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Testing the validity of functional response models using molecular gut content analysis for prey choice in soil predators

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Analysis of predator–prey interactions is a core concept of animal ecology, explaining structure and dynamics of animal food webs. Measuring the functional response, i.e. the intake rate of a consumer as a function of prey density, is a powerful method to predict the strength of trophic links and assess motives of prey choice, particularly in arthropod communities. However, due to their reductionist set-up, functional responses, which are based on laboratory feeding experiments, may not display field conditions, possibly leading to skewed results. Here, we tested the validity of functional responses of centipede predators and their prey by comparing them with empirical gut content data from field-collected predators. Our predator–prey system included lithobiid and geophilomorph centipedes, abundant and widespread predators of forest soils and their soil-dwelling prey. First, we calculated the body size-dependent functional responses of centipedes using a published functional response model in which we included natural prey abundances and animal body masses. This allowed us to calculate relative proportions of specific prey taxa in the centipede diet. In a second step, we screened field-collected centipedes for DNA of eight abundant soil-living prey taxa and estimated their body size-dependent proportion of feeding events. We subsequently compared empirical data for each of the eight prey taxa, on proportional feeding events with functional response-derived data on prey proportions expected in the gut, showing that both approaches significantly correlate in five out of eight predator–prey links for lithobiid centipedes but only in one case for geophilomorph centipedes. Our findings suggest that purely allometric functional response models, which are based on predator–prey body size ratios are too simple to explain predator–prey interactions in a complex system such as soil. We therefore stress that specific prey traits, such as defence mechanisms, must be considered for accurate predictions.

Keywords: allometric scaling, generalist predator, molecular prey detection, predator–prey interaction

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Introduction

Analysis of consumer–resource interactions is key to understand the structure and dynamics of food webs, eventually explaining composition, stability and development of communities and ecological processes coupled with them. Depending on the specific question and scale of feeding interactions, ecologists are able to select from a broad spectrum of methods, from field observations to tracking of nutrients and DNA in the consumer's body (Nielsen et al. 2017). Measuring the functional response, i.e. the intake rate of a consumer (hereafter referred to as predator) as a function of food resource (hereafter referred to as prey) density has been demonstrated to be a powerful method to assess the interaction strength (Holling 1959). Based on a small set of parameters including densities and body sizes of prey and predator, functional response models allow predicting general patterns and mechanisms of trophic interactions in very different systems, spanning from *Daphnia* water fleas feeding on phytoplankton to wolf packs preying on moose (Messier 1994, Sarnelle and Wilson 2008). The approach allows investigating the strength of feeding interactions on a large scale and can be modified to include changes in body size (Hansen et al. 1997, Pawar et al. 2012, Rall et al. 2012), ambient temperature (Hansen et al. 1997, Englund et al. 2011, Rall et al. 2012) as well as habitat structure (Hauzy et al. 2010, Kalinkat et al. 2013a, Kalinkat and Rall 2015).

The simplicity of functional responses, however, may not depict interaction strength of predator–prey links in the field. Functional responses, in particular those of invertebrates, are typically based on single predator–prey laboratory feeding trials, which lack many characteristics of natural settings. Among these are, for example, habitat structure, competitors and alternative prey (but see Skalski and Gilliam 2001, Elliott 2004, 2006, Vucic-Pestic et al. 2010a, b, Kalinkat et al. 2011, Lang et al. 2011, DeLong 2014, Kalinkat and Rall 2015, Barrios-O'Neill et al. 2015, 2016, as overview for exceptions). Especially the labour-intensive characteristics of functional response experiments hindered till now to generate enough data to create an empirical based comprehensive framework unifying combined effects of body mass, habitat structure, mutual interference and the effect of alternative prey. Thus, the majority functional response models based on idealized laboratory settings (one prey type, a fixed number of predators, none to simple habitat structures) may be of limited use to predict feeding interactions in the field. To test the predictive power of functional response models and evaluate the accuracy in reflecting natural feeding interactions, we compare the outcome of a laboratory-based allometric multi-species functional response model ignoring taxonomic differences of predators and prey with empirically measured trophic interactions using DNA-based gut content analysis from the field.

DNA-based molecular gut content analysis offers a state-of-the-art technique (Pompanon et al. 2012, Traugott et al. 2013, Nielsen et al. 2017) to identify trophic links in various systems including sea shores (Peters et al. 2014), arctic tundra (Wirta et al. 2015) and arable soils (Wallinger et al. 2014). Using specifically designed PCR assays targeting prey DNA in a predator's gut, species-specific trophic interactions can be tracked, allowing to unravel trophic links in unprecedented detail (Eitzinger et al. 2013, Traugott et al. 2013, Nielsen et al. 2017). The frequency at which specific prey is detected in field-collected predators provides a good proxy for trophic interaction strength (Traugott et al. 2013, Baker et al. 2014). Hence, molecular gut content analysis allows to empirically assess complex trophic interactions in the field and provides the opportunity to evaluate functional response models under natural settings.

We adopted this approach, for the first time, using a soil predator–prey system in European deciduous forests. We examined the body size-dependent predation frequency on abundant prey of centipedes (Chilopoda, Myriapoda), widespread generalist predators in the litter and soil layers of temperate forests (Lewis 1981, Poser 1988). Moreover, we use predictive models from laboratory functional response experiments and compare these with field-measured trophic links. This allows for evaluating the suitability and effectiveness of functional response models for analysing trophic interactions in a complex soil system.

Centipedes, in particular lithobiid (Lithobiidae) and geophilomorph (Geophilomorpha) species, prey on a variety of prey taxa including Collembola, Diptera larvae and Lumbricidae (Günther et al. 2014). Lithobiids predominantly colonize the litter layer and perform a sit-and-wait strategy of prey capture, whereas geophilomorph centipedes are active hunters in crevices of the mineral soil (Lewis 1981, Poser 1988). Prey capture of centipedes specifically depends on body size, indicating an allometric relationship between predator and prey size (Schneider et al. 2012, Günther et al. 2014). Typically, small predators have narrow diets while large predators feed on a wider range of prey including taxa from the same trophic level, i.e. intraguild prey (Woodward and Hildrew 2002, Riede et al. 2011). Body size-dependent prey-switching, coupled with feeding on intraguild prey may be a key factor reducing dietary niche overlap (Woodward and Hildrew 2002). Moreover, it might explain coexistence of different centipede species and other predators in forest soils. Studies employing functional response models suggest that body size acts as a supertrait, explaining most of the variance in predator–prey interactions in soil systems (Vucic-Pestic et al. 2010a, Kalinkat et al. 2013b). Hence, allometry-based functional response models may be applied to many different predator–prey interactions.

Based on the generalised allometric functional response model by Kalinkat et al. (2013b), we calculated body size-dependent trophic interaction strength of centipede predators as a function of natural abundances of different prey groups present in soil of unmanaged beech forests in central Germany. We then analysed the gut content of field-collected centipedes from the same forests using nine group- and five species-specific primers for DNA of abundant prey taxa.

We hypothesized that 1) the strength of actual feeding interactions of centipedes in the field is driven by predator–prey body size ratios, and that 2) actual feeding interactions in a complex system such as soil may not fully predicted by allometric functional response models.

Material and methods

Sampling of predator and prey individuals

Invertebrate predators were collected in four unmanaged beech forests (>120 years old) within the national park Hainich (Mülverstedt, Thuringia, Germany). Each study plot spanned 1 ha and formed part of the Biodiversity Exploratories, an integrated biodiversity project (Fischer et al. 2010). To investigate trophic links during periods of maximum invertebrate activity, we sampled animals in autumn and spring/early summer, each represented by four sampling dates (8, 20 and 28 October, 3 November 2009; 15, 24 and 29 June and 8 July 2010). Centipedes were collected by sieving litter (i.e. the L horizon consisting of little decomposed plant material; ca 5 cm deep) of the beech forest soil until we had sampled a minimum of 15 individuals per plot and date. Specimens were transferred individually to 1.5 ml microcentrifuge tubes, cooled in transport boxes, freeze-killed and stored at the same day 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 May of 2008 and 2011 (Klarner et al. 2014). 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, earthworms were collected by hand after application of mustard solution (Eisenhauer et al. 2008). Average densities between the two sampling dates were taken to represent prey density at the sampling dates of centipedes. While collection of prey and predators took place in different years, we assume this to be justified as soil arthropod composition and density does not change significantly between years for most prey (Bengtsson 1994, Bluhm et al. 2016, Pollierer and Scheu 2017). A separate analysis of prey animal densities between years showed that soil arthropod composition and density indeed did not change significantly for most prey, except for oribatid mites (one-way ANOVA, $F=25.99$, $p=0.00223$, Supplementary material Appendix 1 Table A1).

A total of 532 field-caught *Lithobius* spp. and 65 geophilomorph centipedes were – if possible – identified to species level using the keys of Eason (1964) and Latzel (1880). Further, we determined developmental stages and body length of each individual. We used several mass–length regressions to calculate predator and prey body masses, which could then be used in the functional response model. Body mass of lithobiid centipedes was calculated using the following equation:

$$\log_{10}M = 2.32784 \times \log_{10}L - 1.24015 \quad (1)$$

with fresh body mass (M) and body length (L) of individuals. The equation is based on body length–body mass relationship of 560 lithobiid individuals used in laboratory studies (Eitzinger et al. 2014). Based on body size of collected specimens from the study plot, the body mass of geophilomorph centipedes and all prey taxa was calculated using mass–length regressions given in Gowing and Recher (1984) and Mercer et al. (2001). Note, that we used order-specific equations, except for lithobiid centipedes and staphylinid beetles, which were based on family level. Body mass (for predator and prey) and prey abundance were $\log_{10}$-transformed prior to statistical analyses.

DNA extraction

We extracted DNA of whole centipedes including prey DNA using a CTAB-based DNA extraction protocol (Juen and Traugott 2005) with modifications given in Eitzinger et al. (2013). DNA extracts were purified using Geneclean Kit (MP Biomedicals, Solon, OH, USA). To test for cross contamination of samples, a blank control (containing DNA-free water instead of animal tissue) was included within each batch of 47 individuals. No contamination was found when testing these controls using the universal invertebrate primer pair LCO1490/HCO2198 (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×)(Geneaxxon, Ulm, Germany), 1.25 mM $MgCl_2$, 0.5 µl bovine serum albumin (BSA, 3%), 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 at 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 separated in 1% ethidium bromide-stained agarose gels and visualized under UV-light.

Screening predators for prey DNA

All centipede DNA extracts were screened for DNA of Araneae, Collembola, Diptera, Gamasida, Isopoda, Lumbricidae, Oribatida and Staphylinidae using group-specific primers (Eitzinger et al. 2013). PCR mixes and thermocycling conditions were the same as above only differing in the primers used, an elongation step at 72°C for 45 s and the primer pair-specific annealing temperature (for primers and annealing temperature see Supplementary material Appendix 1 Table A2). DNA extracts of geophilomorph centipedes were tested additionally for consumption of *Lithobius* spp. intraguild prey. All predator samples scoring positive for Collembola were subsequently tested for *Ceratophysella denticulata*, *Folsomia quadrioculata*, *Lepidocyrtus lanuginosus*, *Pogonognathellus longicornis* and *Protaphorura armata* (the five collembolan species showing highest densities in our plots in Supplementary material Appendix 1 Table A2).

Specificity of the PCR assays was guaranteed by testing against a set of up to 119 non-target organisms present on the study sites (Eitzinger et al. 2013). PCR products were

separated using the capillary electrophoresis system QIAxcel; fragments of the expected size and a relative fluorescent value ≥ 0.1 RFU were scored as positive. DNA extracts showing no amplification were re-tested once.

Statistical analysis of prey DNA detection rates

To compare prey DNA 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). We analysed relationship between prey DNA detection rates and several independent variables using generalized linear models (GLM) in R ver. 2.12.2 (<www.r-project.org>) using the function 'glm' {stats}.

For lithobiid centipedes, independent variables were predator body mass, square of predator body mass, predator development stage (immature or adult) and prey taxon. For geophilomorph centipedes, independent variables were predator body mass, square of predator body mass and prey taxon. We did not include 'predator species' as independent variable in our model, as many (particularly non-adult) individuals could not be identified to species level. Also, we refrained from including 'predator individual' as a random effect in the model, as during the model selection process we saw that the effects of body size would have been captured by such random effect. Prey DNA detection data was coded as binary (prey DNA present or absent). As predator body size and different prey tissue qualities (i.e. sclerotized versu non-sclerotized prey) do not affect prey DNA detection success in centipedes (Eitzinger et al. 2014), comparisons between different predator sizes and prey types is justified.

Starting from a full model, including all these predator and prey traits, we selected the most parsimonious model based on comparisons of Akaike information criterion (AIC, Burnham and Anderson 2004) using the function 'dredge' in the R package 'MuMIn' (Supplementary material Appendix 1 Table A3–A4).

Calculation of relative feeding rates based on functional response models

A multi-prey functional response model was used to calculate feeding rates (*F)* of centipede predator (*i*) and prey (*j*) when alternative prey organisms (*k*) are present (note that *k* includes *j*; Kalinkat et al. 2011):

$$F_{ij} = \frac{b_{ij} N_j^{1+q_{ij}}}{1 + \sum_{k=1}^{k=n} b_{ik} h_{ik} N_k^{1+q_{ik}}} \quad (2)$$

prey density (*N* ;individuals m^{-2}), the number of alternative prey items (*n)*, the handling time (*h* [s]; time for killing, ingesting and digesting prey), the capture coefficient (*b*) and the scaling exponent (*q*) that converts hyperbolic type II ($q = 0$) into sigmoid type III ($q > 0$) functional responses (Kalinkat et al. 2013b). We used prey-specific body masses [g] and values for generalised allometric functional response (Kalinkat et al. 2013b) to calculate *b*, *h* and *q* for each of the eight prey groups, that we tested for in molecular gut content analysis, and added plot-specific prey density data. For each of the four plots, the relative proportion of each of the eight prey-specific feeding rates from the overall prey feeding rates was then calculated, resulting in 32 individual prey- and plot-specific feeding ratios, *Frel*:

$$Frel_{ij} = \frac{F_{ij}}{\sum_{k=1}^{k=n} F_{ik}} \quad (3)$$

Using the molecular gut content data of all lithobiid respectively geophilomorph predators from all plots combined, we then calculated the proportion of predators tested positive for DNA of each one of the eight prey taxa. Then, we compared the relative feeding rates with the proportion of prey DNA-positive predators using Pearson's correlation coefficient in R 2.12.2.

Data deposition

Data available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.31t0k> (Eitzinger et al. 2017).

Results

Centipede community

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

Prey DNA screening

A total of 532 *Lithobius* spp. and 65 geophilomorph centipedes collected at the eight sampling dates were tested for DNA of all of the eight prey taxa. Per sampling date 41–91 *Lithobius* spp. and 4–12 geophilomorph centipedes were investigated.

In 241 *Lithobius* individuals and 32 geophilomorph centipedes DNA of at least one prey taxon could be detected. Lithobiid predators were significantly more often testing positive for Collembola than for any other prey group (Fig. 1A). Detection rates of Diptera and Lumbricidae were significantly higher than those of other prey, such as Isopoda and Oribatida. Intraguild prey, such as Gamasida and Araneae, formed only a minor fraction of lithobiid prey. In 69 predator individuals two or three prey taxa were detected simultaneously. The lithobiids which tested positive with the general Collembola primers ($n = 141$) consumed significantly

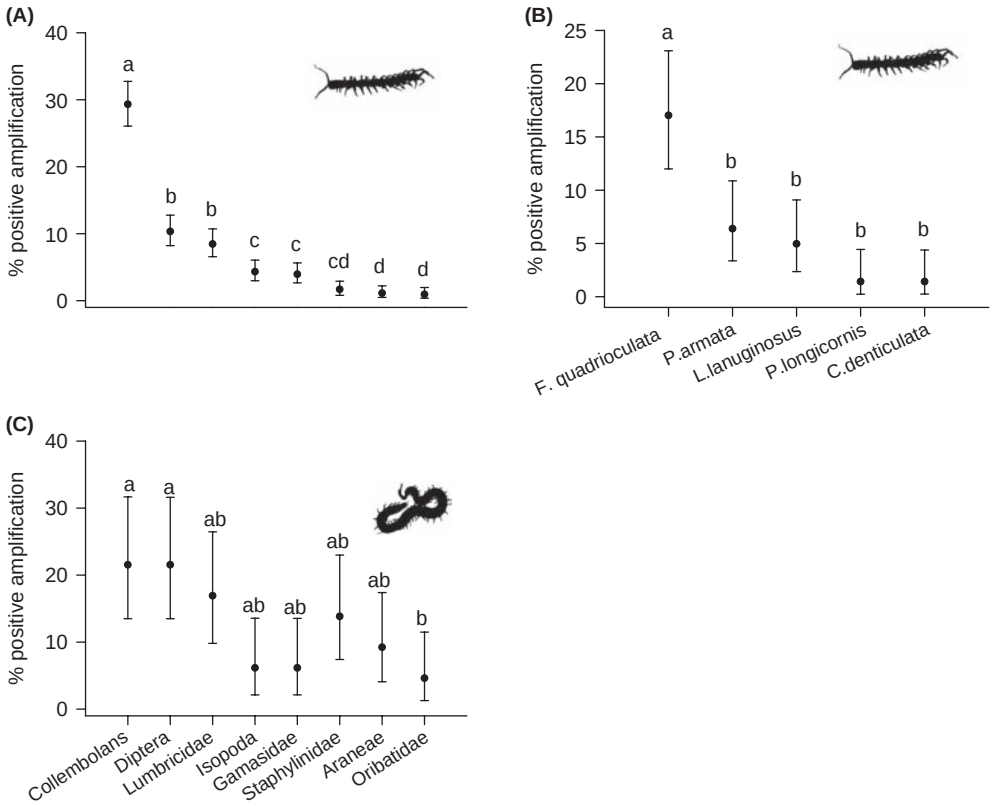

Figure 1. Prey detection rates of lithobiid (A; n = 532) and geophilomorph centipedes (C; n =65) sampled in autumn 2009 and spring 2010. Specimens tested positive for Collembola prey (B; n = 141) further were tested for Collembola prey species. Error bars indicate 95% confidence intervals and letters denote significant differences in DNA detection rates at $p < 0.05$.

more often *Folsomia quadrioculata* than any other of the four tested Collembola species (Fig. 1B).

In geophilomorph centipedes, prey detection rates of Collembola and Diptera were significantly higher than those of Oribatida. Lumbricidae, Isopoda, Staphylinidae, and intraguild prey Araneae and Gamasida were less often detected (Fig. 1C). None of the geophilomorph centipedes were tested positive for potential intraguild prey *Lithobius* or any of the five Collembola species. In 14 geophilomorph centipedes two or three prey taxa were detected simultaneously.

DNA detection frequencies in centipede predators

We selected the most parsimonious model based on AIC comparison within a delta AIC of 2 (Supplementary material Appendix 1 Table A3), thereby rejecting models containing factor development stage. Overall, prey DNA detection frequencies (i.e. the proportion of predators testing positive for a specific prey) of lithobiid centipedes was significantly affected by predator body mass and prey taxon (Table 1). For Collembola and Lumbricidae prey, the proportion of prey-positive predators in relation to predator body mass followed a unimodal curve, peaking at medium lithobiid body masses of 6.3 mg and 4.9 mg, respectively (Fig. 2). In contrast, detection frequency of Diptera prey increased exponentially with predator body mass, indicating that Diptera are increasingly fed on by larger lithobiids while being rejected by smaller ones. The generally very low prey detection frequencies for Oribatida, Gamasida, Staphylinidae and Isopoda also increased with predator body mass, with the curve flattening at 25, 60, 62 and 69 mg predator body mass, respectively. In contrast, low detection

Table 1. Results of generalized linear model (GLM) on the effect of predator body mass, square of predator body mass, prey taxon and the two-way interactions on the prey DNA detection rates in lithobiid predators. Significant effects are highlighted in bold. df: degrees of freedom.

Variable	df	Deviance	Resid. df	Resid. Dev	p(>|χ|)
NULL			4247	2270.2	
Log_{10} predator body mass	1	5.38	4246	2264.8	**0.0204**
Prey taxon	7	386.35	4239	1878.5	**<0.001**
Log_{10} predator body mass^2	1	0.61	4238	1877.9	0.4352
Log_{10} predator body mass × prey taxon	7	18.44	4231	1859.5	**0.0101**

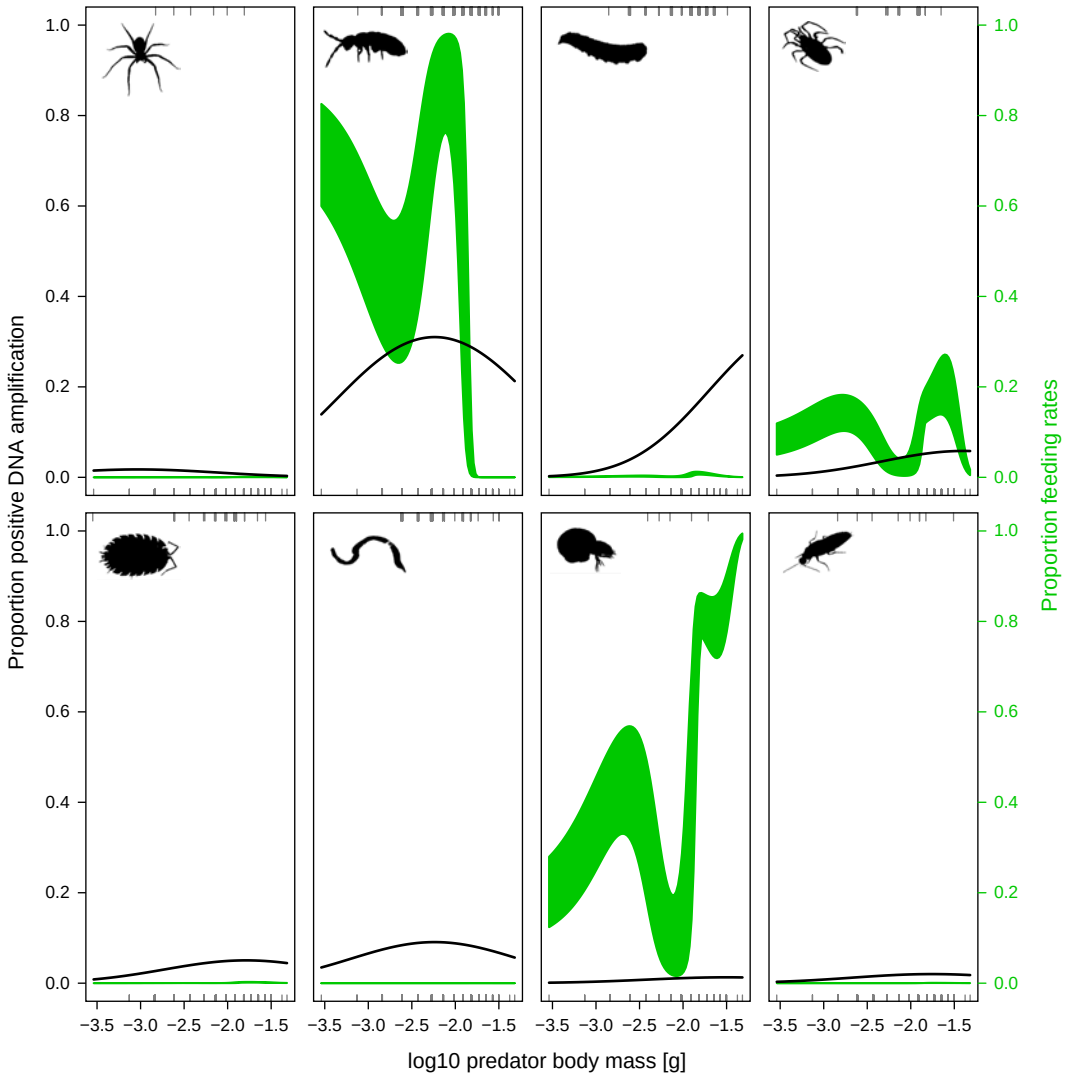

Figure 2. Body-size-dependent probability of positive prey-DNA detection of eight taxa (Araneae, Collembola, Diptera, Gamasida, Isopoda, Lumbricidae, Oribatida, Staphylinidae) in lithobiid centipedes (n = 532) sampled in autumn 2009 and spring 2010 (black line); rugs on top and bottom of each diagram display single data points with values 1 or 0. The body-size-dependent proportion of eight prey taxa in the diet of centipede predators as based on the functional response model using abundance and body-size data of invertebrates sampled in autumn 2009 and spring 2010 is presented in green; upper and lower limit indicate highest and lowest diet proportion in the four forest plots.

frequencies of Araneae, another intraguild prey, i.e. showed a steady decrease with body mass.

Prey DNA detection frequencies of geophilomorph centipedes also varied with predator body mass (Table 2). In contrast to lithobiids, detection rates followed a unimodal curve for each of the prey taxa (Fig. 3) indicating highest feeding rates for medium-sized predator individuals.

Relative prey proportions according to functional response models

Proportions of prey items in the gut of a single predator as predicted by a multi-preyfunctional response model, showed that small mesofauna prey Collembola, Gamasida and Oribatida accounted for most of the diet of lithobiid and geophilomorph centipedes (Fig. 2, 3). However, the proportions of prey varied with predator body mass showing a bimodal relationship. Highest proportion of Collembola prey – almost 100% – were calculated for medium-sized lithobiid and geophilomorph centipedes, while large individuals of both centipede groups had highest prey proportions – also 85–100% – of Oribatida and to a lesser extent – 20–30% – of Gamasida. Other than mesofauna prey, only proportions of Diptera and Isopoda prey increased slightly at high predator body masses, while Araneae, Staphylinidae and Lumbricidae did not form part of the diet of lithobiid and geophilomorph centipede predators at all.

Table 2. Results of generalized linear model (GLM) on the effect of predator body mass, square of predator body mass, prey taxon and the two-way interactions on the detection of prey DNA in geophilomorph centipedes. Significant effects are highlighted in bold. df: degrees of freedom.

Variable	df	Deviance	Resid. df	Resid. Dev	p(>|χ|)
NULL			519	391.84	
Log_{10} predator body mass	1	6.39	518	385.45	**0.0115**
Log_{10} predator body mass^2	1	5.25	517	380.20	**0.0219**
Prey taxon	7	20.89	510	359.31	**0.0039**

Comparison of relative prey proportions with prey proportions of predators testing prey-positive

The visual comparison of body-size dependent relative prey proportions in centipede predators with body size-dependent proportion of centipede predator individuals testing positive for a specific prey DNA (Fig. 2, 3) illustrates major dissimilarities of both approaches. First, high relative prey proportions for mesofauna prey are not reflected in DNA detection frequencies. Second, DNA analysis reveals (though low) feeding on a total of eight prey groups, which is more than the three mesofauna taxa plus Diptera prey as calculated from the functional responses.

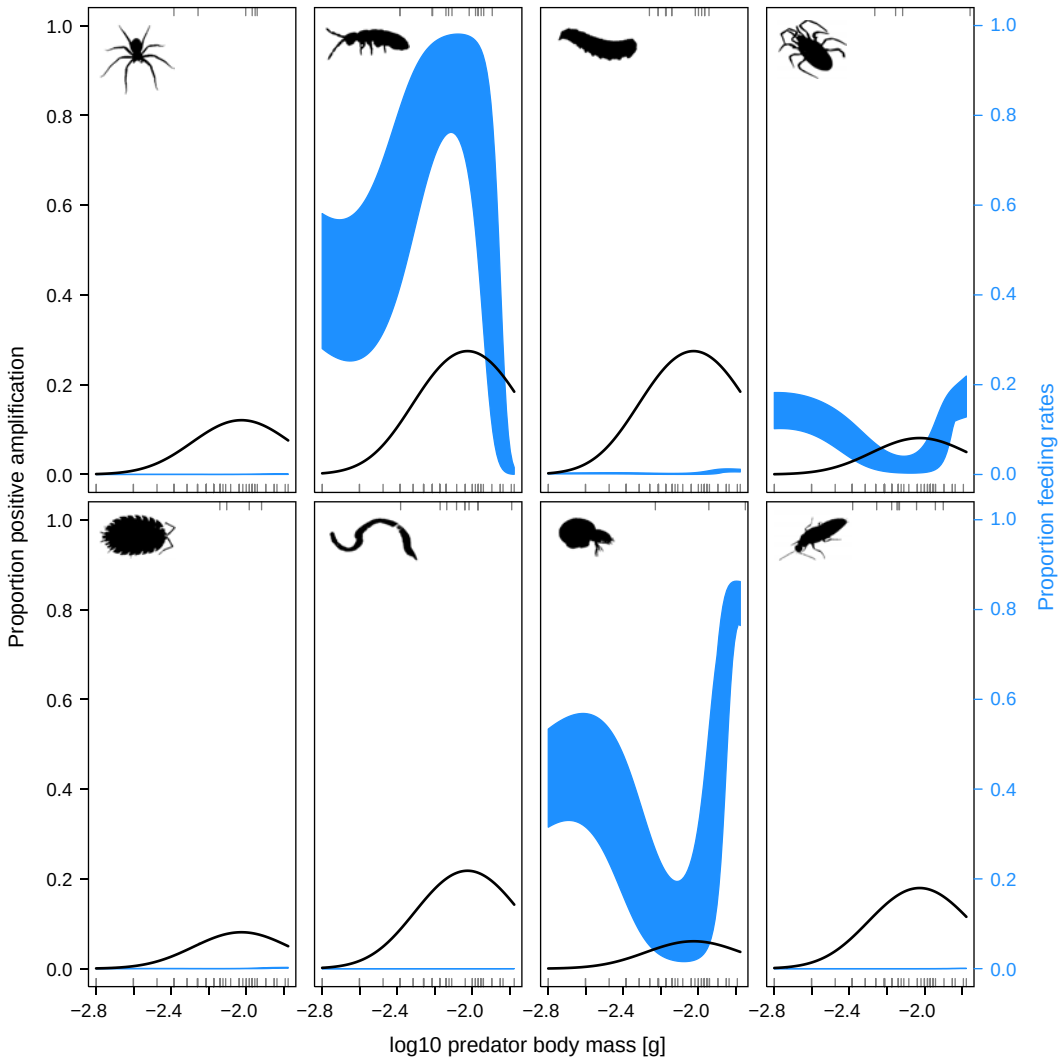

Figure 3. Body-size-dependent probability of positive prey-DNA detection of eight taxa (Araneae, Collembola, Diptera, Gamasida, Isopoda, Lumbricidae, Oribatida, Staphylinidae) in geophilomorph centipedes (n = 65) collected in autumn 2009 and spring 2010 (black line). Rugs on top and bottom of each diagram display single data points with values 1 or 0. The body-size-dependent proportion of eight prey taxa in the diet of centipede predators as based on the functional response model using abundance and body-size data of invertebrates sampled in autumn 2009 and spring 2010 is presented in blue; upper and lower limit indicate highest and lowest diet proportion in the four forest plots.

Table 3. Results of Pearson correlation between body-size dependent relative prey proportions with body-size dependent proportion of centipede predator individuals testing positive for a specific prey DNA in centipede predator groups lithobiidae and geophilomorpha respectively. Significant correlations ($p < 0.05$) are highlighted in bold. df: degrees of freedom.

	Lithobiidae				Geophilomorpha			
Prey group	Pearson correlation coefficient	df	p-value	t-value	Pearson correlation coefficient	df	p-value	t-value
Araneae	–0.5880032	529	**<0.001**	–16.72	–0.04647756	63	0.7131	–0.3693
Collembola	0.6725674	529	**<0.001**	20.903	0.2639361	63	**0.0336**	2.1719
Diptera	0.3086715	529	**<0.001**	7.4639	–0.1412212	63	0.2618	–1.1323
Gamasida	–0.1025666	529	**0.0181**	–2.3715	–0.4392583	63	**<0.001**	–3.881
Isopoda	0.3880475	529	**<0.001**	9.6839	–0.002885686	63	0.9818	–0.022905
Lumbricidae	–0.2935069	529	**<0.001**	–7.0617	0.1688685	63	0.1787	1.3599
Oribatida	0.1268986	529	**0.0034**	2.9425	–0.3002077	63	**0.0151**	–2.498
Staphylinidae	0.4235126	529	**<0.001**	10.753	–0.02655692	63	0.8337	–0.21086

For most prey, the two methods show diverging effects of predator body size. Only in the case of Collembola (in lithobiids and geophilomorphs), and to a lesser extent for Diptera and Gamasida (only in lithobiids) the pattern of body size-dependent feeding was similar.

To check for similarity patterns between the two methods we inspected correlations using Pearson correlation coefficient. In lithobiid centipedes relative prey proportions correlated significantly with the proportion of prey-positive predators for each of the prey groups (Pearson correlation coefficients, $p < 0.001$, Table 3). While we found a positive correlation for the five prey groups Collembola, Diptera, Isopoda, Oribatida and Staphylinidae, the other three prey groups showed negative correlations. In geophilomorph centipedes only correlations with Collembola prey were significantly positive ($p < 0.05$, Table 3), while Gamasida and Oribatida showed significant negative correlations ($p < 0.05$). The other five prey groups did not show any significant correlation.

Discussion

The present study is a first attempt to test the validity of a generalised allometric functional response model to predict predator–prey interactions in a complex soil system, which is characterized by high structural habitat complexity, and includes competitors and alternative prey taxa. We compared the proportion of eight important soil prey groups in the diet of centipedes, based on feeding rates as calculated by functional responses, with empirically quantified prey DNA detection frequencies from field collected centipedes. Model and empirical data positively correlated in five of eight tested prey taxa in lithobiid centipedes while for geophilomorph centipedes we only found a positive correlation for one prey group. The results therefore indicate that functional response models are not always suited to predict real predator–prey interactions in the field. We will first discuss results of each of the two methods and then point to potential reasons and consequences of their mismatch.

The allometric functional response model

The allometric functional response models predicted high relative occurrences of prey in the gut of a single predator of both lithobiid and geophilomorph centipedes on mesofauna prey consisting of Collembola, Oribatida and Gamasida. A combination of high prey abundance, facilitating high encounter rates, and an optimal predator–prey body mass relationship allows the predator to forage on a maximum of prey individuals with a minimum of handling time, thereby reducing energetic costs (Aljetlawi et al. 2004, Brose et al. 2008, Vucic-Pestic et al. 2010a). Results of the model used in this study allow to track shifts from a hyperbolic (type-II) to a sigmoid (type III) functional response. This suggests that with increasing predator body mass, relative feeding rates follow a roller-coaster pattern, peaking at the respective optimal body mass ratios. As metabolism increases with body size, consumers require a higher energy uptake, which is covered by the ingestion of more prey biomass, i.e. more small prey or larger prey individuals (Woodward and Hildrew 2002). We were able to find this pattern in larger proportions of Oribatida and Gamasida prey in the overall diet of large predators. However, we did not find examples for increased feeding on larger than mesofauna prey with the exception of Diptera. Diptera larvae are a prey group with average body masses a magnitude higher than mesofauna and with field densities double than most macrofauna (e.g. Araneae, Lumbricidae), therefore occupying an intermediate position in the eight-species prey spectrum studied here. While this would suggest resulting in recognisable higher feeding rates in larger predator individuals, calculated rates increased only minimally above zero.

Empirical data based on molecular gut content analysis

In contrast to results based on the mathematical model, empirical data showed that centipede predators feed on more than mesofauna prey and that body size-dependent feeding curves match in only one specific case – Collembola – for both predator taxa. Analogous to the model, Collembola and Gamasida constitute an important prey group, which, however, was not the case in the third mesofauna taxon,

Oribatida. While the high abundance and optimal body size of Oribatida suggest them to be ideal prey in the model, other traits, particularly their hard exoskeleton and toxic secretions presumably function as effective defence traits contributing to why they were only rarely consumed (Peschel et al. 2006, Heethoff et al. 2011).

Collembola DNA was detected in most centipedes, particularly medium-sized individuals of both predator taxa. Collembola are abundant in virtually any terrestrial ecosystem and are of high nutritional value, thereby functioning as major prey for a wide range of predators in soil throughout the globe (Marcussen et al. 1999, Bilde et al. 2000, Oelbermann et al. 2008). Using a taxonomic–allometric model, Rall et al. (2011) calculated an optimal body mass ratio of 649 between the lithobiid centipede species *L. forficatus* and the Collembola species *Heteromurus nitidus*. In our study a similar ratio applied to *L. lanuginosus* and *P. armata*, the second and third most often detected Collembola prey species of lithobiid centipedes, respectively.

Lumbricidae, on the other hand, were a far more important prey than expected from the functional response model. Lumbricidae for long have been regarded as major prey of centipedes, in particular geophilomorph species (Lewis 1981), however, their low abundance and big size – even as juveniles – make them an unlikely prey in our allometric model. Using their poison claws, however, centipedes can kill prey far below the optimal body mass ratio (Eason 1964), and this presumably contributed to underestimating the importance of Lumbricidae as prey of centipedes.

Interestingly, we found a strong increase in feeding on Diptera larvae with lithobiid body size, much stronger than predicted by the model. In combination with reduced feeding on other important prey, Collembola and Lumbricidae, this suggests prey switching towards this abundant prey of high nutritional value (Oelbermann and Scheu 2002). Prey switching has been reported in many studies (Hohberg and Traunspurger 2005, Petchey et al. 2008) and its frequency is increasing if predators become larger, presumably due to a combination of effects of habitat structure and optimal foraging processes (Murdoch and Oaten 1975, Kalinkat et al. 2013a).

Habitat structure modifies lithobiid feeding by allowing small prey such as Collembola but also small Lumbricidae, to take refuge from predation, forcing particularly large predator individuals to focus on more accessible prey dwelling in the upper litter layer (Günther et al. 2014). Simultaneously, larger predators have higher energetic demands forcing them to hunt for larger prey, i.e. bigger individuals of species already feeding upon or a new larger species. Higher energetic costs of killing, ingesting and digesting prey (i.e. 'handling time'), such as large tipulid fly larvae or earthworms are more easily balanced by the prey's high nutritional value. However, our results suggest that to meet their nutritional and energetic demands, large lithobiid centipedes cannot be too selective in their prey choice: their spectrum still includes mesofauna prey and also encompasses Isopoda and Staphylinidae. These results confirm earlier studies showing that the prey spectrum of predators broadens with predator body size, suggesting that large predators exploit prey communities more efficiently (Cohen et al. 1993, Woodward and Hildrew 2002). On the other hand, our findings argue against suggestions that at high density of extraguild prey, intraguild predation is negligible (Halaj and Wise 2002, Eitzinger and Traugott 2011). Further, the results contradict findings that the role of intraguild predation is reduced in well-structured habitats providing refuge for intraguild prey (Finke and Denno 2002, Janssen et al. 2007).

Causes of match and mismatch of functional response model and molecular gut content analysis

Comparing feeding rates with data on gut content analysis comes with certain restrictions. Correct quantification of prey DNA in a field caught predator, which would allow to measure prey proportions in a generalist predator, still is difficult (Deagle et al. 2013). Molecular ecologists therefore rely on DNA detection frequencies based on prey DNA presence/absence, favourably on a large set of field-caught predators, which minimises the stochastic effect of time-dependent DNA detection success. Hence, the number of screened geophilomorph centipedes in this study may have been too low for correctly reflecting prey capture events and this may have contributed to the mismatch of model and empirical data for this predator group.

Comparing prey DNA detection frequencies (i.e. number of predators tested positive for prey DNA) with feeding rates (i.e. the number of prey individuals ingested by a predator individual) is possible when assuming that prey DNA detection frequency correlates with predation rates (Traugott et al. 2013, Baker et al. 2014). While the modelled prey proportions sum up to 100%, DNA detection frequencies never reach 100%. This can be caused by low DNA detection rates (e.g. due to low primer performance, low amount of prey DNA) or due to the biology of the predator, such as starving phases. While we were vigilant to reduce the effect of laboratory-derived biases, we cannot rule out that the latter contributed to mismatches between model and empirical results.

Based on gut content analysis, predator body size and prey taxon proved to be the two major drivers of prey capture by centipede predators, corroborating previous studies employing functional response models (Vucic-Pestic et al. 2010b, Rall et al. 2011). Allometry-based feeding has proved to be a universal pattern in many systems (Brose et al. 2006), indicating that body size serves as a 'supertrait' explaining most variance in predator–prey interactions. A positive correlation between DNA results and the allometric functional response model for five out of eight prey of lithobiid centipedes points at the importance of body size in this soil system.

One of the reasons for the lack of correlation between DNA results and the allometric functional response model in three of eight prey of lithobiid centipedes and one of eight prey of geophilomorph centipedes might be due to the fact

that gut content analysis cannot discriminate between feeding on small larval and large adult stages of a given prey taxon. We used average prey body masses from animals collected in the field to calculate feeding rates. Therefore, while we know about the average distribution of prey sizes in the field, we do not know about preferential prey sizes of centipede predators.

Moreover, prey-specific traits, such as sclerotization and toxicity, presumably contributing to reduced prey capture are rarely implemented in functional response models (but see Heethoff and Rall 2015), indicating that the model needs to be extended to include defence traits of prey. Kalinoski and DeLong (2016) stress the importance of prey traits in a simple aquatic system believed to be dominated by allometric constraints. This may be even more important for predator–prey interactions in soil, with its multitude of organism on small spatial scale. Additionally, we have to consider specific predator traits such as use of poison claws, which allows centipedes to attack and kill prey above their optimal body-size ratio. To improve the prediction success of functional response models we therefore call for more data from specific laboratory feeding trials which are calibrated with results from field studies.

Conclusions

The present study, for the first time, tested the power of functional response models to predict allometry-based prey choice in a natural setting by comparing model predictions with empirical data based on molecular gut content analysis. The results suggest the models to correctly predict prey consumption by lithobiid predators in five out of eight prey taxa studied, however, the empirical data also showed that feeding interactions in soil also depend on factors which are not considered in the functional response model. For improving the effectiveness of allometric functional response models in predicting food web interactions in the field, additional traits of prey species, such as defence characteristics, have to be included.

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References

Aljetlawi, A. A. et al. 2004. Prey–predator size-dependent functional response: derivation and rescaling to the real world. – J. Anim. Ecol. 73: 239–252.

Baker, R. et al. 2014. Fish gut content analysis: robust measures of diet composition. – Fish Fish. 15: 170–177.

Barrios-O'Neill, D. et al. 2015. Predator-free space, functional responses and biological invasions. – Funct. Ecol. 29: 377–384.

Barrios-O'Neill, D. et al. 2016. On the context-dependent scaling of consumer feeding rates. – Ecol. Lett. 19: 668–678.

Bengtsson, J. 1994. Temporal predictability in forest soil communities. – J. Anim. Ecol. 63: 653–665.

Bilde, T. et al. 2000. The value of Collembola from agricultural soils as food for a generalist predator. – J. Appl. Ecol. 37: 672–683.

Bluhm, C. et al. 2016. Temporal fluctuations in oribatid mites indicate that density-independent factors favour parthenogenetic reproduction. – Exp. Appl. Acarol. 68: 387–407.

Brose, U. et al. 2006. Consumer–resource body-size relationships in natural food webs. – Ecology 87: 2411–2417.

Brose, U. et al. 2008. Foraging theory predicts predator–prey energy fluxes. – J. Anim. Ecol. 77: 1072–1078.

Burnham, K. P. and Anderson, D. R. 2004. Multimodel inference – understanding AIC and BIC in model selection. – Soc. Methods Res. 33: 261–304.

Cohen, J. E. et al. 1993. Body sizes of animal predators and animal prey in food webs. – J. Anim. Ecol. 62: 67–78.

Deagle, B. E. et al. 2013. Quantifying sequence proportions in a DNA-based diet study using Ion Torrent amplicon sequencing: which counts count? – Mol. Ecol. Resour. 13: 620–633.

DeLong, J. P. 2014. The body-size dependence of mutual interference. – Biol. Lett. 10: 20140261.

Eason, E. H. 1964. Centipedes of the British Isles. – Warne, London.

Eisenhauer, N. et al. 2008. Efficiency of two widespread non-destructive extraction methods under dry soil conditions for different ecological earthworm groups. – Eur. J. Soil Biol. 44: 141–145.

Eitzinger, B. and Traugott, M. 2011. Which prey sustains cold-adapted invertebrate generalist predators in arable land? Examining prey choices by molecular gut-content analysis. – J. Appl. Ecol. 48: 591–599.

Eitzinger, B. et al. 2013. Unveiling soil food web links: new PCR assays for detection of prey DNA in the gut of soil arthropod predators. – Soil Biol. Biochem. 57: 943–945.

Eitzinger, B. et al. 2014. Effects of prey quality and predator body size on prey DNA detection success in a centipede predator. – Mol. Ecol. 23: 3767–3776.

Eitzinger, B. et al. 2017. Data from: Testing the validity of functional response models using molecular gut content analysis for prey choice in soil predators. – Dryad Digital Repository, <http://dx.doi.org/10.5061/dryad.31t0k>.

Elliott, J. M. 2004 Prey switching in four species of carnivorous stoneflies. – Freshwater Biol. 49: 709–720.

Elliott, J. M. 2006 Prey switching in *Rhyacophila dorsalis* (Trichoptera) alters with larval instar. – Freshwater Biol. 51: 913–924.
Englund, G. et al. 2011. Temperature dependence of the functional response. – Ecol. Lett. 14: 914–921.
Finke, D. L. and Denno, R. F. 2002. Intraguild predation diminished in complex-structured vegetation: implications for prey suppression. – Ecology 83: 643–652.
Fischer, M. et al. 2010. Implementing large-scale and long-term functional biodiversity research: The Biodiversity Exploratories. – Basic Appl. Ecol. 11: 473–485.
Folmer, O. et al. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. – Mol. Mar. Biol. Biotechnol. 3: 294–299.
Gowing, G. and Recher, H. F. 1984. Length–weight relationships for invertebrates from forests in southeastern New South Wales. – Aust. J. Ecol. 9: 5–8.
Günther, B. et al. 2014. Variations in prey consumption of centipede predators in forest soils as indicated by molecular gut content analysis. – Oikos 123: 1192–1198.
Halaj, J. and Wise, D. H. 2002. Impact of a detrital subsidy on trophic cascades in a terrestrial grazing food web. – Ecology 83: 3141–3151.
Hansen, P. J. et al. 1997. Zooplankton grazing and growth: scaling within the 2–2000-micrometer body size range. – Limnol. Oceanogr. 42: 687–704.
Hauzy, C. et al. 2010. Spatial heterogeneity and functional response: an experiment in microcosms with varying obstacle densities. – Oecologia 163: 625–636.
Heethoff, M. and Rall, B. C. 2015. Reducible defence: chemical protection alters the dynamics of predator–prey interactions. – Chemoecology 25: 53–61.
Heethoff, M. et al. 2011. Tasty but protected – first evidence of chemical defense in Oribatid mites. – J. Chem. Ecol. 37: 1037–1043.
Hesterberg, T. et al. 2003. Bootstrap methods and permutation tests. – In: Moore, D. S. and McCabe, G. P. (eds), Introduction to the practice of statistics, 5th edn. W. H. Freeman Co., pp. 4–74.
Hohberg, K. and Traunspurger, W. 2005. Predator–prey interaction in soil food web: functional response, size-dependent foraging efficiency, and the influence of soil texture. – Biol. Fertility Soils 41: 419–427.
Holling, C. 1959. Some characteristics of simple types of predation and parasitism. – Can. Entomol. 91: 385–398.
Janssen, A. et al. 2007. Habitat structure affects intraguild predation. – Ecology 88: 2713–2719.
Juen, A. and Traugott, M. 2005. Detecting predation and scavenging by DNA gut-content analysis: a case study using a soil insect predator–prey system. – Oecologia, 142: 344–352.
Kalinkat, G. and Rall, B. C. 2015. Effects of climate change on the interactions between insect pests and their natural enemies. – In: Björkman, C. and Niemelä, P. (eds), Climate change and insect pests. CABI Climate Change Ser., pp. 74–91.
Kalinkat, G. et al. 2011. The allometry of prey preferences. – PloS One 6: e25937.
Kalinkat, G. et al. 2013a. Habitat structure alters top-down control in litter communities. – Oecologia 172: 877–887.
Kalinkat, G. et al. 2013b. Body masses, functional responses and predator–prey stability. – Ecol. Lett. 16: 1126–1134.
Kalinoski, R. M. and DeLong, J. P. 2016. Beyond body mass: how prey traits improve predictions of functional response parameters. – Oecologia 180: 543–550.
Kempson, D. et al. 1963. A new extractor for woodland litter. – Pedobiologia 3: 1–21.
Klarner, B. et al. 2014. Trophic shift of soil animal species with forest type as indicated by stable isotope analysis. – Oikos 123: 1173–1181.
Latzel, R. 1880. Die Myriapoden der österreichisch–ungarischen Monarchie. 1. Bd.: Die Chilopoden. – Holder, Wien.
Lang, B. et al. 2011. Warming effects on consumption and intraspecific interference competition depend on predator metabolism. – J. Anim. Ecol. 81: 516–523.
Lewis, J. G. E. 1981. The biology of centipedes. – Cambridge Univ. Press.
Marcussen, B. M. et al. 1999. The value of two Collembola species as food for a linyphiid spider. – Entomol. Exp. Appl. 92: 29–36.
Mercer, R. D. et al. 2001. Invertebrate body sizes from Marion Island. – Antarct. Sci. 13: 135–143.
Messier, F. 1994. Ungulate population models with predation: a case study with the North American moose. – Ecology 75: 478–488.
Murdoch, W. W. and Oaten, A. 1975. Predation and population stability. – Adv. Ecol. Res. 9: 1–131.
Nielsen, J. M. et al. 2017. Diet tracing in ecology: method comparison and selection. – Methods Ecol. Evol. 2017: 1–14.
Oelbermann, K. and Scheu, S. 2002. Effects of prey type and mixed diets on survival, growth and development of a generalist predator, *Pardosa lugubris* (Araneae: Lycosidae). – Basic Appl. Ecol. 291: 285–291.
Oelbermann, K. et al. 2008. Utilization of prey from the decomposer system by generalist predators of grassland. – Oecologia 155: 605–617.
Pawar, S. et al. 2012. Dimensionality of consumer search space drives trophic interaction strengths. – Nature 486: 485–489.
Peschel, K. et al. 2006. Do oribatid mites live in enemy-free space? Evidence from feeding experiments with the predatory mite *Pergamasus septentrionalis*. – Soil Biol. Biochem. 38: 2985–2989.
Petchey, O. L. et al. 2008. Size, foraging and food web structure. – Proc. Natl Acad. Sci. USA 105: 4191–4196.
Peters, K. J. et al. 2014. Fine-scale diet of the australian sea lion (Neophoca cinerea) using DNA-based analysis of faeces. – Mar. Ecol. 36: 347–367.
Pollierer, M. M. and Scheu, S. 2017. Driving factors and temporal fluctuation of Collembola communities and reproductive mode across forest types and regions. – Ecol. Evol. 7: 4390–4403.
Pompanon, F. et al. 2012. Who is eating what: diet assessment using next generation sequencing. – Mol. Ecol. 21: 1931–1950.
Poser, T. 1988. Chilopoden als Prädatoren in einem Laubwald. – Pedobiologia 31: 261–281.
Rall, B. C. et al. 2011. Taxonomic versus allometric constraints on non-linear interaction strengths. – Oikos 120: 483–492.
Rall, B. C. et al. 2012. Universal temperature and body-mass scaling of feeding rates. – Phil. Trans. R. Soc. B 367: 2923–2934.
Riede, J. O. et al. 2011. Size-based food web characteristics govern the response to species extinctions. – Basic Appl. Ecol. 12: 581–589.
Sarnelle, O. and Wilson, A. E. 2008. Type III functional response in *Daphnia*. – Ecology 89: 1723–1732.
Schneider, F. D. et al. 2012. Body mass constraints on feeding rates determine the consequences of predator loss. – Ecol. Lett. 15: 436–443.
Skalski, G. T. and Gilliam, J. F. 2001. Functional responses with predator interference: viable alternatives to the Holling type II model. – Ecology 82: 3083–3092.

Traugott, M. et al. 2013. Empirically characterising trophic networks: what emerging DNA-based methods, stable isotope and fatty acid analyses can offer. – Adv. Ecol. Res. 49: 177–224.
Vucic-Pestic, O. et al. 2010a. Allometric functional response model: body masses constrain interaction strengths. – J. Anim. Ecol. 79: 249–256.
Vucic-Pestic, O. et al. 2010b. Habitat structure and prey aggregation determine the functional response in a soil predator–prey interaction. – Pedobiologia 53: 307–312.
Wallinger, C. et al. 2014. How generalist herbivores exploit belowground plant diversity in temperate grasslands. – Mol. Ecol. 23: 3826–3837.
Wirta, H. K. et al. 2015. Exposing the structure of an Arctic food web. – Ecol. Evol. 5: 3842–3856.
Woodward, G. and Hildrew, A. G. 2002. Body-size determinants of niche overlap and intraguild predation within a complex food web. – J. Anim. Ecol. 71: 1063–1074.

Supplementary material (available online as Appendix oik-04885 at <www.oikosjournal.org/appendix/oik-054885>). Appendix 1.