	9.2	10	no	NA	NA	
Dipt_803	Diptera	88	medium	10.5	L. muticus	Male	8.1	8.7	yes	NA	NA	
Dipt_827	Diptera	88	medium	10.5	L. muticus	Female	13.7	17.7	no	4.03E-05	1.96E+05	
Dipt_852	Diptera	88	medium	10	L. nodulipes	?	9.4	10.3	no	NA	NA	
Dipt_870	Diptera	88	medium	8	L. crassipes	Male	5	5.9	no	NA	NA	
Dipt_878	Diptera	88	medium	9	L. mutabilis	Male	10	10.4	no	NA	NA	
Dipt_893	Diptera	88	medium	10.5	L. sp.	Female	10	10.6	no	No Ct	No Ct	ambiguous qPCR results
Dipt_765	Diptera	104	medium	11.5	L. aulacopus	Male	12.4	12.9	no	NA	NA	
Dipt_766	Diptera	104	medium	9.5	L. aulacopus	Male	10.6	11.2	no	NA	NA	
Dipt_772	Diptera	104	medium	10.5	L. mutabilis	Male	11.9	12.6	no	NA	NA	
Dipt_776	Diptera	104	medium	10	L. aulacopus	Male	11	12.1	yes	NA	NA	
Dipt_791	Diptera	104	medium	10	L. mutabilis	Male	10.6	11.2	no	NA	NA	
Dipt_828	Diptera	104	medium	9	L. aulacopus	Male	9.9	11	no	NA	NA	
Dipt_843	Diptera	104	medium	11	L. mutabilis	Male	13.7	14.4	no	NA	NA	
Dipt_874	Diptera	104	medium	9	L. crassipes	Female	8.2	8.9	no	NA	NA	
Dipt_896	Diptera	104	medium	11.5	L. mutabilis	Male	12.3	12.9	no	NA	NA	
Dipt_906	Diptera	104	medium	10	L. mutabilis	Male	6.5	7.3	no	NA	NA	
Dipt_270	Diptera	120	medium	10.5	L. mutabilis	Female	12.7	12.9	no	NA	NA	
Dipt_777	Diptera	120	medium	10	L. crassipes	Male	10.9	11.3	no	NA	NA	
Dipt_805	Diptera	120	medium	NA	NA	NA	9.7	10.1	no	NA	NA	
Dipt_815	Diptera	120	medium	11.5	L. muticus	Male	10.8	11.1	no	NA	NA	
Dipt_860	Diptera	120	medium	8	L. crassipes	Female	6.5	7.2	no	NA	NA	
Dipt_864	Diptera	120	medium	11	L. sp.	Male	11.4	12.4	no	NA	NA	
Dipt_873	Diptera	120	medium	11.5	L. muticus	Male	12.1	13.2	no	No Ct	No Ct	ambiguous qPCR results
Dipt_889	Diptera	120	medium	13.5	L. muticus	Male	22.2	22.8	no	NA	NA	

Dipt_891	Diptera	120	medium	10	L. mutabilis	Female	9.9	10.6	no	NA	NA	
Dipt_901	Diptera	120	medium	NA	NA	NA	14	14.8	no	No Ct	No Ct	ambiguous qPCR results
Dipt_322	Diptera	144	medium	9	L. crassipes	Male	11.9	12.8	no	No Ct	No Ct	ambiguous qPCR results
Dipt_778	Diptera	144	medium	10.5	L. muticus	Male	12	13	no	No Ct	No Ct	ambiguous qPCR results
Dipt_779	Diptera	144	medium	10.5	L. muticus	Male	10.3	11	no	NA	NA	
Dipt_780	Diptera	144	medium	11	L. sp.	Female	8	8.6	yes	NA	NA	
Dipt_786	Diptera	144	medium	9	L. sp.	Female	8	9	no	NA	NA	
Dipt_790	Diptera	144	medium	8.5	L. nodulipes	Female	5.5	6.1	no	NA	NA	
Dipt_813	Diptera	144	medium	11	L. muticus	Male	14.8	15.7	no	No Ct	No Ct	ambiguous qPCR results
Dipt_848	Diptera	144	medium	8	L. crassipes	Male	5.7	6.6	no	NA	NA	
Dipt_861	Diptera	144	medium	7.5	L. sp.	Male	5.4	6.3	no	NA	NA	
Dipt_882	Diptera	144	medium	11.5	L. sp.	Female	12.1	12.7	yes	NA	NA	
Dipt_251	Diptera	168	medium	11	L. aulacopus	Male	12.1	12.5	no	NA	NA	
Dipt_340	Diptera	168	medium	9	L. crassipes	Female	12.4	16.3	no	NA	NA	
Dipt_408	Diptera	168	medium	10	L. mutabilis	Male	9.3	10.2	no	No Ct	No Ct	ambiguous qPCR results
Dipt_444	Diptera	168	medium	9	L. crassipes	Male	8	9.2	no	NA	NA	
Dipt_468	Diptera	168	medium	11	L. mutabilis	Female	13.9	14.5	no	NA	NA	
Dipt_496	Diptera	168	medium	10.5	L. muticus	Female	8.1	9.5	no	NA	NA	
Dipt_557	Diptera	168	medium	8	L. crassipes	Male	7.1	8.2	no	NA	NA	
Dipt_351	Diptera	168	medium	NA	NA	NA	6.3	7.1	no	NA	NA	
Dipt_560	Diptera	168	medium	9	L. sp.	Female	7	7.6	no	NA	NA	
Dipt_396	Diptera	168	medium	9.5	L. sp.	Male	12.3	13.4	no	NA	NA	

Appendix Chapter 4

Table A1: Number and percentage of *Lithobius* sp. and *Philonthus carbonarius* tested positive for prey DNA in exploratories Hainich (HEW) and Schorfheide (SEW). Columns marked with a star (*) are percentages in relation to the number of tested predators. Number of predators tested positive for 1, 2 or 3 prey groups are presented in the last column. Individuals of L. curtipes (n= 32; SEW only) were included in number of L. crassipes

		Prey						Predators	Multiple prey DNA detection		
Area	Predator	N Collembolan	% Collembolan*	N Dipteran	% Dipteran*	N Lumbricid	% Lumbricid*	N tested	1	2	3
HEW	*Lithobius* sp. overall	79	40.9	21	10.9	29	15.0	193	79	22	2
	L. crassipes	21	30.4	3	4.3	7	10.1	69	21	5	0
	L. mutabilis	58	46.8	18	14.5	22	17.7	124	58	17	2
	P. carbonarius	20	90.9	2	9.1	7	31.8	22	12	7	1
SEW	*Lithobius* sp. overall	56	44.8	58	46.4	69	55.2	125	40	43	19
	L. crassipes	30	69.8	27	62.8	21	36.8	57	18	15	10
	L. mutabilis	26	38.2	31	45.6	48	70.6	68	22	28	9
	P. carbonarius	8	53.3	3	20.0	9	60.0	15	4	5	2
In total	*Lithobius* sp. overall	135	42.5	79	24.8	98	30.8	318	119	65	21
	L. crassipes	51	45.5	30	26.8	28	22.2	126	39	20	10
	L. mutabilis	84	43.8	49	25.5	70	36.5	192	80	45	11
	P. carbonarius	28	75.7	5	13.5	16	43.2	37	16	12	3

Table A2: Summary of generalized linear model (GLM) on the effect of exploratory, predator species, prey type, predator body mass (mg), prey abundance, litter mass and soil pH in *Lithobius* predators. SE represents the standard error of the estimated coefficient of the model. Significant effects are indicated with (*).

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	4.4419	2.2273	1.994	0.04612 *
Schorfheide	0.5343	0.8123	0.658	0.51071
Diptera prey	-7.5428	3.5877	2.102	0.03552 *
Lumbricidae prey	-1.7930	3.4284	-0.523	0.60098
Predator body mass ²	1.0329	1.5566	0.664	0.50697
Soil pH	-0.6875	0.2433	- 2.826	0.00472 **
Predator body mass	-1.4734	3.1283	- 0.471	0.63765
Schorfheide × Diptera prey	2.2553	0.5427	4.156	3.24e-05 ***
Schorfheide × Lumbricidae prey	2.6785	0.5459	4.907	9.26e-07 ***
Diptera prey × Predator body mass ²	-1.3549	2.5890	- 0.523	0.60075
Lumbricidae prey × Predator body mass²	7.2120	2.5281	2.853	0.00433 **
Diptera prey × Predator body mass	4.8833	5.4438	0.897	0.36971
Lumbricidae prey × Predator body mass	-12.4776	5.1011	- 2.446	0.01444 *
Schorfheide × Predator body mass	-1.0691	0.6614	- 1.616	0.10602
Diptera prey × Soil pH	0.3228	0.3788	0.852	0.39416
Lumbricidae prey × Soil pH	0.7690	0.3730	2.061	0.03926 *

Table A3: Summary of generalized linear model (GLM) on the effect of predator species, predator body mass (mg), prey abundance, litter mass and soil pH in *Lithobius* predators and dipteran prey in the Hainich exploratory. SE represents the standard error of the estimated coefficient of the model. Significant effects are indicated with (*).

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	-68.779	26.953	-2.552	0.0107 *
L. mutabilis	-9.229	3.743	-2.466	0.0137 *
Predator body mass	33.402	25.063	1.333	0.1826
Predator body mass²	-13.157	10.575	-1.244	0.2135
Prey abundance	20.434	8.860	2.306	0.0211 *
Litter mass	7.754	5.493	1.412	0.1581
L. mutabilis x Litter mass	-13.966	6.025	-2.318	0.0204 *

Table A4: Summary of generalized linear model (GLM) on the effect of predator species, predator body mass (mg), prey abundance, litter mass and soil pH in Lithobius predators and collembolan prey in the Hainich exploratory. SE represents the standard error of the estimated coefficient of the model. Significant effects are indicated with (*).

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	19.8531	7.4969	2.648	0.00809 **
L. mutabilis	-13.3966	8.7765	-1.526	0.12690
Predator body mass	-8.4593	4.6798	-1.808	0.07066
Predator body mass²	4.1918	2.2958	1.826	0.06788 .
Prey abundance	-3.3777	1.6220	-2.082	0.03730 *
Litter mass	6.6102	2.8597	2.312	0.02080 *
Soil pH	0.6688	0.8798	0.760	0.44718
L. mutabilis x Prey abundance	5.7919	2.0353	2.846	0.00443 **
L. mutabilis x Litter mass	-8.2022	3.3140	-2.475	0.01332 *
L. mutabilis x Soil pH	-3.0617	1.0766	-2.844	0.00446 **

Table A5: Summary of generalized linear model (GLM) on the effect of predator species, predator body mass (mg), prey abundance, litter mass and soil pH in Lithobius predators and lumbricid prey in the Hainich exploratory. SE represents the standard error of the estimated coefficient of the model. Significant effects are indicated with (*).

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	-3.1153	0.6335	-4.918	8.76e-07 ***
Predator body mass²	1.1231	0.4572	2.456	0.014 *

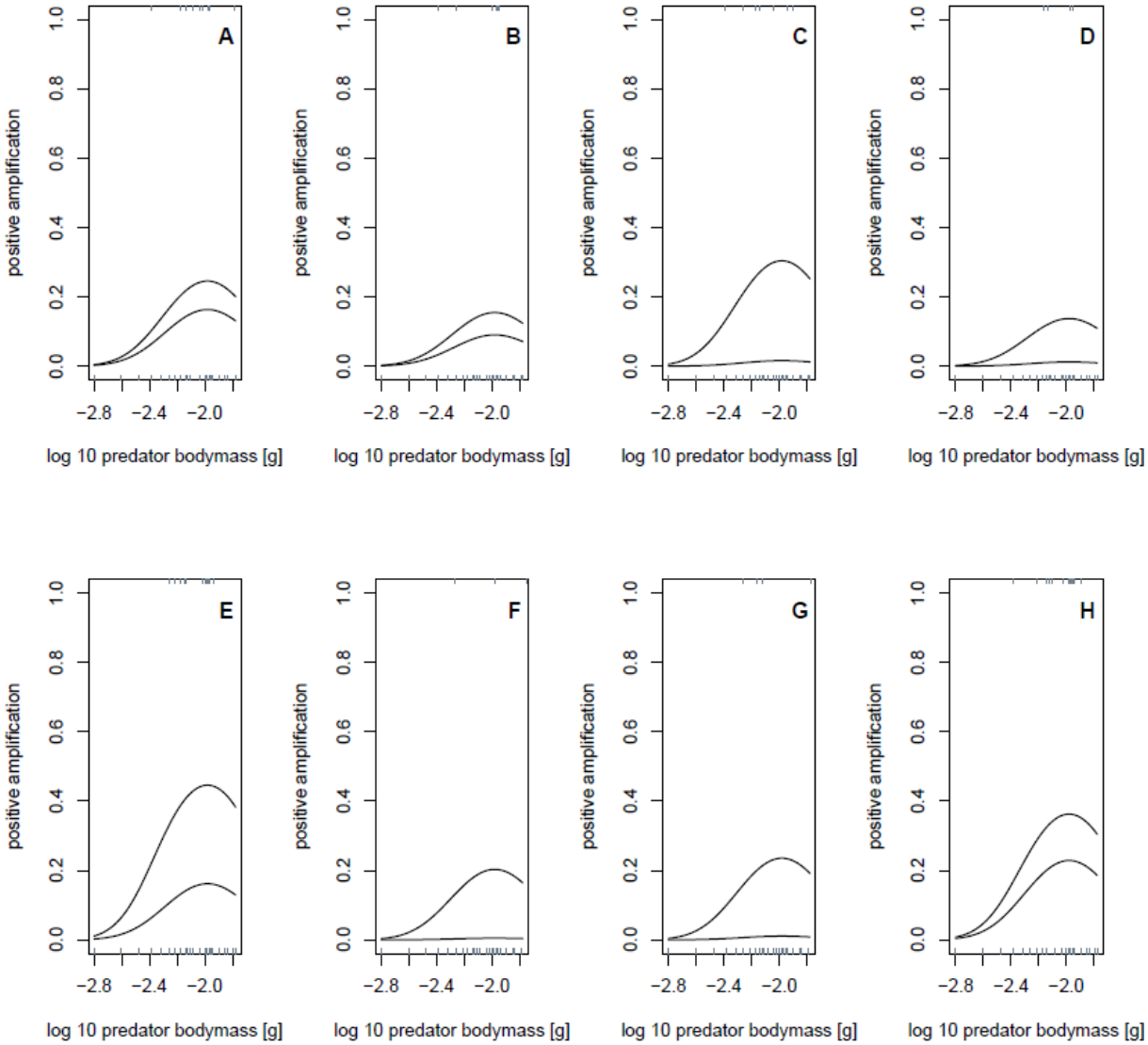
Table A6: Summary of generalized linear model (GLM) on the effect of predator species, predator body mass (mg), prey abundance, litter mass and soil pH in Lithobius predators and collembolan prey in the Schorfheide exploratory. SE represents the standard error of the estimated coefficient of the model. Significant effects are indicated with (*).

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	0.1054	0.2653	0.397	0.691
L. mutabilis	-0.5849	0.3642	-1.606	0.108

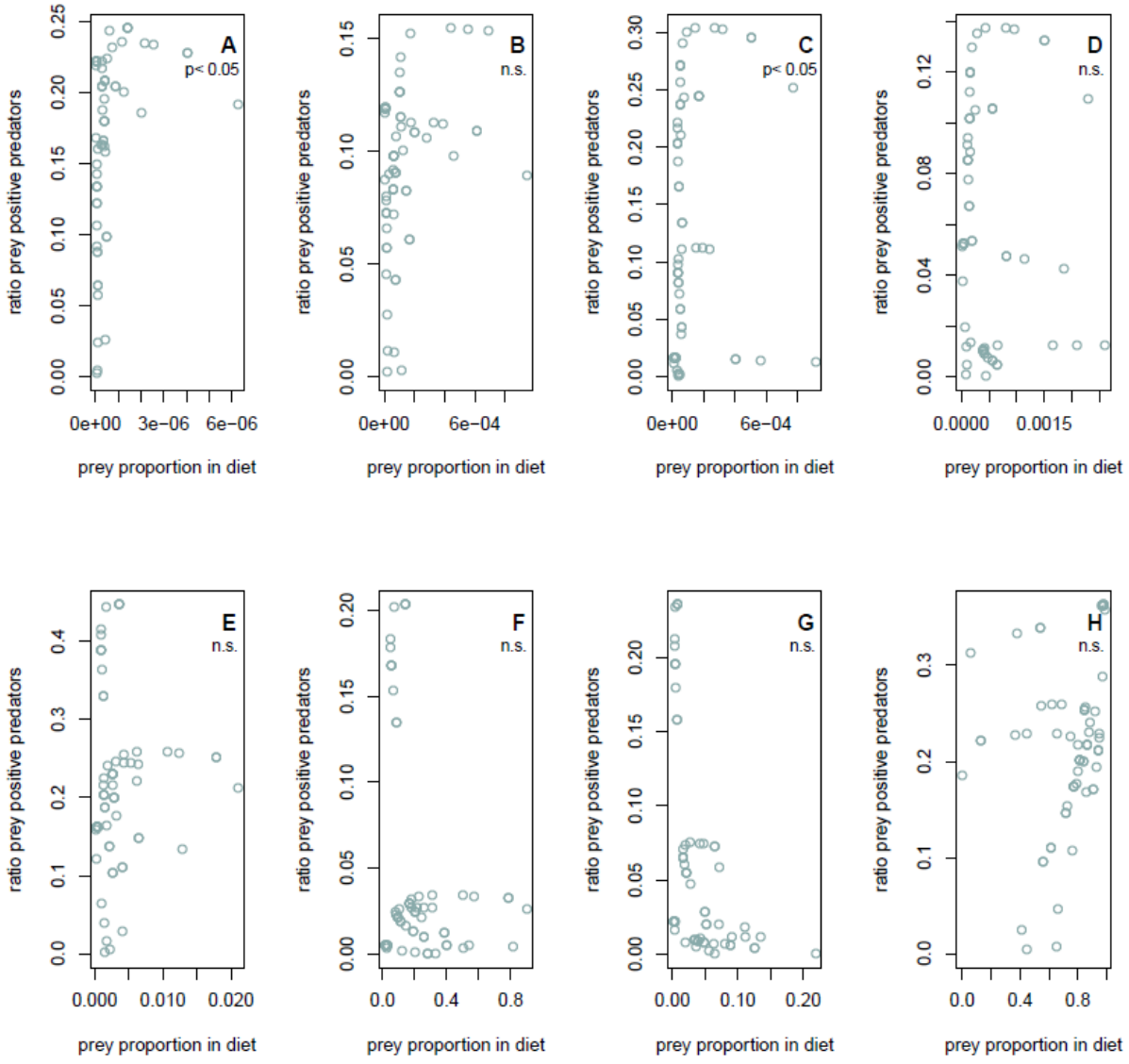
Appendix Chapter 5

Appendix S1. Generalized linear model (GLM) on the effect of predator body mass, square of predator body mass, prey type and the two-way interactions on the detection of prey DNA in geophilomorph predators. Significant effects are highlighted in bold.

Variable	Df	Deviance	Resid. Df	Resid. Dev	P(>|Chi|)
NULL			519	391.84	
Log_{10} predator body mass	1	6.3881	518	385.45	**0.011489**
Log_{10} predator body mass^2	1	5.2536	517	380.20	**0.021901**
Log_{10} prey abundance	1	0.8910	516	379.31	0.345219
Prey type	7	23.2663	509	356.04	**0.001532**
Prey type× Log_{10} prey abundance	7	15.9512	502	340.09	**0.025566**



Appendix S2. Body-size dependent probability of positive detection of eight prey taxa in geophilomorph predators (n=65) collected in the Hainich forest in autumn 2009 and spring 2010. **A** Lumbricidae, **B** Araneae, **C** Staphylinidae, **D** Isopoda, **E** Diptera, **F** Oribatida, **G** Gamasida, **H** Collembola. Upper line indicates feeding at maximum, lower line at minimum prey abundance. Rugs on top and bottom of each diagram display single data points with values 1 or 0.



Appendix S3. Pearson correlation coefficients between functional response models (output: prey proportion in diet) and results of general linear model with molecular gut content data (output: proportion of prey-positive tested predators) of centipede predator Geophilomorpha and eight prey groups. **A** Lumbricidae, **B** Araneae, **C** Staphylinidae, **D** Isopoda, **E** Diptera, **F** Oribatida, **G** Gamasida, **H** Collembola

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Scientific publications

(1) Eitzinger, B., Micic, A., Körner, M., Traugott, M. & Scheu, S. (2013). Unveiling soil food web links: New PCR assays for detection of prey DNA in the gut of soil arthropod predators. *Soil Biology and Biochemistry*, 57, 943–945.

(2) Eitzinger, B. & Traugott, M. (2011). Which prey sustains cold-adapted invertebrate generalist predators in arable land? Examining prey choices by molecular gut-content analysis. *Journal of Applied Ecology*, 48, 591–599.

Scientific presentations

(3) Eitzinger B, Rall BC, Traugott M, Scheu S (2013): *Does size matter? Impact of predator body mass and prey type on feeding patterns of litter dwelling centipedes*. MTI-2. 2nd International Symposium on the Molecular Detection of Trophic Interactions May 13-17 2013, Lexington, Kentucky, USA (oral presentation)

(4) Eitzinger B & Scheu S (2010): *Analysing trophic links in the litter food web of temperate forests: A molecular approach*. The GfÖ 40th Anniversary Meeting 2010, 30 August- 3. September, Gießen (poster presentation)

(5) Eitzinger B & Traugott M (2009):*"Serve chilled": Trophic interactions of autumn-and winter active beetle larvae*. Entomologentagung 16. -19. März, Göttingen (oral presentation)

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