GEORG-AUGUST-UNIVERSITÄT
GÖTTINGEN

Plant diversity and landscape-scale effects on multitrophic interactions involving invertebrates

Dissertation

zur Erlangung des mathematisch-naturwissenschaftlichen Doktorgrades

"Doctor rerum naturalium"

der Georg-August-Universität Göttingen

im Promotionsprogramm Biologische Diversität und Ökologie

der Georg-August University School of Science (GAUSS)

vorgelegt von

Julia Tiede

aus Hildesheim

Göttingen, 2017

Betreuungsausschuss

Prof. Dr. Christoph Scherber	Arbeitsgruppe Tierökologie und Multitrophische Interaktionen, Institut für Landschaftsökologie, Westfälische Wilhelms-Universität Münster
Prof. Dr. Teja Tscharntke	Abteilung Agrarökologie, Department für Nutzpflanzenwissenschaften, Georg-August-Universität Göttingen
Prof. Dr. Rolf Daniel	Genomische und Angewandte Mikrobiologie und Göttinger Genomlabor, Institut für Mikrobiologie und Genetik, Georg-August-Universität Göttingen

Mitglieder der Prüfungskommission

Referent:	**Prof. Dr. Christoph Scherber**
Korreferent:	**Prof. Dr. Teja Tscharntke**
2. Korreferent:	**Prof. Dr. Rolf Daniel**

Weitere Mitglieder der Prüfungskommission

Prof. Dr. Stefan Scheu	Arbeitsgruppe Tierökologie, Blumenbach-Institut für Zoologie und Anthropologie, Georg-August-Universität Göttingen
Prof. Dr. Johannes Isselstein	Abteilung Graslandwissenschaften, Department für Nutzpflanzenwissenschaften, Georg-August-Universität Göttingen
Prof. Dr. Stefan Vidal	Abteilung Agrarentomologie, Department für Nutzpflanzenwissenschaften, Georg-August-Universität Göttingen

Tag der Disputation: 15.11.2017

“What escapes the eye, however, is a much more insidious kind of extinction: the extinction of ecological interactions.”

Daniel H. Janzen, 1974, The deflowering of Central America. Nat. Hist. 83:48–53

“Round and round as the nature flows
It's like one big ring
Caterpillars eat plants
Small birds eat caterpillars
Big birds eat small birds
Bacteria eats the dead- big bird
From there plants grow
Again, caterpillar eats the plant!
How natural, no waste
It is an endless chain
What an amazing, wonderful, and excellent!
How harsh, not careless
It's an endless chain
What an amazing, wonderful, and excellent food... food chain!”

The food chain song, Adventure Time, Season 6, Episode 7

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ABBREVIATIONS

µ	Micro
µL	Microliter
µM	Micromole
a.s.l.	Above Sea Line
AIC	Akaike's Information Criterion
ANOVA	Analysis of Variance
ARISA	Automated Ribosomal Intergenic Spacer Analysis
BLAST	Basic Local Alignment Search Tool
BLASTN	BLAST search using a nucleotide query
bp	Base Pairs
°C	Degree Celsius
CA	California
ca.	Circa
CDL	Cropland Data Layer
CLMM	Cumulative Link Mixed-Effects Models
cm	Centimeter
COI	Mitochondrial Cytochrome C Oxidase Subunit I
CT	Connecticut
denom. df	Denominator Degrees of Freedom
DEPC	Diethylpyrocarbonate
df	Degrees of Freedom
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic Acid
dNTP	Deoxynucleotide Triphosphates
dsDNA	Double Stranded DNA
E	East
e.g.	Exempli Gratia, for Example
eDNA	Environmental DNA
EDTA	Ethylenediaminetetraacetic Acid
Eqs.	Equations
ESRI	Environmental Systems Research Institute
Fig.	Figure
Figs.	Figures
g	Gram
GLM	Generalized Linear Model
hr	Hour(s)
HS	High Sensitivity
IGP	Intraguild Predation
ITS	Internal Transcribed Spacer
km	Kilometer
km^2	Square Kilometer
L	Liter
m	Meter
M	Molar
Mg	Milligram
MID	Multiplex Identifier
min	Minute
ml	Milliliter
mm	Millimeter
mM	Millimole
N	North
n	Number
NASS	National Agricultural Statistics Service
NCBI	National Centre for Biotechnology Information
NGS	Next Generation Sequencing
nlme	Non-Linear Mixed-Effects Models
NMDS	Non-Metric Multidimensional Scaling
nt	Nucleotide
OUT(s)	Operational Taxonomic Unit(s)
PCR	Polymerase Chain Reaction
perMANOVA	Permutational Multivariate Analysis of Variance
PERMDISP	Permutational Dispersion
pH	Power of Hydrogen
QIIME	Quantitative Insights into Microbial Ecology
RAD	Rank-Abundance Distribution
rDNA	DNA Coding for Ribosomal DNA
REML	Restricted Maximum Likelihood
RNA	Ribonucleic Acid
ROX	6-Carboxyl-X-Rhodamine
rRNA	Ribosomal RNA
SDS	Sodium Dodecyl Sulfate
SE	Standard Error
sec	Second
SEM	Structural Equation Modeling
SILVA	A Database for rRNA
SRA	Sequence Read Archive
SS	Sums of Squares
SSU	Small Subunit of the Ribosome
Tab.	Table
TAE	Tris-Acetate-EDTA
TBE	Trait-Based Experiment
TE	Tris EDTA
TP	Trophic Position
TRIS	Trishydroxymethylaminomethane
UCHIME	UCHIME is an algorithm for detecting chimeric sequences
UCLUST	UCLUST is an algorithm that divides a set of sequences into clusters
UNITE	User-friendly Nordic ITS Ectomycorrhiza Database
UPARSE	UPARSE is a method for generating OTUs from NGS reads
USA	United States of America
USDA	United States Department of Agriculture
USEARCH	USEARCH is a sequence analysis tool
VT	Vermont
WI	Wisconsin

<u>Abbreviations for nucleotides</u>

A	Adenine
C	Cytosine
G	Guanine
R	Purines (Adenine or Guanine)
T	Thymine (5-Methyluracil)
Y	Pyrimidines (Cytosine, Thymine, or Uracil)

SUMMARY

Biodiversity in terrestrial ecosystems is declining due to increasing anthropogenic pressure. Urban and agricultural expansion lead to shrinking natural or seminatural habitats. In addition, management intensification of existing agricultural land further reduces the quality of agroecosystems as habitat for many species. Both, local reduction of plant diversity and the regional degradation of seminatural vegetation have consequences for consumer species. The consequences of plant species loss for the diversity of consumer species and ecosystem functioning have been addressed over the past decades in numerous studies, ranging from controlled experiments with manipulated plant communities to systems with natural occurring gradients in plant diversity on a local and landscape scale. These studies have found that plant and habitat diversity can have cascading effects on the faunal community, shift its trophic structure and influence species-mediated ecosystem-processes, such as predation. Although it is clear that interspecific interactions are the drivers of such community shifts and ecosystem processes, our knowledge on how changing diversity of basal resources impacts species interactions is currently still limited.

The major aim of my thesis was to contribute to a deeper understanding of the impact of plant diversity on the multitude of interactions that species in complex communities are involved in. This thesis focuses on interactions of mobile generalist omnivores and predators that can be found across the whole gradient from natural to strongly modified habitats. Although this group of invertebrates may not be particularly threatened by the loss of plant diversity, they are of great interest as model organisms, because their dietary plasticity allows them to interact with different sets of co-occurring species in their local environment. A further aim of my thesis was to expand the focus from trophic to non-trophic interactions by including microorganisms in my studies that are, despite their tremendous diversity and importance for many ecosystem functions, rarely considered in the analysis of aboveground interactions.

In the first research chapter (chapter 2), I studied the effects of landscape context on the gut bacterial community and body condition of predatory insects (lady beetles) in a mensurative experiment in the Midwest of the USA. Insects were sampled across a landscape complexity gradient (increasing amount of cropland), and across two field types with a pronounced difference in plant diversity (soybean monocultures vs. restored prairies). Unexpectedly, predators collected in soybean fields had a more diverse set of gut microbes than predators from prairies. However, predators from soybean had lower fat content than predators collected in prairies, suggesting greater resource availability in prairies. Whether the lady beetle species were native or exotic to the USA influenced the effect of landscape context, suggesting differences in foraging preferences between these groups at a landscape scale. Another key finding was that lady beetle species have distinct microbial communities. Overall, my study highlights complex interactions among gut microbiota, predator identity, and landscape context.

The studies in chapter 3 and 4 were conducted within the framework of a biodiversity experiment (The Jena Experiment) with manipulated taxonomic and functional plant diversity to study if plant diversity *per se* has an impact on microbial and trophic interactions involving invertebrates. In a pilot study (chapter3), I evaluated metabarcoding of gut contents of

invertebrates as a novel approach to analyze biotic interactions in species-rich communities. In an extended study spanning the full experimental design (chapter 4), I subsequently used this approach to analyze DNA of plants, animals, fungi, and bacteria in gut contents of three invertebrate species that vary in their degree of omnivory. The results the richness and composition of detected taxa is only little affected by plant diversity directly and mainly driven by indirect effects of plant diversity via the performance of the plant or animal community. A key finding was that vegetation cover shifts the trophic position of omnivores but the direction of the effects depended on the species identity of the omnivore. Further, the consumers were associated with different sets of animal and microbial taxa, reflecting their different food preferences.

The final research chapter (chapter 5) assessed the efficiency of pitfall trapping, which is one of the most frequently used approaches to assess aboveground invertebrate diversity, under different scenarios by employing an ecological simulation approach. An individual-based model for simulating the movement and pitfall trap sampling of arthropods was developed and factors that are assumed to affect the trapping efficiency were systematically assessed at the species and community level. Body mass, temperature, and pitfall trap number strongly increased the sampling efficiency. This has implications on the study of communities, as the strong impact of body mass could result in an overestimation of large-sized species in the arthropod community and imply wrong conclusions about its trophic structure. It is therefore proposed to conduct a bias correction and a correction factor that requires only information on species body mass is provided to derive reliable abundance estimates from pitfall trap sampling.

This thesis revealed that species interactions are driven by a multitude of direct and indirect effects of plant diversity on a local and landscape scale. This is further complicated by the contrasting responses of consumer species that are often treated as one functional group and highlights the need to further investigate the response of individual key species instead of focusing solely on whole communities. Overall, my thesis is a first step to integrate novel approaches that allow the empirical assessment of multi-level species interactions into biodiversity research.

CHAPTER 1

General introduction and overview of this thesis

1.1 General introduction

The loss of biodiversity and its consequences for associated communities and ecosystem processes has become a major concern (Sala et al. 2000; Hooper et al. 2012; Newbold et al. 2015). How declining diversity affects ecosystem functioning, is studied most often for plant diversity loss, including both, systems with already excising diversity gradients (Grace et al. 2014; Allan et al. 2015) and controlled experiments with manipulated plant communities (Haddad et al. 2009; Weisser et al. 2017).

For decades plant diversity experiments have focused on primary productivity (Hooper et al. 2005; 2012), while more recent research investigates the cascading effects from primary producer diversity to higher trophic levels (Cardinale et al. 2006; Barnes et al. 2014). These studies show, that plant diversity has bottom-up effects on the abundance and richness of consumer species (Knops et al. 1999; Koricheva et al. 2000; Borer et al. 2012), induces shifts in the functional structure and diversity across trophic levels (Haddad et al. 2009; Ebeling et al. 2017), and affects ecosystem processes (Scherber et al. 2010; Allan et al. 2015; Hertzog et al. 2016b; Meyer et al. 2017).

In addition to plant diversity in the local habitat, the availability and diversity of suitable habitats in the surrounding landscape can act as a source and refugium for mobile consumer species (Tscharntke et al. 2005; 2012; Bianchi et al. 2013). Landscape context has been shown to shape the richness and structure of consumer communities (Gardiner et al. 2009b; Woltz & Landis 2014), affect the body condition of predators (Östman et al. 2001) and change, for instance predation and parasitism rates (This et al. 2005; Gardiner et al. 2009a; Lire et al. 2015).

Ecosystem processes on a local or landscape scale are often mediated by trophic interactions but only few studies have empirically measured interactions in response to plant diversity because appropriate methods were lacking. Direct measurements are usually limited to interactions that can be easily observed such as plant-pollinator interactions (Venjakob et al. 2016) or specialized herbivores feeding on aboveground plant parts (Meyer et al. 2017). Resolving feeding links of omnivores and carnivores has remained challenging, especially when they are polyphagous. A further complication arises, if organisms are highly mobile, nocturnal, or fluid feeders (Traugott et al. 2013). In consequence, the effects of altered resource diversity on species interactions are often measured only indirectly as the numerical response of species or changes in ecosystem process rates (Thies et al. 2005; Tscharntke et al. 2005; Gardiner et al. 2009a; Chaplin-Kramer et al. 2011).

Metabarcoding of gut contents is a promising new approach to study trophic interactions. For the last two decades DNA of food items has been widely used as a marker molecule to resolve feeding interactions in various ecosystems (e.g., Andrew King et al. 2011; Symondson 2012; Traugott et al. 2013). Two basic approaches exist. In the first approach, food-specific PCR primers are used to test samples for the presence of specific to *a priori* defined species or broader taxonomic groups (Harper et al. 2005; Eitzinger & Traugott 2011; Lundgren & Fergen 2014). This approach is highly sensitive but usually limited to a limited number of food types of interest and not practical to assess the dietary spectrum of generalist feeders in species-rich ecosystems. The second approach uses general PCR primers for all potential food types that are in a final step identified based on their DNA sequence. The traditional DNA

sequencing (Sanger et al. 1977) of samples with DNA from multiple species, however, requires a time-consuming cloning step in which DNA of different species is separated (Zeale et al. 2011). Next generation sequencing (NGS) technologies have overcome this limitation and allow to sequence samples with mixed DNA types without *a priori* knowledge on the ingested taxa. In combination with primers for common barcoding regions for which extensive sequence databases exist, this approach allows the simultaneous detection of feeding events from a wide range of potential interaction partners in complex and species-rich ecosystems (Valentini et al. 2009; Piñol et al. 2013; Pompanon & Samadi 2015).

Animal guts comprise not only food remains but harbor a vast diversity of microbes (Bahrndorff et al. 2016; Engel & Moran 2013; Gibson & Hunter 2010) that can affect host fitness in many ways including host nutrition, or protection against predators or pathogens (Dillon & Dillon 2004; Douglas 2009; Henry et al. 2015; Ruokolainen et al. 2016). As the gut microbial community of many arthropod species is affected by host diet (Broderick et al. 2004; Lundgren & Lehman 2010; Mason & Raffa 2014), integrating microbial interaction partners into the study of trophic interactions can yield important insights into ecological interactions. Moreover, mobile polyphagous consumers encounter a great number of microorganisms in their environment and can serve as sampling devices for the local microbial diversity (Boyer et al. 2015).

1.2 Study systems and regions

1.2.1 Landscape approach - Agroecosystems in the Midwestern USA

Insect predator communities and pest predation rates are strongly affected by the features of the surrounding landscapes, like the availability of suitable habitats (Tscharntke et al. 2005; Layman et al. 2007; Bianchi et al. 2013). It is currently, however, neither known how landscape context affects the prey spectrum of mobile generalist predators, nor how or whether the predators gut microbiota respond to changes in landscape composition. The first part of this thesis (chapter 2) was conducted in southern Wisconsin, USA, in 2012. The landscapes across southern Wisconsin vary considerably in composition, from dominated by agricultural row crops (mainly corn and soybean) to high proportions of seminatural habitat such as forests, prairies, and wetlands, thus making this a useful region in which to study the effects of landscape context on mobile predators. I selected 10 prairies and 10 conventially managed soybean fields as two field types with contrasting diversity of plants and associated consumer species (Fig. 1). The fields were positioned along a landscape gradient from seminatural dominated to crop-land dominated in the region around Madison on a scale of several thousand km².

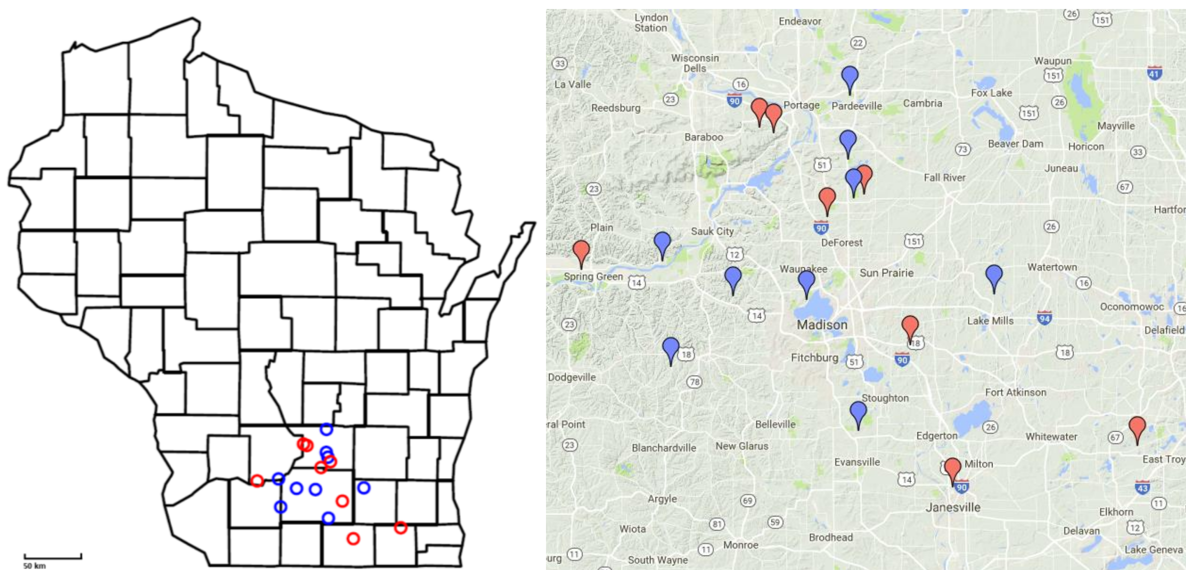

Figure 1. Map of sampling locations. Locations of soybean fields (red points) and prairies (blue points) around Madison, Wisconsin, USA, in which the lady beetle specimen included in our study were collected between July and August 2012. (right side: Kartendaten © 2017 Google)

Aphidophagous lady beetles were used as model organism, as they are abundant, locally widespread, and important natural enemies of aphids in agricultural crops (Obrycki et al. 2009; Snyder 2009) and seminatural habitats (Bianchi et al. 2013). Although aphids are their preferred prey, the lady beetles' food spectrum includes a broad range of other arthropods, and fungal or plant resources (Dixon 2000; Evans 2009; Hodek & Honěk 1996; Trilitsch 1999; Weber & Lundgren 2009). As highly mobile predators, lady beetles may forage on a landscape scale and represent a useful study system to examine the effects of landscape context on the gut bacterial community and body condition of predatory insects (Fig. 2).

Figure 2. Foraging lady beetles and their prey (left column), examples for prairie study sites (middle column), and examples for soybean fields and crop dominated regions in southern Wisconsin (right column).

1.2.2 Experimental approach - The Jena Experiment

Biodiversity experiments with an experimentally manipulated gradient of plant species richness exclude many confounding factors of descriptive field studies such as management or land-use intensity (Loreau et al. 2001; Hooper et al. 2005) and offer a great opportunity to study the importance of plant species richness *per se* on trophic interactions. The second and third study (chapter 3 and 4) used the Jena Experiment as a platform to assess how plant diversity shapes interspecific interactions involving omnivores.

The Jena Experiment was established on a former arable field at the flood plain of the Saale river in 2002 (Germany, 50°95′ N, 11°63′ E, 130 m above sea level; Roscher et al. 2004). I used the experimental plant communities of the Trait-Based Experiment (TBE; Fig. 3; Ebeling et al. 2014), that were sown in 2010 and assembled from a total of 20 Central European grass and non-legume herbaceous species to cover a gradient of plant species richness (1, 2, 3, 4, and 8) and plant functional diversity (1, 2, 3, and 4) on 138 plots (3.5 m x 3.5 m). The gradient of plant functional diversity was based on plant traits known to be important for spatial and temporal resource use and represents the levels from low (1) to high (4) trait complementarity in the plant community. The experimental plots were maintained by biannual mowing and weeded three times per year to remove unwanted species and maintain the sown communities.

As model organism to study interspecific interactions three locally abundant, geographically wide spread and ecological relevant invertebrate species were used; the ground beetles *Pterostichus melanarius* Illiger, and *Harpalus rufipes* DeGeer (Coleoptera: Crabidae), and the field slugs of the genus *Deroceras* Rafinesque (Pulmonata: Agriolimacidae). All three model organism are polyphagous feeders on a wide range of food types but include varying degrees of plant matter in their diet (Thiele 1977; Barker & Efford 2004) with the potential to adapt their feeding behavior to changing environmental conditions.

Figure 3. Experimental plots of the Trait-Based Experiment at the Jena Experiment with invertebrate enclosures in the pilot study in 2013 (top left), and in the full study in 2014 (top right and bottom).

1.2.3 Virtual ecologist approach - A simulation

To understand the impact of plant diversity on ecological communities, it is essential to quantify animal population densities. Surface-active invertebrates are routinely sampled with pitfall traps (Greenslade 1964; Zhao et al. 2013). It is well known that the resulting data are biased by arthropod mobility, activity, and environmental factors (Lang 2000, Perner & Schueler 2004, Woodcock 2005, Saska et al. 2013, Brown & Matthews 2016), but comprehensive studies that analyze which factors are mostly responsible for the bias are yet missing.

Computational simulations can reduce such knowledge gaps by simulating ground arthropods sampling across a range of different scenarios (Crist & Wiens 1995; Perner & Schueler 2004; Ellis & Bedward 2014). The "virtual ecologist" (Zurell et al. 2010), is an approach in simulation modeling to evaluate an experimental design by simulating data and observer models to mimic real species and their sampling. The last chapter (5) applied the virtual ecologist approach. We use an allometric individual-based model that simulates movement and pitfall sampling of virtual arthropods in simulation experiments (Fig 3) to systematically compare the known "true" abundance to the "observed" abundance in trap catches.

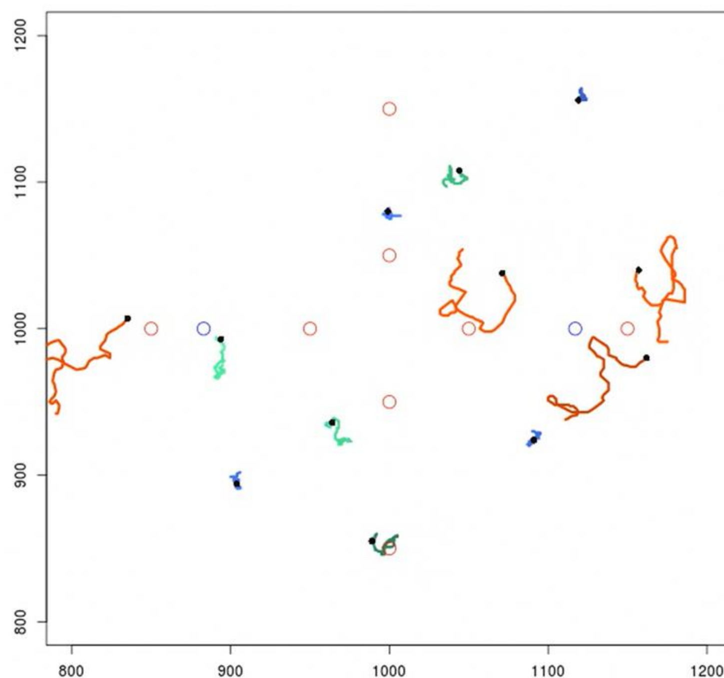

Figure 3. Simulated movement of 12 ground arthropods within the central 4 x 4 m square of the simulated area (20 x 20 m). Individuals are presented as black dot, with a colored tail attached. The tail visualizes the movement path across the previous 11 minutes and 40 seconds. A red tail indicates large species, green medium species, and blue small species. Red and blue circles represent a spatial arrangement of pitfall traps.

1.3 Research objectives and chapter outline

This thesis comprises one mensurative experiment that I conducted in the Midwest of the USA to analyze the effects of landscape context on the bacterial community in the guts of mobile predatory insects (**chapter 2**). The second and the third study (**chapter 3**, and **chapter 4**) were conducted in the framework of the Jena Experiment (Roscher et al. 2004; Ebeling et al. 2014). In **chapter 3**, I demonstrate in a pilot study that DNA metabarcoding of insect gut contents is a valuable tool to simultaneously analyze plant diversity effects on trophic and non-trophic interactions of a highly polyphagous consumer. In **chapter 4**, I use this approach to investigate direct and cascading plant diversity effects on interactions involving three invertebrate omnivores and a broad range of interaction partners. Measuring the impact of plant diversity on higher trophic levels requires methods that reliably quantify invertebrate communities. Therefore, in **chapter 5** the pitfall trap sampling bias was systematically analyzed with an individual-based model in a virtual ecologist approach (Zurell et al. 2010).

Chapter 2

I studied the effect of landscape context on the gut microbiome of mobile predatory insects in a mensurative field experiment. Landscape context is known to affect predator–prey interactions (Layman et al. 2007; Bianchi et al. 2013; Tscharntke et al. 2005) and therefore likely influences the diet of individual consumers (Schmid et al. 2016). Diet composition, in turn, is a strong predictor for the gut microbial community in many species (Lundgren & Lehman 2010; Mason & Raffa 2014; Wang et al. 2011). In this study, I aimed to test if landscape composition affects the gut microbiomes of mobile, predatory insects. I tested the effects of landscape context at two spatial scales by sampling lady beetles in two field types with contrasting plant diversity: (1) plant species-rich prairies and soybean monocultures that (2) were systematically selected to be surrounded by landscapes ranging from low to high proportion of land covered by annual crops in southern Wisconsin, USA. I used DNA-based community fingerprinting techniques to investigate patterns in the gut bacterial community richness and composition. Further, gut microbiota are a determinant of physiology and condition (Bahrndorff et al. 2016; Borer et al. 2013; Gibson & Hunter 2010; Ruokolainen et al. 2016). I therefore examined whether landscape-mediated changes in gut microbiota are associated with differences in body condition, assessed using estimates of body fat content.

Hypothesis for chapter 2

1. Mobile predators that forage in prairies will have a greater access to a broader range of prey types compared to beetles foraging in soybean and will therefore have a richer gut community.
2. Predators will have a relatively simpler gut community when the collection sites are surrounded by crop-dominated landscape compared to sites surrounded by more natural habitats.
3. Prairies and landscapes with low proportions of arable land will foster greater body condition in mobile predators.

Chapter 3

In this study, I used the framework of a grassland biodiversity experiment to test the potential of DNA metabarcoding for the direct and simultaneous assessment of trophic and non-trophic interactions. Sequence-based identification of food DNA using next generation sequencing (here after NGS) was so far mainly used to describe the dietary spectrum of species (Piñol et al. 2013; Vesterinen et al. 2013; Clare et al. 2014) but is underexploited in research on biodiversity and ecosystem functioning. Most studies on consumer communities in biodiversity experiments have measured the numerical response of species or ecosystem process rates (Scherber et al. 2010; Ebeling et al. 2014; Allan et al. 2015) but few have empirically analyzed species interactions. The recent advances in DNA sequencing offer new possibilities to examine interactions empirically (Traugott et al. 2013; Clare 2014; Vacher et al. 2016; Kamenova et al. 2017). Here, I sampled regurgitates of omnivorous beetles and analyzed DNA of food remains and gut microbiota by DNA metabarcoding (Roche 454 sequencing platform) to identify trophic and non-trophic interactions and analyze how these interactions are affected by plant biodiversity.

Hypothesis for chapter 3

1. Metabarcoding of gut contents will allow new insights into different types of interactions in biodiversity experiments.
2. Regurgitates are a good source material to study interactions as they contain only little consumer DNA.
3. An increase in plant species richness will result in a higher number of interactions.

Chapter 4

Based on the findings described in **chapter 3**, I empirically assessed trophic and microbial interactions of surface-active invertebrates with omnivorous feeding habits in response to biodiversity manipulations within a grassland biodiversity experiment (The Jena Experiment; Roscher et al. 2004; Ebeling et al. 2014). In particular, I identified DNA in gut contents and feces of three model consumer species with varying degree of omnivory by DNA metabarcoding (Illumina MiSeq platform) of PCR products from common barcoding regions for plants, animals, fungi, and bacteria (Pompanon et al. 2012; Traugott et al. 2013; Tiede et al. 2016). Our study is the first to simultaneously examine the direct and indirect impact of plant diversity on the multitude of interactions involving omnivores in a controlled grassland biodiversity experiment.

Hypothesis for chapter 4

1. Omnivores from plots inhabiting many plant and animal species will interact with a greater number of species.
2. Plant diversity will have greater direct effects on omnivores that consume mostly plant material and more indirect effects via the trophic chain on more predatory species.
3. The ratio of beneficial to harmful microbes (e.g., symbionts, pathogens) increases along the plant diversity gradient.

Chapter 5

This study addressed a major uncertainty in diversity inventories: the difference between the experimentally sampled and the real population density (Collins et al. 2003; Hutchison 2007; Woodcock 2005). Pitfall traps are routinely used to assess the community of ground arthropods although they are known to generate data that are biased by species-specific differences in mobility and activity (Lang 2000; Perner & Schueler 2004; Woodcock 2005; Saska et al. 2013; Brown & Matthews 2016). In this study, we use an allometric individual-based model that simulates movement and pitfall sampling in a simulation experiments to systematically quantify the effect of pitfall trap number, spatial trap arrangement, temperature, arthropod body mass, and population density on sampling bias.

Hypothesis for chapter 5

1. The pitfall trap bias will decrease with increasing arthropod body mass due to a higher mobility of larger species.
2. The pitfall trap bias will decrease with increasing temperature through greater locomotory activity of arthropods.
3. The sampling bias decreases with increasing number of pitfall traps and is affected by their spatial arrangement.

1.4 Original articles

Chapter 2

Gut microbiomes of mobile predators vary with landscape context and species identity

Julia Tiede[1,2,3], Christoph Scherber[1,2], James Mutschler[4], Katherine D McMahon[4], Claudio Gratton[3]

Author contributions: JT, CG, and KDM conceived and designed the study; JT performed the laboratory experiments and collected field samples; JT and JM performed molecular analysis and processed the data; JT, CG, and CS analyzed output data. JT wrote the first draft of the manuscript, and all authors were substantially involved in discussions and editing.

Published in Ecology and Evolution, 2017, 00:1–13. https://doi.org/10.1002/ece3.3390 © 2017 Tiede et al.

Chapter 3

Trophic and non-trophic interactions in a biodiversity experiment assessed by next-generation sequencing

Julia Tiede[1,2,3], Bernd Wemheuer[5,6], Michael Traugott[7], Rolf Daniel[5], Teja Tscharntke[2], Anne Ebeling[8], Christoph Scherber[1,2]

Author contributions: JT, BW, MT, RD, TT, AE, and CS conceived and designed the experiments; JT and BW performed the experiments; JT, CS, and BW analyzed the data; TT, MT, and RD contributed reagents/materials/analysis tools; JT wrote the first draft of the manuscript, and all authors were substantially involved in discussions and editing.

Published in PLoS ONE 11(2): e0148781. https://doi.org/10.1371/journal.pone.0148781
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Chapter 4

Plant diversity effects on multitrophic interactions analyzed by gut content metabarcoding

Julia Tiede[1,2,3], Bernd Wemheuer[5,6], Michael Traugott[7], Rolf Daniel[5], Teja Tscharntke[2], Anne Ebeling[8], Christoph Scherber[1,2]

Author contributions: JT, BW, MT, RD, TT, AE, and CS conceived and designed the experiments; JT and BW performed the experiments; JT, CS, and BW analyzed the data; TT, MT, and RD contributed reagents/materials/analysis tools; JT wrote the first draft of the manuscript, and CS and BW were involved in editing.

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Chapter 5

Pitfall trap sampling bias depends on body mass, temperature, and trap number: insights from an individual-based model

Jan Engel[8,9]*, Lionel Hertzog[9,10,]*, **Julia Tiede**[1,2,3], Cameron Wagg[11], Anne Ebeling[8], Heiko Briesen[12], Wolfgang W Weisser[9]

Author contributions: JE, LH, and WWW designed the study; JE and LH developed the model and analyzed the data; JE implemented the model and wrote the first draft of the manuscript, and all authors were substantially involved in discussions and editing.

Published in Ecosphere 8(4): e01790. https://doi.org/10.1002/ecs2.1790 © 2017 Engel et al.

Author affiliations

[1] Institute of Landscape Ecology, University of Muenster, Muenster, Germany

[2] Agroecology, Department of Crop Sciences, University of Goettingen, Goettingen, Germany,

[3] Department of Entomology, University of Wisconsin-Madison, Madison, WI, USA

[4] Departments of Civil and Environmental Engineering and Bacteriology, University of Wisconsin-Madison, Madison, WI, USA

[5] Institute of Microbiology and Genetics, Department of Genomic and Applied Microbiology, University of Goettingen, Goettingen, Germany

[6] Centre for Marine Bio-Innovation (CMB), UNSW Sydney, Australia

[7] Mountain Agriculture Research Unit, Institute of Ecology, University of Innsbruck, Innsbruck, Austria

[8] Institute of Ecology, University of Jena, Jena, Germany

[9] Department of Ecology and Ecosystem Management, University of Munich, Freising, Germany

[10] Department of Biology, Ghent University, Gent, Belgium

[11] Institute of Evolutionary Biology and Environmental Studies, University of Zurich, Zurich, Switzerland

[12] Department of Process Systems Engineering, University of Munich, Freising, Germany

CHAPTER 2

Gut microbiomes of mobile predators vary with landscape context and species identity

Julia Tiede, Christoph Scherber, James Mutschler, Katherine D McMahon, Claudio Gratton

Published in Ecology and Evolution, 2017, 00:1–13. doi:10.1002/ece3.3390 © 2017 Tiede et al.

Abstract

Landscape context affects predator–prey interactions and predator diet composition, yet little is known about landscape effects on insect gut microbiomes, a determinant of physiology and condition. Here, we combine laboratory and field experiments to examine the effects of landscape context on the gut bacterial community and body condition of predatory insects. Under laboratory conditions, we found that prey diversity increased bacterial richness in insect guts. In the field, we studied the performance and gut microbiota of six predatory insect species along a landscape complexity gradient in two local habitat types (soybean fields vs. prairie). Insects from soy fields had richer gut bacteria and lower fat content than those from prairies, suggesting better feeding conditions in prairies. Species origin mediated landscape context effects, suggesting differences in foraging of exotic and native predators on a landscape scale. Overall, our study highlights complex interactions among gut microbiota, predator identity, and landscape context.

2.1 Introduction

Animal guts harbor a vast diversity of microbes, as revealed by modern DNA-based methods (Bahrndorff et al. 2016; Engel & Moran 2013; Gibson & Hunter 2010). The gut microbiome may affect host fitness in many ways including host nutrition, regulating growth rate and stress tolerance, through protection against natural enemies, or by mediating host–pathogen interactions (Dillon & Dillon 2004; Douglas 2009; Ferrari et al. 2004; Henry et al. 2015; Ruokolainen et al. 2016). Gut microbes can be vertically transmitted or acquired from the environment (horizontal transmission; Gibson & Hunter 2010; Mason & Raffa 2014). In addition, the total gut community also includes transient species that cannot permanently colonize the gut (Dillon et al. 2005; Erkosar & Leulier 2014) but may represent a supplementary food source, or contribute to digestion (Bouchon et al. 2016). Understanding factors influencing animal gut microbiome composition can thus yield important insights into ecological interactions.

Laboratory studies have found that the gut microbial community of many arthropod species is affected by host diet (Broderick et al. 2004; Lundgren & Lehman 2010; Mason & Raffa 2014; Wang et al. 2011), either through effects of food substrates on the persistence of specific microbes, or directly from the acquisition of associated microbes (Bili et al. 2016; Chandler et al. 2011). In addition, gut microbiota of wild insect populations vary geographically, suggesting that differences in the local environment can shape microbial assemblages (Adams et al. 2010; Coon et al. 2016; Toju & Fukatsu 2011; Yun et al. 2014). The gut microbiome of wild insect populations likely represents a sample of microbiota from local food and other sources in their surrounding environment (Borer et al. 2013). On a local scale (small quadrats of 0.025 m^2), correlations among gut microbial richness of two ground-dwelling cricket species and prey richness in the habitat have been reported (Schmid et al. 2015); yet, the landscape-level consequences for mobile organisms such as flying predators have remained largely unexplored.

Predator–prey interactions have frequently been shown to be influenced by landscape composition and structure. A multitude of studies has investigated numerical responses of predators to the surrounding landscape (Chaplin-Kramer et al. 2011; Gardiner et al. 2009; Liere

et al. 2015), including predator movement (Blitzer et al. 2012; Forbes & Gratton 2011; Schellhorn et al. 2014). If predators use multiple prey items located in different habitat types, landscape complexity should be positively correlated with diet items consumed (Bianchi et al. 2013; Bianchi et al. 2009; Layman et al. 2007; Tscharntke et al. 2005), resulting in a greater variety of food-related or environmental microbes in the predators' guts. Yet, systematic studies on the effects of landscape context on predator gut microbiota are lacking.

Ideally, studies investigating landscape configuration and composition are performed in experimental landscapes, where landscape attributes are controlled by the experimenter (Hadley & Betts 2016, p. 59). However, such studies are often performed within only a single habitat type and cover often cover less than 1 km² (Haddad et al. 2015); such scales are considerably smaller than the foraging range of many insects, including pollinators or predatory beetles. Here, we report results from a mensurative experiment, in which study sites are selected a priori on a meaningful biological scale. We present evidence for landscape-level effects on insect gut microbiota on a scale of several thousand km².

Predator fitness may be affected by landscape context directly through variability in food quality and quantity. Prior work has shown that landscape context is associated with fitness-related measures of body condition, such as body size or fat content, in ground-dwelling predators (Bommarco 1998; Öberg 2009; Östman et al. 2001), but this relationship has not been examined in mobile arthropod predators and the role of gut microbes has remained elusive. As the microbiome can directly affect the nutritional state and health of an organism (Bahrndorff et al. 2016; Borer et al. 2013; Gibson & Hunter 2010; Ruokolainen et al. 2016), changes in the microbiome associated with the landscape could also have indirect microbe-mediated effects on body condition.

In this study, we examined the effects of landscape context on the gut bacterial community and body condition of predatory insects. We used aphidophagous lady beetles as our study system, as they are locally widespread and important natural enemies of aphids in agricultural crops (Obrycki et al. 2009; Snyder 2009) and seminatural habitats (Bianchi et al. 2013). Although aphids are their preferred prey, the lady beetles' food spectrum includes a broad range of other soft-bodied arthropods, as well as fungal or plant resources (Dixon 2000; Evans 2009; Hodek & Honěk 1996; Trilitsch 1999; Weber & Lundgren 2009). In a proof-of-concept laboratory experiment, we first show that even a single meal can increase the richness and alter the community composition of gut bacteria in individual beetles, indicating that diet diversity can affect gut communities. In a mensurative field experiment (Hadley & Betts 2016), we sampled six lady beetle species that differ in their phylogenetic relatedness (including three in the same genus), origin (native and exotic), and body size to explore the contribution of host-specific factors to differences in the gut microbiome and physiological response to landscape context. We tested the effects of landscape context at two spatial scales by sampling beetles in two field types with contrasting plant diversity: (1) species-rich prairies and soybean monocultures that (2) were systematically selected to be surrounded by landscapes ranging from low to high proportion of land covered by annual crops in southern Wisconsin, USA. We expected that mobile predators that forage in prairies have access to a broader range of prey types compared to beetles foraging in soybean and therefore would have a richer gut community. Because mobile predators may forage on a landscape scale, we further predicted

that lady beetles would have a relatively simpler gut community when the collection sites are surrounded by crop-dominated landscape compared to sites surrounded by more natural habitats. In addition, we examined whether landscape-mediated changes in predator gut microbiota were associated with differences in body condition, assessed using estimates of beetle fat content. Fat content reflects the available energy reserves for survival and reproduction and resistance to nutritional stress (Arrese & Soulages 2010; Roma et al. 2010). We predicted that prairies and landscapes with low proportions of arable land would foster greater body condition. We show that changes at the field and landscape scale affected the gut bacterial community and physiological response of predators, but the direction of the effect differed significantly between exotic and native species, raising the possibility of inherent differences in habitat use and foraging preferences among these groups.

2.2 Material and Methods

2.2.1 Feeding experiment

In a laboratory feeding experiment, we tested whether a single meal has the potential to alter the gut bacterial community of lady beetles. Adult *Coleomegilla maculata* De Greer (pink spotted lady beetle) were collected in April 2012 in Arlington, Wisconsin (USA), from dandelion flowers where they commonly aggregate in the spring (Harmon et al. 2000; Figure 1d). Beetles were maintained in the laboratory on dandelion flowers and moistened cotton balls for 7 days to allow their gut bacteria to equilibrate to similar diet environments. Prior to testing, beetles were starved for 48 hr. The beetles were randomly allocated to three treatments: (1) no food (control), (2) a meal consisting of one individual of *Acyrthosiphon pisum* Harris (pea aphid), and (3) a meal consisting of five different prey species (one individual each of *A. pisum, Rhopalosiphum padi* L. (bird cherry-oat aphid), *Aphis gossypii* Glover (cotton aphid), and *Aphis glycines* Matsamura (soybean aphid), and three eggs of *Spodoptera frugiperda* JE Smith ([Lepidoptera], beet armyworm). These species represent common prey of lady beetles in Wisconsin and the Midwestern USA. Beetles that finished their meal completely within 1 hr (n = 19 beetles) were transferred into 1.5-ml microtubes containing 70% ethanol and frozen at −20 °C (n = 7 for the control, n = 5 for the 1-species diet, and n = 7 for the 5-species diet).

2.2.2 Field study

We sampled wild populations of lady beetles in southern Wisconsin, USA, in 2012. The region is dominated by agricultural row crops (mainly corn [*Zea mays* L.] and soybean [*Glycine max* L.]) with remaining patches of seminatural habitat (i.e., forest, grasslands, wetlands). We initially selected 10 prairies and 10 conventionally managed soy fields as two field types with contrasting diversity of plants and likely associated prey species. The fields were at least 2.6 km apart (Fig. S1 in Appendix S1). We analyzed the landscape composition within a 2 km radius of each field, which is an ecological meaningful distance for foraging flights in lady beetles (Woltz & Landis, 2014). The proportions of land cover types within each sector were analyzed with ArcGIS (10.0, ESRI, Redlands, CA, USA) and the Geospatial Modeling Environment software (Beyer, 2012) with the Cropland Data Layer (CDL, USDA, NASS 2012). As a metric for landscape

complexity, we used the proportion of annual crop monocultures (0.16–0.77; cropland hereafter) as it represents a habitat that is frequented by lady beetles but is intrinsically species poor and, in contrast to seminatural habitat, is easy to unambiguously categorize. The proportion of cropland and seminatural habitat were negatively correlated (Pearson's $r = -.88$, $p < .001$) and the later produced essentially the same results when used in the analysis instead.

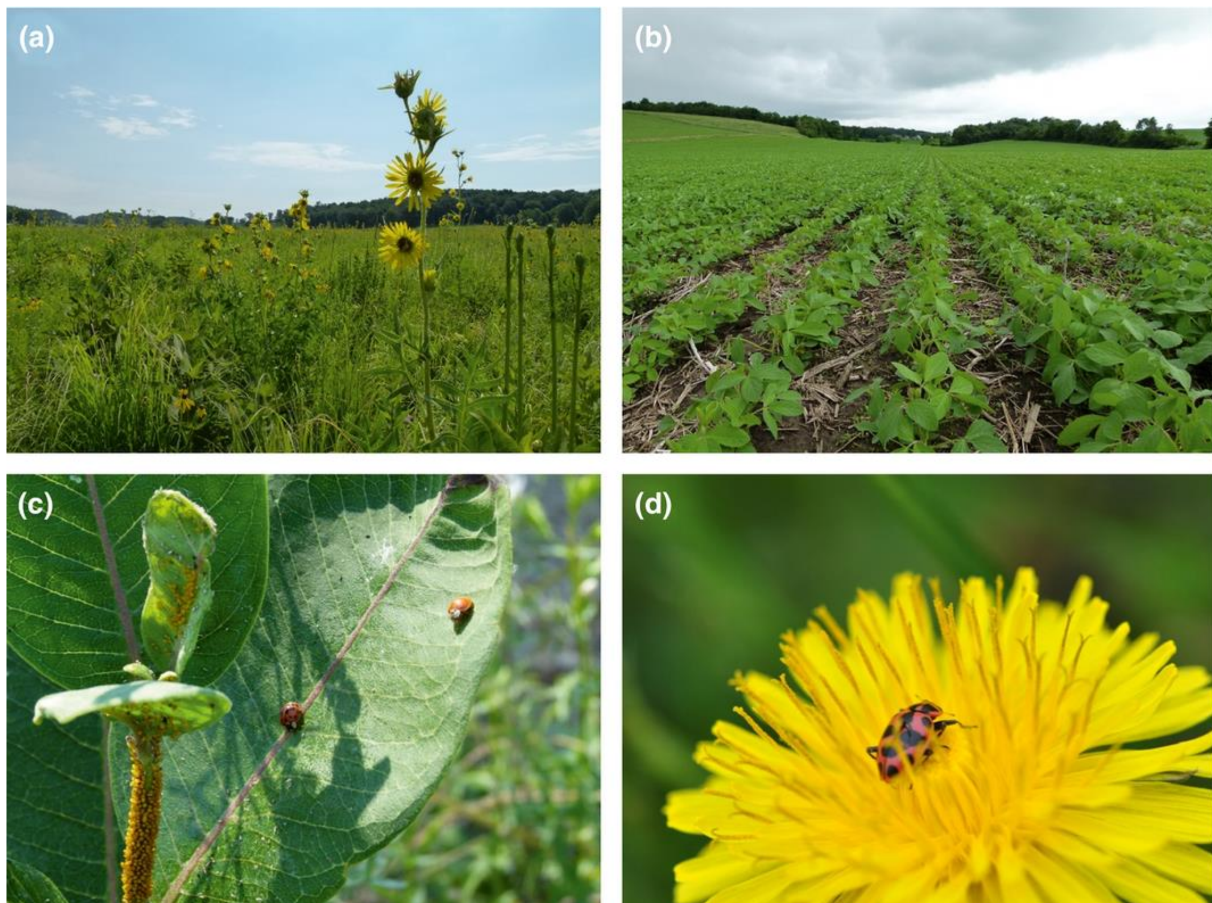

Figure 1. Examples for field study sites. (a) Restored prairie; (b) soybean field; (d) *Harmonia axyridis* on aphid-infested milkweed (*Asclepias syriaca* L.) in a prairie (photo by J. Dreyer); (c) *Coleomegilla maculata* on dandelion (*Taraxacum officinale* L.)

We sampled each field multiple times by sweep netting or hand collection from July through mid-August. During this time, soybean aphid (*A. glycines*) populations usually reach high densities, but in 2012, they remained exceptionally low likely due to the severe drought in the Midwest (Liere et al. 2015). It was also difficult to find lady beetles (compared to our previous experience), and we succeeded in only eight soy fields and nine prairies. In total, we collected 243 beetles ($n = 139$ in prairie, $n = 104$ in soy) belonging to six aphidophagous species (Coccinellidae: Coccinellinae: Coccinellini) including the exotic *Coccinella septempunctata* L. ($n = 49$), *Harmonia axyridis* Pallas ($n = 72$), and *Hippodamia variegata* Goeze ($n = 59$), and the native *Cycloneda munda* Say ($n = 16$), *Hippodamia convergens* Guérin-Méneville ($n = 25$), and *Hippodamia parenthesis* Dejean ($n = 22$; Gardiner et al. 2009). Collected beetles were

immediately placed separately into microtubes containing 70% ethanol, transported to the laboratory on ice and preserved at −20°C until later analysis.

2.2.3 Sample processing

2.2.3.1 Gut dissections

For both the beetles from the laboratory experiment and field collected specimens, the analysis of gut bacteria was conducted on dissected alimentary tracts. The beetles were carefully opened ventrally with sterilized fine-tipped forceps in individual Petri dishes. Complete guts were isolated and stored in new 1.5-ml microtubes containing 70% ethanol at −20°C. The ethanol was removed before DNA extraction with the PowerSoil Kit (MoBio Laboratories, Carlsbad, USA).

2.2.3.2 Analysis of gut bacteria

We characterized the total gut bacterial community of lady beetles with Automated Ribosomal Intergenic Spacer Analysis (ARISA), a cost- and time-efficient fingerprinting technique. ARISA detects bacterial phylotypes based on the length heterogeneity of the intergenic spacer region between the 16S and 23S rRNA genes (Fisher & Triplett 1999). ARISA-PCR was performed with 1406f/23Sr (Borneman & Triplett 1997), a bacteria-specific primer set with high taxonomic coverage (Purahong et al. 2015), as previously described (Shade et al. 2007; Yannarell et al. 2003).

We analyzed up to four technical PCR replicates for each sample of the feeding experiment due to the low number of biological replications. No technical replications were used for wild populations. Reagent-only controls were included from the PCR step onwards. The PCR fragments were separated with a capillary sequencer (ABI 3730 DNA Analyzer, Applied Biosystems, Foster City, USA). The fragment sizes were determined by comparison with a custom internal 100–2,000 bp ROX-labeled standard (BioVentures, Murfreesboro, USA) using GeneMarker v 1.5 (Soft Genetics LLC, State College, USA). Fragments were binned into operational taxonomic units (OTUs). The bin size was expanded from 1 bp for small fragments (200–550 bp) to 2 bp (551–700 bp), 3 bp (701–950 bp) and 5 bp for large fragments (951–1,200 bp) to account for the decreasing resolution with increasing fragment size (Abdo et al. 2006). Peaks that resulted from fluorescently labeled fragments were distinguished from the background noise by a custom R script (R Development Core Team 2012) developed by Jones and McMahon (2009) based on Abdo et al. (2006).

Operational taxonomic units were treated as distinct bacterial taxa, and their relative fluorescence intensity was used as a proxy for relative taxon abundance within a sample to compare bacterial diversity and community structure between samples. ARISA can fail to accurately separate bacterial taxa at species level when multiple species have the same sequence length of the intergenic spacer and the method tends to underestimate diversity when species richness is high. Despite these limitations, other studies have demonstrated that patterns detected with ARISA are similar to those observed with sequencing-based analysis at a fraction of the cost (van Dorst et al. 2014; Jami et al. 2014).

2.2.3.3 Estimation of body fat content

We visually estimated the fat content in individual beetles during gut dissections. Beetles were assigned to the categories low, medium, and high fat content (Anderson 1981): "Low": little visual fat, mainly accumulated in the parietal layer; "Medium", clearly visible fat accumulations also in regions of the gut or reproductive organs; "High": fat filling and expanding the abdomen. Compared to whole body fat extraction, visual estimates of body fat do not provide quantitative data but allowed us to distinguish between storage fat and accumulated lipids in reproductive organs. Considering the fluctuations in total body fat in females during egg laying, estimates of storage fat provide a suitable assessment of the nutritional state.

2.2.4 Statistical analyses

All statistical analyses were performed in R (version 3.3.1, R Development Core Team, 2016) and R-Studio (version 0.99.903, RStudio Team 2015; Data files and R scripts in Appendices S2, S3, and S4). Means are reported ±1 *SD*.

2.2.4.1 Feeding experiment

For the feeding experiment, technical replications existed for all but three samples and were averaged prior to the analysis. The relationship between bacterial richness and the number of prey species in the meal (zero in the control, 1-species diet, 5-species diet) was analyzed with linear regression. The number of bacterial taxa in a sample was log-transformed, and the model included number of technical replicates per sample as known prior weights, giving more weight to samples with more replications.

We analyzed the gut bacterial community assemblage using bacterial taxon relative abundances and calculating Bray–Curtis similarities (*vegan: vegdist*; Oksanen et al. 2017). We tested the effects of meal type (control, 1-species diet, 5-species diet) on community composition with permutational multivariate analysis of variance (perMANOVA; *adonis*; Oksanen et al. 2017) and permutation tests for the between group homogeneity in multivariate dispersions (*vegan: betadisper*, *permutest*; Oksanen et al. 2017; Anderson, 2006; McArdle & Anderson, 2001). Similarities between samples were visualized by NMDS (*metaMDS*; Oksanen et al. 2017).

2.2.4.2 Field study

2.2.4.2 | .1 Bacterial richness

We tested the effects of host-specific factors, sex, field type, and proportion of annual cropland in the surrounding 2 km on the log-transformed gut bacterial richness using linear mixed-effects models (*nlme: lme;* Pinheiro & Bates 2000). Alternative distributions for count data (Poisson, negative binomial) had higher AICc values (Akaike's information criterion corrected for small sample size; *stepAICc* function, *MASS* package, corrected for small sample sizes by C. Scherber 2009, http://www.christoph-scherber.de/stepAICc.txt), and we therefore decided for a log-transformation of the response. For the host-specific factors, we constructed a custom contrast matrix that compared the six species according to three different attributes: origin (exotic vs. native), size (small vs. large), and genus (genus *Hippodamia* vs. non-*Hippodamia*; Table 1). Models further included sex within species within collection site as a random effect.

Variance heterogeneity between species was accounted for by introducing a variance function with different variances estimated for each species. Models were simplified based on AICc, starting with a model including the three-way interaction. For the reported output, parameters were estimated based on restricted maximum likelihood (REML).

Table 1. Custom contrast matrix for lady beetle species

Lady beetle species	Genus group	Origin	Body size
Coccinella septempunctata	non-*Hippodamia*	exotic	big
Cycloneda munda	non-*Hippodamia*	native	small
Harmonia axyridis	non-*Hippodamia*	exotic	big
Hippodamia convergens	*Hippodamia*	native	big
Hippodamia variegata	*Hippodamia*	exotic	small
Hippodamia parenthesis	*Hippodamia*	native	small

Small versus large body size refers to average measures of species elytron length (small <4.0 mm vs. big >4.5 mm; Julia Tiede (JT) & Claudio Gratton (CG), unpublished data).

2.2.4.2 | .2 Bacterial community structure

Bacterial community composition in wild collected species was visualized as in the laboratory experiment with NMDS based on Bray–Curtis distances and by mean relative abundance of bacterial taxa per beetle species and habitat type (Fig. S2 in Appendix S1). We tested the effect of species, and species grouped by genus, origin, and body size on bacterial composition using separate (one-way) perMANOVA (*adonis*; Oksanen et al. 2017). Species, as the best predictor, was included in a model testing the interactions between species and field type, and species and proportion cropland. Additionally, we tested the interaction between species and sex. All models included sex within species within collection site as random effect. Homogeneity of sample dispersion was tested (*vegan*: *betadisper*, *permutest*; Oksanen et al. 2017).

2.2.4.2 | .3 Body fat content

We analyzed the proportion of beetles in three ordinal categories (low, medium, and high fat content) using cumulative link mixed-effects models (*ordinal: CLMM*; Christensen 2015) as a function of beetle species contrasts, field type, proportion cropland, and bacterial richness as fixed effects and beetle species within collection site as random effects. The full models included all two-way interactions, and models were simplified as described above. To assess the effect of sex, three-way interactions with sex were included in the best fit model and deleted from maximal models based on AICc.

2.3 Results

2.3.1 Feeding experiment

In guts of the 19 beetles from the feeding experiment, we found 313 bacterial phylotypes (OTUs). The bacterial richness in individual beetle guts increased with the number of prey species in the meal (Table 2; Figure 2a) from 28 ± 7 (mean ± *SD*) in the beetles in the unfed (control) diet, to 31 ± 5 in the 1-species diet, and 39 ± 11 in the 5-species diet. Overall, we detected a significant but weak effect of the meal type on the bacterial community (perMANOVA; Table 2a; Figure 2b). In pairwise tests (Table 2b–d), the gut communities between beetles from the 1-species diet and the 5-species diet differed from the control but not from each other. Nonsignificant differences in sample dispersion (Table 2) indicated that the effects were driven by differences in the group centroids.

Table 2. Laboratory experiment results on the effect of meal type on gut bacteria in the gut of *C. maculata*

Linear model		*df*	Estimate ± *SE*	*t* value	*p* value
(Intercept)		1	3.301 ± 0.07	45.61	**< 2e-16**
Number of prey species		1	0.078 ± 0.02	3.41	**0.003**
Residuals		17			
perMANOVA		***df***	**SS**	***F* value**	***p* value**
a)	All meal types	2	1.13	1.74	**0.014**
	Residuals	16	5.2		[R^2 = 0.18]
b)	Control vs. 1-species diet	1	0.67	2.13	**0.003**
	Residuals	10	3.13		[R^2 = 0.18]
c)	Control vs. 5-species diet	1	0.67	2.12	**0.008**
	Residuals	12	3.79		[R^2 = 0.15]
d)	1-species diet vs. 5-species diet	1	0.35	1	0.393
	Residuals	10	3.49		[R^2 = 0.09]
PERMDISP		***df***	**SS**	***F* value**	***p* value**
Meal type		2	0.01	0.43	0.659
Residuals		16	0.14		

Horizontal lines separate the different analysis. Linear model parameter estimates and standard errors on the effect of meal type on log-transformed bacterial richness. PerMANOVA results on the effect of meal type on gut bacterial community in multiple (a) and pairwise contrasts (b–d). PERMDISP results on homogeneity of multivariate sample dispersion. *p* values <.05 are reported in bold numbers.
df, degrees of freedom; *SE*, standard errors; SS, sums of squares.

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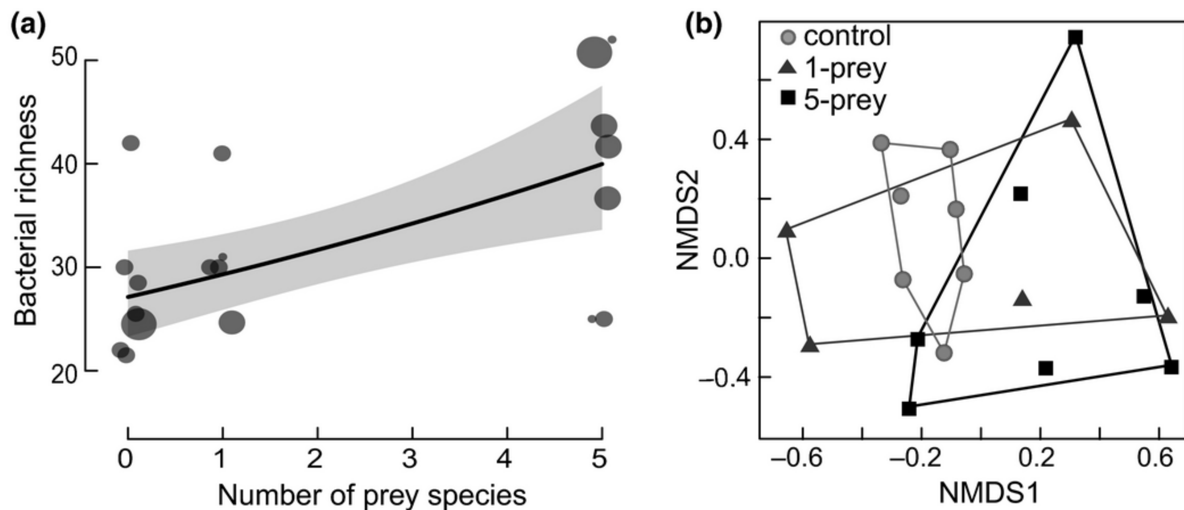

Figure 2. Bacterial (OTU) community richness and composition in feeding experiments. (a) Bacterial richness in guts of *C. maculata* as a function of the number of prey species in the meal (zero in the control, 1-species diet, 5-species diet). Points represent individual beetles and are scaled based on the number of averaged technical replicates, the black line and gray area show the predictions and 95% confidence interval of the linear regression model, respectively. (b) Community composition of bacteria in guts of *C. maculata* shown as NMDS (2D, stress = 0.19) based on Bray–Curtis dissimilarities of the relative abundance of bacterial taxa. Symbols represent individual beetles; colors and enclosing polygons refer to meal types.

2.3.2 Field study

3.2.1 Bacterial richness

In total, we found 551 bacterial taxa (OTUs) in the guts of 243 field collected beetles; the mean bacterial richness was 80 ± 20. Most of the variance in richness was explained by the differences between beetle species, which was higher in the three exotic species than in the three native species (Table 3 and Figure 3a; Table S3 in Appendix S1). Moreover, exotic and native species responded differently to landscape context: the bacterial richness in native species guts increased with increasing proportion of cropland surrounding the collection side, but decreased for exotic species (Tables 3 and Figure 3a; Table S3 in Appendix S1). Further, there was an effect of field type with higher bacterial richness in beetles collected in soy than in prairies (Table 3; Table S3 in Appendix S1). Sex had no effect.

2.3.2.2 Bacterial community structure

The bacterial assemblages were largely associated with beetle species identity (perMANOVA; Table 3a and Figure 3b). Origin, genus, and body size, also, had significant effects on the community structure, but the fit of the models was weaker (Table 3b–d). Sex, field type (corn vs. soy), and proportion cropland did not explain additional variability (Table 3e,f). The detected effects on the bacterial community might be partly driven by variances in sample dispersion between species (Table 3), but species also had distinct sets of abundant bacteria indicating compositional differences among species (Fig. S2 in Appendix S1).

Table 3. Field study results on gut bacteria and fat content of wild populations of lady beetles

Linear mixed model*		*df*	*denom. df*	χ2	*p* value
Species		3	312	177.55	**<0.001**
Field type		1	14	12.22	**<0.001**
Proportion crop		1	14	3.04	*0.081*
Species x proportion crop		3	31	13.27	**0.004**
perMANOVA		***df***	**SS**	***F* value**	***p* value**
a)	Species	5	27.51	26.89	**0.001**
	Residuals	237	48.49		[R^2 = 0.36]
b)	Origin	1	5.54	18.95	**0.001**
	Residuals	241	70.46		[R^2 = 0.07]
c)	Genus	1	5.39	18.39	**0.001**
	Residuals	241	70.61		[R^2 = 0.07]
d)	Size	1	5.14	17.48	**0.001**
	Residuals	241	70.86		[R^2 < 0.02]
e)	Sex	1	0.31	1.53	0.148
	Species x sex	5	1.11	1.08	0.413
	Residuals	231	47.10		[R^2 = 0.38]
f)	Field type	1	0.56	2.85	1
	Species x field type	4	1.38	1.78	0.147
	Residuals	226			
g)	Proportion crop	1	0.32	1.66	0.722
	Species x proportion crop	5	1.25	1.30	0.485
	Residuals	226	44.04		[R^2 = 0.42]
PERMDISP		***df***	**SS**	***F* value**	***p* value**
Species		5	1.39	39.02	**<.001**
Residuals		237	1.69		
Cumulative link mixed model**		***df***	***denom. df***	**χ2**	***p* value**
Bacterial richness (log)		1	153	0.51	0.476
Species		3	34	12.04	**0.007**
Field type		1	13	4.33	**0.037**
Proportion cropland		1	13	0.10	0.753
Bacterial richness (log) x species		3	153	10.32	**0.016**
Bacterial richness (log) x proportion crop		1	153	4.20	**0.043**
Field type x proportion crop		1	13	2.97	*0.085*

Horizontal lines separate the different analysis. Wald chi-square tests from linear mixed model on the effect of species contrasts (native vs. exotic origin, small vs. big size; *Hippodamia* vs. other genera), sex, field type, and proportion cropland on log-transformed bacterial richness. PerMANOVA results on the effects of species (a) and species grouped by origin, and size, (b–d), and sex (e), field type and proportion cropland after accounting for the effect of species and their interactions with species (f) on the bacterial community. PERMDISP results on homogeneity of multivariate sample dispersion. Likelihood-ratio tests from cumulative link mixed model results on the effect of beetle species contrasts, log-transformed bacterial richness, field type, and proportion cropland on beetle fat content. *p* values <.05 are reported in bold numbers and *p* <.10 in italics. Details on parameter estimates and standard errors are reported in Table S3 and S4 in Appendix S1.
*Mixed effects model *denom. df* = 159.
** Cumulative link mixed model *denom. df* = 153.
df, degrees of freedom; *denom. df*, denominator degrees of freedom; *SE*, standard errors; SS, sums of squares.

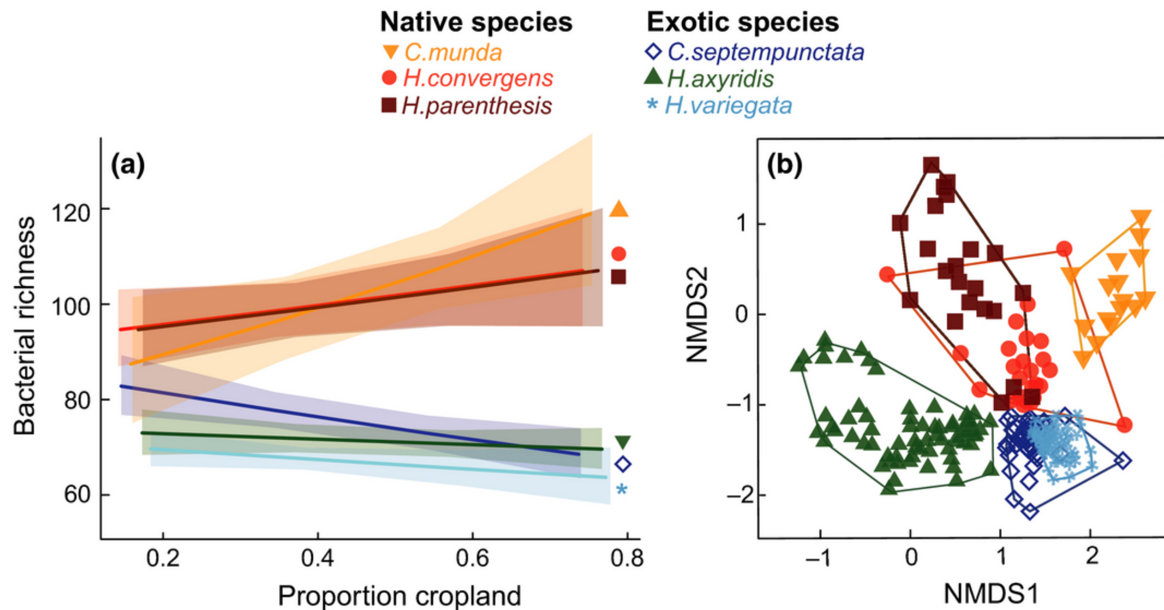

Figure 3. Bacterial (OTU) community richness and composition in wild beetle populations. (a) Effect of the interaction of beetle species and proportion cropland on the log-transformed bacterial richness (back-transformed for illustrative purposes). Lines and shaded regions show response predictions and 95% confidence intervals from the mixed-effects model. (b) Community composition of bacteria in gut samples of six wild populations of lady beetles visualized as NMDS (2D, stress = 0.20) based on Bray–Curtis dissimilarities of the relative abundance of bacterial taxa. Symbols and enclosing polygons represent individuals of different beetle species.

2.3.2.3 Body fat content

The relative fat content of beetles was associated with species identity (Tables 3 and Table S4 in Appendix S1). Most beetles of the genus *Hippodamia* contained low body fat. Fat content of the two native *Hippodamia* species, *H. convergens* and *H. parenthesis,* increased with their gut bacterial richness, but this pattern was not observed in the exotic *H. parenthesis*. Conversely, in the exotic *C. septempunctata* and *H. axyridis*, 3.2.2 Bacterial community structure

The bacterial assemblages were largely associated with beetle species identity (perMANOVA; Table 3a and Figure 3b). Origin, genus, and body size, also, had significant effects on the community structure, but the fit of the models was weaker (Table 3b–d). Sex, field type (corn vs. soy), and proportion cropland did not explain additional variability (Table 3e,f). The detected effects on the bacterial community might be partly driven by variances in sample dispersion between species (Table 3), but species also had distinct sets of abundant bacteria indicating compositional differences among species (Fig. S2 in Appendix S1).

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when the beetles were collected in crop-dominated areas (Tables 3 and Figure 4b; Table S4 in Appendix S1). Further, beetles collected in prairie had a higher fat content compared to soy (Table 3; Table S4 in Appendix S1) and tended to be fatter when the prairie was surrounded by cropland, but this interaction was only marginally significant (Table 3 and Figure 4c; Table S4 in Appendix S1). When sex was included as a fixed effect in the analysis, the interaction between prairie and the proportion of cropland also became significant. Additionally, we found an interaction between crop and sex with only females responding positive to increasing proportions of cropland. Further, there was an interaction between species and sex (Table S5 and S6 in Appendix S1).

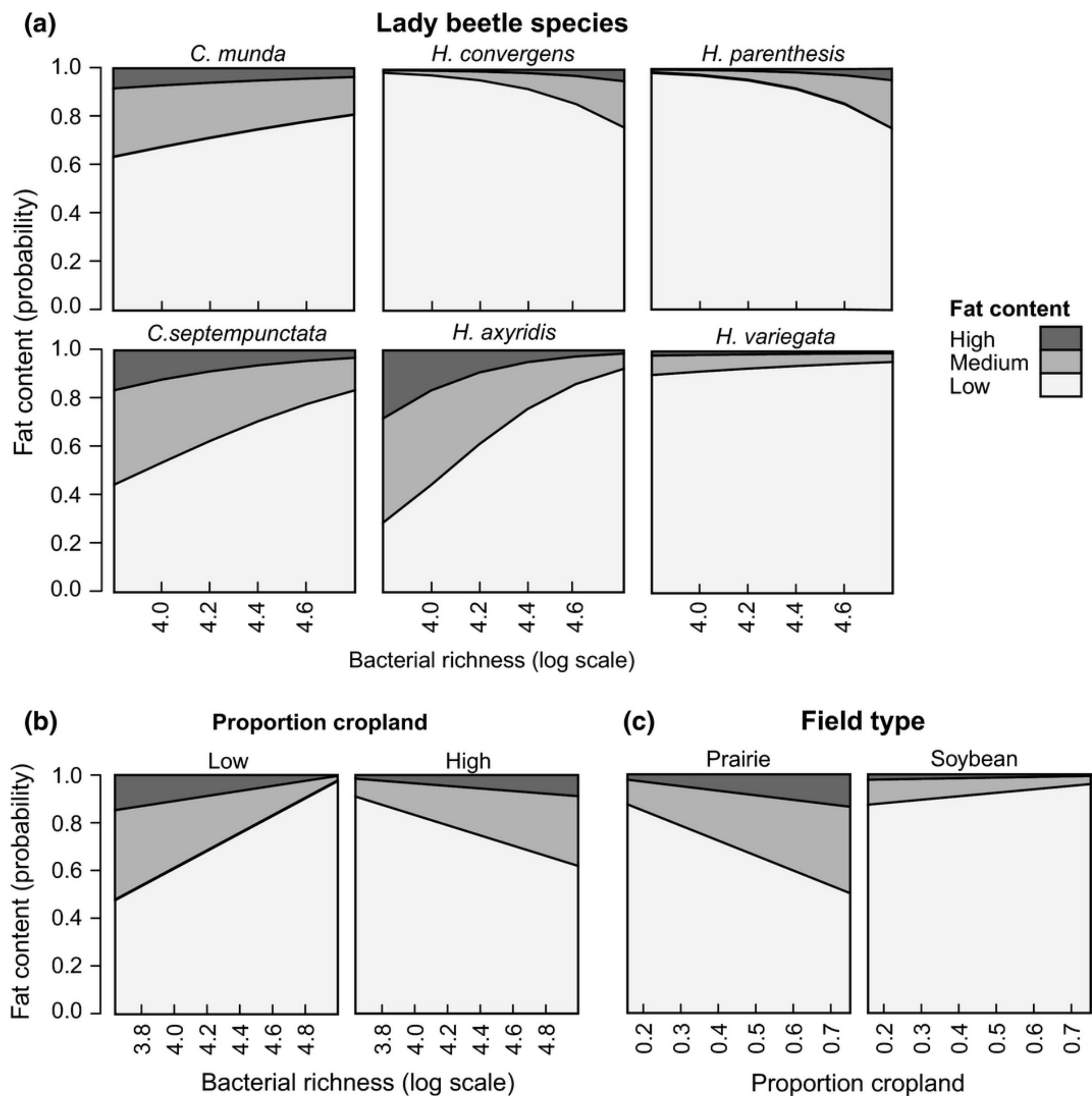

Figure 4. Body fat content in wild beetle populations. (a) Effects of the interactions of beetle species and log-transformed gut bacterial taxon richness (OTUs), (b) proportion cropland and log-transformed gut bacterial taxon richness (cropland was a continuous variable in the model but is shown as low and high for illustrative purposes), and (c) field type and proportion cropland on the proportion of beetles with low, medium, or high body fat as predicted by a cumulative link mixed model.

2.4 Discussion

We hypothesized that the diversity and composition of gut microbes in mobile arthropod predators would be affected by landscape context, both at the local (field) and at broader (among field, landscape) scale. Consistent with this prediction, we found that changes in landscape composition were associated with changes in richness of bacterial OTUs in the guts of beetles, but this effect was strongly species-dependent. In fact, one of the strongest patterns observed in this study was the distinct difference in abundance and composition of gut bacteria across species of lady beetles. Moreover, a significant amount of bacterial community variation, and the response of microbes to landscape composition, was related to whether species were native or exotic, an unexpected finding. Native lady beetles had a richer gut bacterial community, and this richness increased as the landscape became more crop-dominated; in contrast, the gut bacterial richness of exotic beetles was generally lower than that of natives and decreased as the amount of cropland increased in the landscape.

2.4.1 Species effects on bacterial richness and composition

The significant effect of lady beetle species on the gut bacterial community composition raises three nonmutually exclusive hypotheses about drivers of the composition the gut microbiome. That conspecific beetles had similar gut communities, even if they were sampled in different field types at distant collection sites, suggest that there may be a core group of species-specific bacteria. Lady beetles are frequently infected with male-killer bacteria (Majerus & Hurst 1997; Weinert et al. 2007) but specific associations with gut microbes are largely unexplored, as is the case for most predatory insects. Shotgun-sequencing of gut contents of lady beetles revealed potential symbionts (Paula et al. 2016). However, facultative gut symbionts were also detected in omnivorous ground beetles (Lundgren et al. 2007) and distinct gut communities in predatory ants (Anderson et al. 2012) and wasps (Mrázek et al. 2008).

Another potential explanation is that species-specific chemo-physical characteristics of the gut select for colonization by certain bacteria (Dillon & Dillon 2004; Nelson et al. 2012). However, if this was a strong influencing factor, then we would expect that shared evolutionary history of beetles would result in the gut bacterial communities of closely related species to be more similar than distantly related species (Sanders et al. 2014). However, this was not the case for the three species of the genus *Hippodamia* in our study which had distinct bacterial assemblages more associated with whether they were exotic or native to the Midwestern USA. Although this study was not specifically designed to test for systematic differences in bacterial communities as a function of evolutionary relatedness or their exotic vs. native status, the patterns found in the most widespread beetle species in this area were strong and warrant additional study.

A third explanation for our findings of species-specific differences in gut bacteria relates to differences in their diets, which could result in different sets of prey-related bacteria. The laboratory experiment demonstrated that beetle gut communities could change relatively rapidly even within one species. Similar to our findings, *H. axyridis* gut microbes were enriched by aphid symbionts shortly after aphid ingestion (Paula et al. 2015). This hypothesis is further supported by a study on fruit fly species with distinct feeding habits, whose gut communities

were different in wild populations but became similar on the same diet under laboratory conditions (Chandler et al. 2011). Thus, it is likely that at least some of the bacterial variation between lady beetle species was due to dietary differences maybe as a result of resource partitioning through differences in the dietary breadth, prey preferences, the ability to locate prey, preferred areas on a plant to forage, and the likelihood of switching habitats (Forbes & Gratton 2011; Hodek & Honěk 1996; Iperti 1999; Schellhorn & Andow 2005; Sloggett & Majerus 2000). Studies that simultaneously identify food remains and microbes in gut contents (Paula et al. 2015; Tiede et al. 2016) could further illuminate the relation between diet and the gut microbiome.

2.4.2 Landscape effects on bacterial richness and composition

Other studies have shown that exotic species often dominate lady beetle communities in arable land. In this region, native species are mainly found in perennial grasslands and other seminatural habitats (Gardiner et al. 2009; Diepenbrock & Finke 2013; Grez et al. 2013). A similar pattern was found for native and exotic spider communities. An increasing amount of arable land is often associated with seminatural habitat fragmentation and more distant remnant patches are expected to harbor more dissimilar communities than close ones (Tscharntke et al. 2012). Thus, native beetles might have sampled a greater beta diversity of microbes from isolated natural habitat patches when located in landscapes with a high proportion of cropland. The preference of exotic beetles for homogenous agricultural habitats (i.e., crops fields) could have led to a reduced exposure to bacteria in the environment and therefore a lower gut bacterial richness. Additionally, a higher pathogen load in agricultural landscapes combined with higher antimicrobial defense in exotic species could contribute to the pattern of increasing microbial richness with increasing amount of cropland in native but not exotic lady beetles. Along these lines, farmland frogs harbored more potentially harmful bacteria in their guts than frogs from natural habitats (Chang et al. 2016). A strong antimicrobial defense has been detected in the exotic *H. axyridis* (Beckert et al. 2015; Gross et al. 2010; Vilcinskas et al. 2013) and is suggested as a potential mechanism driving invasive predator success (enemy release hypothesis; Roy et al. 2011).

The specific habitat type in which beetles were collected, soy compared to prairie, was another strong predictor for bacterial richness. In contrast, to our expectation that beetles from prairie would have a richer gut community, we found more bacterial diversity in the guts of beetles from soy. This finding could be partly attributed to a drought that affected the soybean plants and aphid populations in southern Wisconsin (Mallya et al. 2013). The low availability of soybean aphids, the principal prey of lady beetles in this crop, likely increased the consumption of alternative prey (Iperti et al. 2000). A broader diet in soybean would expose the beetles to a greater variability of environmental bacteria compared to a diet of mainly aphids. In *H. axyridis*, aphid–symbionts were detected up to 96 hr after aphid consumption (Paula et al. 2015). Prairie plant communities were more resilient to the drought than row crops (Joo et al. 2016) and likely allowed the aphidophagous lady beetles in our study to be more selective in their prey choice.

Additionally, differences in local food availability between the two habitat types could have led to differences in residency time. The beetles we collected in soybean might have switched from another (crop-) habitat not long before (Forbes & Gratton 2011) and carried over bacteria and higher food availability in prairie could have increased small-scale foraging. The lack of information on how much time a beetle has spent in the field where it was sampled may to some degree confound the local and the landscape scale used in our study.

Studies that compare samples from multiple seasons and years could help to further elucidate what shapes the gut community. Our results indicate that the total gut community of lady beetles can be divided into a stable and a variable part. The core OTUs that form similar gut communities in conspecific beetles collected from different habitats and at distant collection sites are likely also relatively stable between seasons and years. More transient, food-related bacterial taxa should be highly variable and respond to annual and seasonal changes in food availability, and the variations might be more extreme in crop-dominated regions with many ephemeral food sources. For example, in a year with high aphid abundance in soy we would expect the pattern we found to be reversed, with lower bacterial richness found in beetles from soy as compared to beetles that forage in prairies.

2.4.3 Microbe and landscape effects on ladybeetle fat content

We posit that the higher gut bacterial richness in beetles from soy fields compared to prairies is an indicator of consumption of mixed alternative resources in absence of soybean aphids. This interpretation is consistent with the findings that beetles collected in prairie had a higher fat content compared to soy-collected beetles, indicating superior feeding conditions and a better outcome for body condition in prairie compared to aphid–depauperate soy. Landscape context on a broad scale had no effect itself but mediated the effect of bacterial richness on body fat of beetles: As bacterial richness increased, beetles became fatter in agriculturally dominated landscapes, while for beetles collected in landscapes with few crops, higher bacterial richness was associated with lower fat content. Generalist predators can benefit from some proportion of cropland, which periodically provides abundant food resources (Rand & Tscharntke 2007) but may benefit more from the inclusion of alternative resource with complementary nutrients in simplified landscapes in which they mainly find crop pests. Other studies on predatory beetle body condition found positive effects of landscape heterogeneity (Östman et al. 2001) and succession-related food supply and diversity of wildflower habitats (Barone & Frank 2003).

Although landscape context clearly had an impact on gut microbiota, and landscape context and gut microbial richness together affected the fat content of lady beetles, the ultimate causal mechanisms remain to be explored. We propose that food resource abundance and diversity in the local habitat could be one of the main drivers for both gut bacterial richness and host fat content. Further, diet-related bacteria can potentially affect host fitness directly when they serve as a supplemental food source, temporarily contribute to digestion processes (Bouchon et al. 2016) or facilitate adaption to novel food sources (Chu et al. 2013). However, if and to what extend a predator benefits from a mixed diet (Evans et al. 999; Harwood et al. 2009; Lefcheck et al. 2012; Lundgren 2009) and diverse gut bacteria depends on host

species: In our study, the two native beetles *H. convergens* and *H. parenthesis* had more body fat when their guts harbored many different bacterial taxa. In contrast, the exotic *C. septempunctata* and *H. axyridis* were fatter when their gut bacterial communities were species poor. This finding might reflect that exotic species are better adapted to homogenous conditions in cropland than native species and therefore often dominate coccinellid communities in cultivated habitats (Bahlai et al. 2013).

2.5 Conclusion

A key finding of this study is that mobile predatory insects have a species-specific set of gut bacteria that is stable over a range of environmental conditions. However, landscape and habitat-associated differences in where they are collected can alter this base assemblage. Although the mechanisms for these patterns are not resolved, the strong differences between exotic and native species and the contrasting effects of landscape context on gut bacteria suggest inherent differences in habitat and prey use among these groups. Moreover, that landscape context can also affect host performance as indicated by fat content, both directly and indirectly via gut microbiota, potentially indicates a novel mechanism through which human-altered landscapes can affect invertebrate predators. The method we used to analyze gut bacterial communities allowed us to rapidly compare samples from multiple species and locations but does not provide information on taxon identity. Sequencing-based technologies in combination with reference databases for taxon identification are an ideal next step. This could help identify the core microbes of different species, their relationship to the host and response to environmental factors. We focused on bacterial microbes which are thought to comprise the greatest fraction of organisms in the guts of many insect (Engel & Moran, 2013), but further studies could expand the range to other potential interaction partners, like fungi, protists, and archaea. Overall, our study illustrates the importance of both resource and landscape-based influences on gut microbiota and their interactions with species-specific traits including foraging behavior and physiology.

2.6 Acknowledgements

We thank the landowners who allowed us to sample lady beetles on their land and the field assistants who helped to collect samples (C. Bergstrom, C. Fritz, A. Rudie, C. Schwantes, and A. Wenninger). We also thank C. Mason and L. Beversdorf for help with sample preparation and analysis and A. Douglas and B. Spiesman for helpful comments on an earlier version of the manuscript. This research was funded by USDA grant 2011-67009-30022 to CG. We acknowledge support by Open Access Publication Fund of University of Muenster.

2.7 Data accessibility

The data and R scripts used for data analysis are provided in the in Appendices S2, S3, and S4.

2.8 Supporting Information

The following supporting information is available for this article online http://onlinelibrary.wiley.com/doi/10.1002/ece3.3390/full

S1 Additional Figures and tables. ece33390-sup-0001-AppendixS1.docx

S2 R Script used for statistical analyses. ece33390-sup-0002-AppendixS2.docx

S3 Data lab experiment. ece33390-sup-0003-AppendixS3.csv

S4 Data field study. ece33390-sup-0004-AppendixS4.txt

S1 Additional Figures and tables

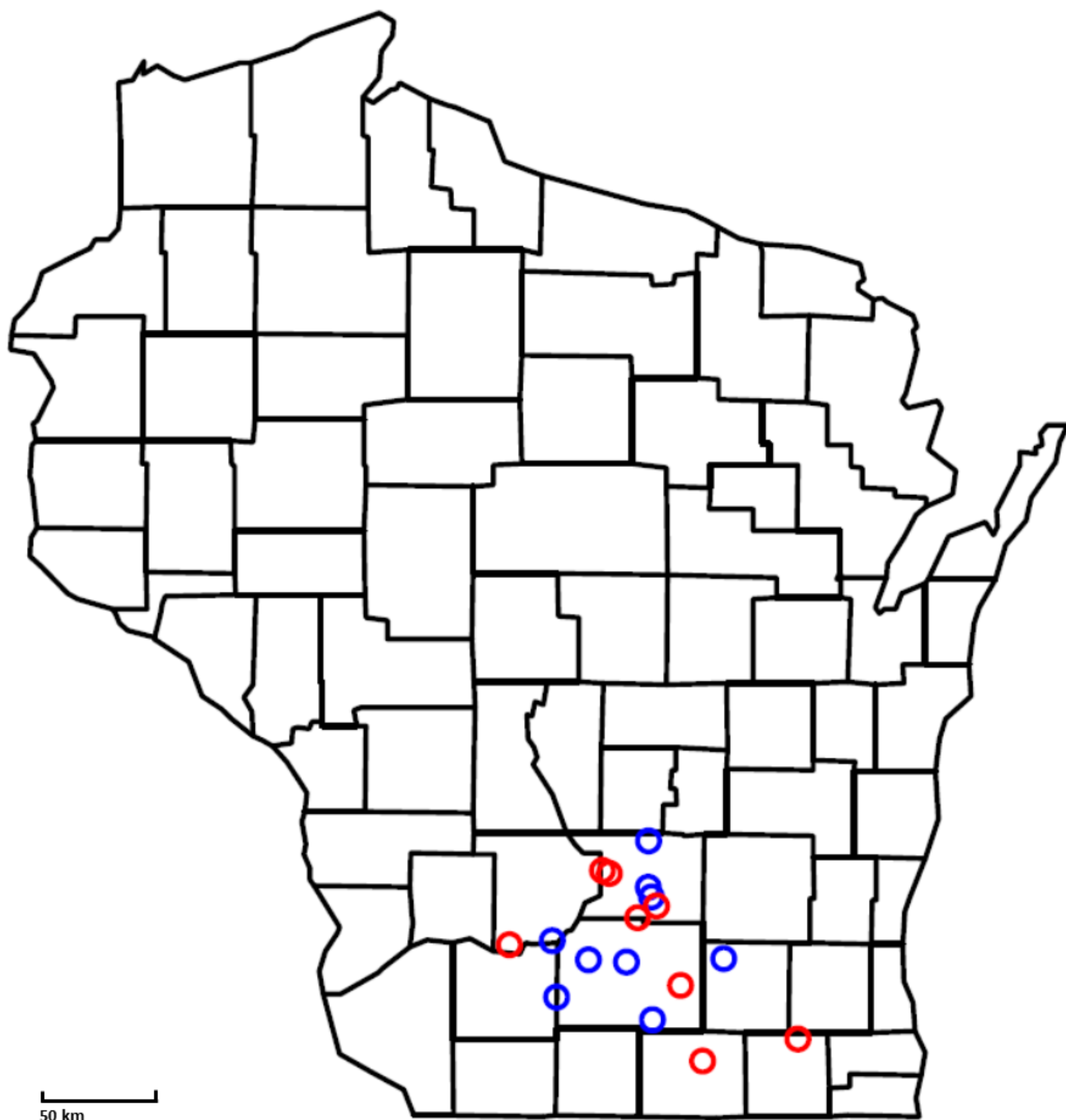

Figure S1. Map of sampling locations. Locations of soybean fields (red points) and prairies (blue points) around Madison, Wisconsin, USA, in which the lady beetle specimen included in our study were collected between July and August 2012.

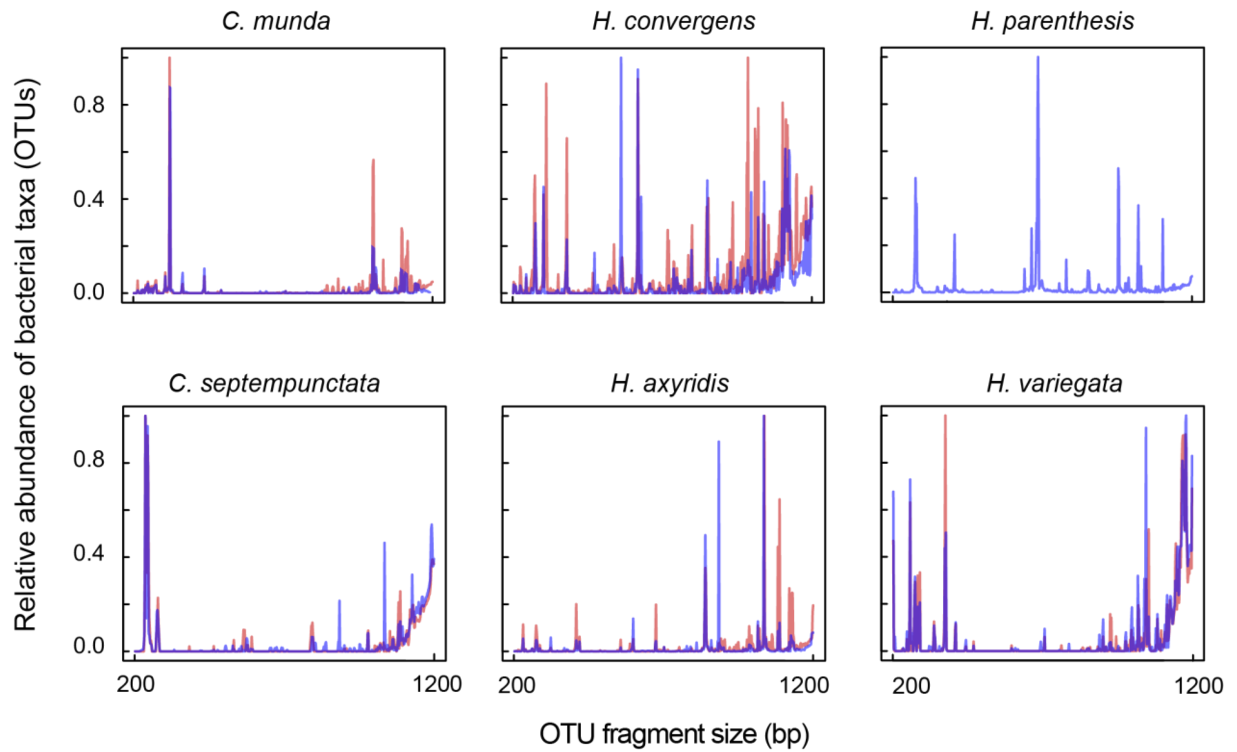

Figure S2. Effect of beetle species and field type on relative abundance of bacterial taxa. Mean relative abundance of bacterial taxa represented by operational taxonomic units (OTUs) of different size (base pairs) in different species of lady beetles. The blue and red lines represent the bacterial community in beetles from prairie and soy, respectively.

Table S3. Field study results for effects on gut bacterial richness. Parameter estimates and standard errors from the linear mixed model (corresponding to Table 2) on the effect of species contrasts (native vs exotic origin, small vs big size; *Hippodamia* vs other genera), field type, and proportion cropland in 2 km on log-transformed richness of gut bacterial taxa. The model included beetle sex within species within collection site as random effect (n = 243 beetles, n = 17 sites, n = 54 beetles within sites, n = 84 sex within species within sites). The estimated variances of the random effects were essentially 0, with a residual variance of $(0.26)^2$. Variance heterogeneity between species was accounted for by introducing a variance function with different variances estimated for each species (*C. munda* = 1, *H. convergens* = 1.053, *H. axyridis* = 1.002, *H. parenthesis* = 1.467, *C. septempunctata* = 0.903, and *H. variegata* = 0.778). *P*-values <0.05 are reported in bold numbers, while *P* <0.10 are in italics.

Term	Value	SS	*df*	*t* value	*p* value
(Intercept)	4.369	0.035	159	124.609	**<0.001**
Species-origin	-0.044	0.043	31	-1.039	0.307
Species-genus	0.018	0.029	31	0.623	0.538
Species-size	0.084	0.032	31	2.579	**0.015**
Field type-soy	0.094	0.027	14	3.495	**0.004**
Proportion crop	0.064	0.080	14	0.803	0.435
Species-origin x proportion crop	-0.304	0.088	31	-3.469	**0.002**
Species-genus x proportion crop	0.036	0.064	31	0.566	0.576
Species-size x proportion crop	-0.122	0.064	31	-1.891	*0.068*

SS= Sums of squares
df = Degrees of freedom

Table S4. Field study results for effects on beetle fat content. Parameter estimates and standard errors from the cumulative link mixed model (corresponding to Table 3) on the effect of beetle species contrasts, log-transformed bacterial richness, field type, and proportion cropland on fat content of lady beetles. The model included beetle sex within species within collection site as random effect (n = 242 beetles, n = 17 sites, n = 54 species within sites, n= 84 sex within beetles within sites). The estimated variances of the random effects were 0.601 for sex within species within site, 0 for species within site, and 0.510 for site. Model selection was based on stepwise deletion of predictors based on AICc. *P*-values <0.05 are reported in bold numbers, while *P* <0.10 are in italics.

Term	Estimate	*SE*	*z* value	*p* value
Low|medium	-14.344	7.526	-1.906	
Medium|low	-12.122	7.497	-1.617	
Bacterial richness (log)	-3.122	1.714	-1.821	*0.069*
Species-origin	5.512	4.538	1.215	0.225
Species-genus	6.280	4.205	1.494	0.135
Species-size	-3.778	4.512	-0.837	0.402
Field type-soy	0.810	1.384	0.585	0.559
Proportion crop	-28.426	15.685	-1.812	*0.070*
Bacterial richness (log) x Species-origin	-1.264	1.011	-1.250	0.211
Bacterial richness (log) x Species-genus	-1.232	0.971	-1.268	0.205
Bacterial richness (log) x Species-size	0.895	1.027	0.872	0.383
Bacterial richness (log) x Proportion crop	7.322	3.628	2.018	**0.044**
Proportion crop x Field type-soy	-5.413	2.940	-1.841	*0.066*

SE = Standard errors

Table S5. Field study results for effects on beetle fat content - Likelihood-ratio tests from CLMM model including sex as fixed term. Likelihood-ratio tests from cumulative link mixed model results on the effect of beetle species contrasts, log-transformed bacterial richness, field type, and proportion cropland on beetle fat content. *P*-values <0.05 are reported in bold numbers. Details on parameter estimates and standard errors reported in Table S6.

Term	*df*	χ^2	*p* value
Bacterial richness (log)	1	0.58	0.448
Species	3	13.25	**0.004**
Field type	1	4.45	**0.035**
Proportion cropland	1	0.17	0.680
Bacterial richness (log) x species	3	12.78	**0.005**
Bacterial richness (log) x proportion crop	1	5.32	**0.021**
Field type x proportion crop	1	3.95	**0.047**
Species x sex	3	10.51	**0.015**
Proportion cropland x sex	1	5.18	**0.023**

df = Degrees of freedom

Table S6. Field study results for effects on beetle fat content - Parameter estimates from clmm model including sex as fixed term. Parameter estimates and standard errors from the cumulative link mixed model (corresponding to Table S5) on the effect of beetle species log-transformed bacterial richness, field type, and proportion cropland on fat content of lady beetles. The model included beetle species within collection site as random effect (n = 242 beetles, n = 17 sites, n = 54 species within sites, n= 84 sex within species within site). The estimated variances of the random effects were essentially 0. Model selection was based on stepwise deletion of predictors based on AICc. *P*-values <0.05 are reported in bold numbers, while *P* <0.10 are in italics.

Term	Estimate	*SE*	*z* value	*p* value
Low|medium	-16.102	7.755	-2.076	
Medium|low	-13.802	7.724	-1.787	
Bacterial richness (log)	-3.554	1.770	-2.008	**0.045**
Species-origin	4.973	4.576	1.087	0.277
Species-genus	7.568	4.310	1.756	*0.079*
Species-size	-4.829	4.550	-1.061	0.288
Field type-soy	1.237	1.456	0.849	0.396
Proportion crop	-32.140	16.360	-1.965	**0.049**
Bacterial richness (log) x Species-origin	-1.212	1.018	-1.190	0.234
Bacterial richness (log) x Species-genus	-1.595	0.997	-1.599	0.110
Bacterial richness (log) x Species-size	1.069	1.036	1.032	0.302
Bacterial richness (log) x Proportion crop	8.568	3.802	2.254	**0.024**
Proportion crop x Field type-soy	-6.581	3.088	-2.131	**0.033**
Proportion crop x Sex-male	-2.457	0.742	-3.313	**0.001**
Species-origin x Sex-male	0.297	0.392	0.758	0.449
Species-genus x Sex-male	0.951	0.356	2.667	**0.008**
Species-size x Sex-male	0.642	0.364	1.763	*0.078*

SE = Standard errors

CHAPTER 3

Trophic and non-trophic interactions in a biodiversity experiment assessed by next-generation sequencing

Julia Tiede, Bernd Wemheuer, Michael Traugott, Rolf Daniel, Teja Tscharntke, Anne Ebeling, Christoph Scherber

Published in PLoS ONE 11(2): e0148781. doi:10.1371/journal.pone.0148781 © 2016 Tiede et al.

Abstract

Plant diversity affects species richness and abundance of taxa at higher trophic levels. However, plant diversity effects on omnivores (feeding on multiple trophic levels) and their trophic and non-trophic interactions are not yet studied because appropriate methods were lacking. A promising approach is the DNA-based analysis of gut contents using next generation sequencing (NGS) technologies. Here, we integrate NGS-based analysis into the framework of a biodiversity experiment where plant taxonomic and functional diversity were manipulated to directly assess environmental interactions involving the omnivorous ground beetle *Pterostichus melanarius*. Beetle regurgitates were used for NGS-based analysis with universal 18S rDNA primers for eukaryotes. We detected a wide range of taxa with the NGS approach in regurgitates, including organisms representing trophic, phoretic, parasitic, and neutral interactions with *P. melanarius*. Our findings suggest that the frequency of (i) trophic interactions increased with plant diversity and vegetation cover; (ii) intraguild predation increased with vegetation cover, and (iii) neutral interactions with organisms such as fungi and protists increased with vegetation cover. Experimentally manipulated plant diversity likely affects multitrophic interactions involving omnivorous consumers. Our study therefore shows that trophic and non-trophic interactions can be assessed via NGS to address fundamental questions in biodiversity research.

3.1 Introduction

Biodiversity in terrestrial ecosystems is declining due to intensified land use and other human-driven environmental changes (Sala et al. 2000; Hooper et al. 2012; Newbold et al. 2015). How such a decline in diversity affects ecosystem functioning is studied most often for plant diversity loss, including both natural systems (Grace et al. 2014) and controlled experiments with manipulated plant communities (e.g. Hooper et al. 2012). For decades, plant diversity experiments have focused on productivity (Hooper et al. 2005; 2012), while more recent research investigates how the diversity of primary producers affects higher trophic levels (Cardinale et al. 2006; Barnes et al. 2014). These studies show that plant species richness has cascading, bottom-up effects on abundance and species richness of higher trophic levels (Knops et al. 1999; Haddad et al. 2009; Scherber et al. 2010; Ebeling et al. 2014). However, the assignment of organisms to trophic groups (such as herbivores, carnivores, or omnivores) is so far mostly based on literature data (Bohan et al. 2016), combined with information on morphology and ecology (Gibb et al. 2015). In addition, it is difficult to relate organism abundances to process rates such as herbivory or predation, because a species may not consume food proportional to its abundance (Wimp et al. 2012; Davey et al. 2013; Wallinger et al. 2014). A further complication arises if consumers are omnivores that feed at more than one trophic level. While omnivores are abundant in many systems (Wolkovich et al. 2014), their responses to plant diversity remain elusive.

A promising approach to directly assess trophic interactions is the DNA-based detection of food remains in gut contents, which is widely used to study trophic interactions in various ecosystems (Pompanon et al. 2012; Symondson et al. 2012; Traugott et al. 2013; Lundgren et al. 2014; Symondson & Harwood 2014). Sequence-based identification of food DNA using next

generation sequencing (NGS), combined with universal primers for common barcoding regions, allows simultaneous detection of feeding events from a wide range of potential interaction partners (Valentini et al. 2009; Clare 2014; Pompanon & Samadi 2015). In addition to food items, NGS-based methods often co-sequence DNA of other organisms encountered in the environment (Symondson et al. 2012). Information on interactions involving these organisms is usually discarded in dietary studies (O´Rorke et al. 2012; Clare et al. 2014), but may indicate non-trophic interactions, such as commensalism or neutralism that are often completely ignored in ecological networks (Kéfi et al. 2015). This approach, albeit ideally suited to empirically assess interactions in biodiverse communities, has not yet been applied to study the effects of plant diversity on trophic and non-trophic processes.

Here, we use the framework of a grassland biodiversity experiment to test the potential of NGS for the direct and simultaneous assessment of trophic and non-trophic interactions and analyze how these interactions are affected by plant biodiversity. We use the omnivorous ground beetle *Pterostichus melanarius* Illiger (Coleoptera; Carabidae) as a model species, as it is geographically widespread, locally abundant and present in many natural and agricultural ecosystems. *Pterostichus melanarius* primarily feeds on a wide range of invertebrates from various trophic levels but its diet also includes plant material (Thiele 1977; Hengeveld et al. 1979; Lovei et al. 1996). Furthermore, *P. melanarius* regurgitates its gut content in response to mechanical or thermal stress, allowing non-invasive and non-lethal collection of gut contents (Waldner & Traugott 2012). Another advantage of using regurgitates instead of whole body DNA extracts of beetles is that they may be ideally suited for sequence-based identification of ingested organisms using universal primers without the need to include blocking primers because only little DNA of the consumer should be present in this sample type (Raso et al. 2014). Blocking primers are the most commonly used approach to overcome the problem that universal primers, which also amplify consumer DNA, primarily generate amplicons of the consumer that limit the detection of less abundant and/or highly digested DNA of food remains (O´Rorke et al. 2012). Blocking primers are consumer-specific oligonucleotides that inhibit the amplification of specific DNA sequences (Vestheim & Jarman 2008). In addition to consumer DNA, however, blocking primers can co-block related non-target species (Piñol et al. 2013) and testing the specificity of blocking primers is often impractical in field studies with many, also unknown, prey species. An alternative approach is to compensate for consumer co-amplification by increasing sequencing depth (Piñol et al. 2013, 2014). However, if regurgitates are used, blocking primers might not be necessary because regurgitates may contain much less consumer DNA. Regurgitates of invertebrates are successfully used in combination with prey-specific primers (Waldner & Traugott 2012; Raso et al. 2014) but their potential for NGS-based diet analysis with universal primers is not yet tested.

The aim of this study is to assess the potential of NGS-based gut content-analysis to study multitrophic interactions in response to changes in biodiversity. Within the framework of a plant diversity experiment, we test if regurgitates of an abundant omnivore can be analysed with NGS by applying universal primers without blocking primers. By simultaneously analysing trophic and non-trophic interactions, we exploit the full potential of NGS to assess the impact of biodiversity on interspecific interactions.

3.2 Material and Methods

3.2.1 Ethics statement

Arthropod sampling was conducted with the permission of the city council of Jena, Germany.

3.2.2 Study site

This study was conducted within the framework of a grassland biodiversity experiment (The Jena Experiment; Thuringia, Germany, 50°950 N, 11°630 E, 130 m above sea level) (Roscher et al. 2004) in experimental plots of the Trait-Based Diversity Experiment (TBE; (Ebeling et al. 2014). The species pool in the TBE consists of 20 Central European grass and non-legume herbaceous species. Plant communities were manipulated to cover a gradient of plant species richness (1, 2, 3, 4, and 8) and plant functional diversity (1, 2, 3, and 4) on 138 plots (3.5 m x 3.5 m). The gradient of plant functional diversity was based on plant traits known to be important for spatial and temporal resource use such as plant height, rooting depth, or phenology, and represents the levels from low (1) to high (4) trait complementarity in the plant community (Ebeling et al. 2014). The experimental plots were maintained by biannual mowing and weeded three times per year to remove unwanted species. In addition to the experimentally manipulated variables (plant species richness and plant functional diversity), we visually estimated vegetation cover (in percent) in mid-August 2013. For logistic reasons only a subset of the 138 plots was used for this study. 33 plots were selected at random: including 10 monocultures, five two-species mixtures, five three-species mixtures, ten four-species mixtures, and all three eight-species mixtures. Thus, our sampling design had more replicates at low (1) and high (4,8) plant species richness, which minimizes the standard error of the slope in subsequent statistical analyses (Draper & Smith 1998). Plant biomass data from the previous year was used to show that the 33 plots selected did not introduce a systematic bias compared to the full 138 plots. Every plot was fenced with an enclosure for a period of two weeks in August 2013 to prevent inter-plot movement of *P. melanarius* and other ground-dwelling organisms. For the enclosures, transparent construction foil (PE, 20 µ, Rajapack, Ettlingen) was wrapped around the four corner poles of each plot (~50 cm height) and sunk into the soil using PVC panels (~15 cm depth) (Fig 1A and 1B).

3.2.3 Study organism

Adult *P. melanarius* were collected in the weeks preceding the experiment using dry pitfall traps at different locations around Göttingen (Germany; 51°550 N, 9°950 E) in July 2013 as well as in the surrounding grass margins of the Jena Experiment in August 2013. Beetles were kept in plastic containers on a substrate of moist clay pebbles in a dark room at 18°C and maintained on cat food (K-Classic Adult, Kaufland AG, Germany) but starved 48 h before the experiment. On August 15, five beetles were released per plot; each beetle was marked with an individual pattern of colored dots on its elytra (Fig 1C). After allowing the released beetles to acclimatise to the plot conditions for four days, we repeatedly recaptured them over a period of 10 days in one central dry pitfall trap (4.5 cm diameter). The traps were filled with clay pebbles and emptied in the morning and evening to minimize within-trap predation events. If remains of

other organisms were found in a trap, all beetles caught in this trap were excluded from analyses. To sample the beetles' gut contents, we placed them individually headfirst in 1.5 mL reaction tubes and exposed the tubes for a few seconds to hot water (~60°C) to induce regurgitation (Fig 1D). Regurgitates were immediately frozen at -18°C and subsequently stored at -80°C. Afterwards, the beetles were released on the original plot. We were not able to recapture beetles from all plots because only one trap per plot was used for a comparatively short recapture period of 10 days, due to other experiments conducted on the same plots. Additionally, some of the beetles failed to regurgitate or the amount of stomach content was too low for analysis. Several other samples dropped out during the analysis process, so that our final dataset represented 13 plots.

Fig 1. Setup of field experiment and regurgitate sampling. (A) Overview of plots of the Trait-Based Experiment with enclosures. (B) Enclosures were made of construction foil sunk into the soil using PVC panels. (C) Marked beetles were released and recaptured to sample regurgitates (D) sampling regurgitates. Photographs by J. Tiede.

3.2.4 DNA extraction

Total DNA was extracted from regurgitates in a molecular diagnostic laboratory at the Institute of Ecology, University of Innsbruck, Austria. Each regurgitate sample was mixed with 200 µL lysis buffer containing 5 µL Proteinase K (10 mg/mL, AppliChem, Darmstadt, Germany) and TES-buffer (0.1 M TRIS, 10 mM EDTA, 2% SDS, pH 8) and was incubated at 56°C for 3 h. The DNA was extracted from the lysate on a BioSprint 96 robotic DNA extraction platform using the MagAttract DNA Blood M96 Kit (Qiagen, Hilden, Germany). Four negative extraction controls (DNA extraction blanks) were included to monitor for carry-over DNA contamination during the extraction process and were subsequently tested in PCR reactions for NGS.

3.2.5 Next generation sequencing and sequence processing

Next generation sequencing of regurgitates was conducted at the Department of Genomic and Applied Microbiology (University of Goettingen, Germany). To analyse a broad spectrum of ingested organisms from the regurgitates of *P. melanarius* without a priori decisions on focal groups, we used universal primers amplifying a ~600 bp region of the eukaryotic 18S rDNA gene: F515 (5'GTGCCAGCMGCCGCGGTAA-3') and R1119 (5'-GGTGCCCTTCCGTCA-3') (Bates et al. 2012). Taxon coverage of the primer pair was previously tested in silico using Primer Prospector (Walters et al. 2011) and reference sequences derived from the SILVA database Quast et al. 2012). The primers included a Roche 454 pyrosequencing adaptor, a library key sequence, and a multiplex identifier (MID). Each 50 µL PCR reaction contained 10 µL of 5x Phusion GC buffer (Finnzymes, Vantaa, Finland), 0.2 mM of each dNTP, 4 µM of each primer, 1.5 µL dimethyl sulfoxide (DMSO), 1 U Phusion Hot Start DNA polymerase (Finnzymes), 1 µL template DNA, and 32 µL diethylpyrocarbonate (DEPC) water. The thermocycling protocol was 98°C for 30 s, 35 cycles of 98°C for 10 s, 60°C for 20 s, 72°C for 20 s, and 72°C for 5 min once. One template-free control was included in every PCR run. Samples that showed PCR products on agarose gel were amplified in three technical replicates, purified with the peqGOLD Gel Extraction Kit (Peqlab, Erlangen, Germany) and pooled at equal DNA concentrations. DNA concentration was quantified using a Qubit fluorometer (Invitrogen, Carlsbad, USA) with the Quant-iT dsDNA HS assay kit; 20 regurgitates with a sufficient DNA concentration (≥ 2 ng μl^{-1}) were sequenced.

The sequencing was carried out on a GS-FLX+ 454 pyrosequencer using Titanium chemistry (Roche, Branford, CT), with a targeted surveying effort of 5,000 reads per sample. Short reads (<200 bp), and low quality reads (homopolymer stretches >8 bp; primer mismatches >5 bp) were removed using QIIME v1.6 (Caprosa et al. 2010). The sequences were denoised using Acacia v1.52 (Bragg et al. 2012) and cutadapt was used to truncate remaining primer sequences (Martin 2011). Chimeric sequences were removed using UCHIME (Edgar et al. 2010) in reference mode with SILVA (SSURef 119 NR database as reference data set (Quast et al. 2012)). Using the UCLUST algorithm (Edgar 2010), the remaining sequences were clustered in operational taxonomic units (OTUs) at 99% genetic similarity. The consensus sequences were calculated using USEARCH (v. 7.0.1090). OTUs were subsequently classified by blast alignment against the SILVA database (Camacho et al. 2009). The taxonomy of the best hit was assigned to the respective OTU. DNA sequences were deposited in the Sequence Read Archive (SRA) of the National Center for Biotechnology Information under accession SRA282133.

3.2.6 Data processing

Two samples were excluded because of low numbers of total sequences or high numbers of consumer (*P. melanarius*) sequences. For the analysis of taxa composition in the remaining 18 regurgitate samples, we removed all OTUs classified as consumer (1 OTU, 1 sample), human (1 OTU, 7 samples), vertebrate (1 OTU, 1 sample), tree species (5 OTU, 1–5 samples) and aquatic species (6 OTU, 1–2 samples). DNA of aquatic species might have originated from a flooding event in June 2013 (Wright et al. 2015), and tree DNA likely originated from pollen of trees growing nearby in northern and eastern direction. Human and vertebrate DNA (squirrel) likely

represented contaminations. In addition, we excluded OTUs that could not be classified to order level (4 OTUs, 1–4 samples), singletons and doubletons (46 OTU, 1–4 samples) from the analysis. A complete list of removed OTUs is provided in S1 Table.

For the analysis of interaction types, all remaining OTUs were aggregated at genus level and grouped based on literature information on their most likely interaction with *P. melanarius* (Table 1). We differentiated between trophic interactions that are beneficial (+) to *P. melanarius* but negative (-) for the interaction partner, and non-trophic interactions that are neutral (0) or negative for the beetle and beneficial or neutral for the interaction partner.

Trophic interactions (+/-):

- Total feeding interactions: all organisms that were likely actively consumed by *P. melanarius*
- Plant derived food: higher plant taxa
- Prey: all animal taxa except phoretic mites
- Intraguild predation: prey with predatory or omnivorous nutrition

Non-trophic interactions:

- Parasitism (-/+): organisms that presumably parasitize *P. melanarius*
- Phoresy (0/+): mites that use insects as phoretic carriers and whose DNA could either originate from mites or mite remains that have fallen off during sampling
- Neutralism (0/0): organisms without known interaction with *P. melanarius* that were likely passively consumed together with food

For the analysis of plant diversity effects on taxa detection in regurgitates, the number of OTUs in each group was calculated for each sample (S1 R-Script, S1 and S2 Data). Four plots were represented by two or three samples. For these, the number of taxa and the number of sequences per group were averaged and rounded to the smallest following integer (ceiling function). The resulting 13 independent data points represented 13 plots, including three monocultures, two two-species mixtures, three three-species mixtures, three four-species mixtures, and two eight-species mixtures.

3.2.7 Statistical analysis

Data were analysed using R (version 3.1.2, R Development Core Team, 2014). We used generalized linear models (GLM) with negative binomial or quasipoisson errors to analyse the effects of the explanatory variables on the richness of OTUs for each group. Models included either plant species richness, functional diversity, or vegetation cover as explanatory variables, as these variables were colinear when entered together in single models; this resulted in a total of three individual models per OTU group. To account for potential effects of the number of sequences per OTU, we additionally ran quasipoisson models with number of sequences per OTU as known prior weights, giving more weight to samples with a high

number of sequences. Note that the number of sequences cannot be used as a measure of consumed biomass as it is affected by the time since consumption and characteristics of the prey tissue that affect digestion time (O´Rorke et al. 2012; Deagle et al. 2010; Deagle et al. 2013; Piñol et al. 2014).

3.3 Results

With NGS, we found a total of 90 OTUs in regurgitates of *P. melanarius*, covering a range of five kingdoms within the Eukaryotes (Ruggerio et al. 2015): Animalia, Chromista, Fungi, Plantae, and Protozoa. 77 OTUs were assigned to family level, covering 73 different families, and 67 to genus level, covering 63 different genera (Table 1).

3.3.1 Detection of trophic and non-trophic interactions with NGS

Of these 90 OTUs, 24 were categorized as feeding interactions, comprising 12 plant and 12 animal taxa. Four of the identified plant taxa were locally present as part of the Trait-Based Experiment: the genera *Plantago* (Lamiales), *Ranunculus* (Ranunculales), and *Rumex* (Caryophyllales), and the family Poaceae (Poales). Other plant taxa, such as the stinging nettle *Urtica* (Rosales), were locally present in the vegetation matrix surrounding the plots and were occasional weeds in the experimental plots.

Animal prey detected using NGS included herbivores and detritivores, such as gastropods (Stylommatophora: *Deroceras*, and *Xerolenta*), mites (Trombidiformes: Microtrombidium; Sarcoptiformes: Glcyyphagidae, and *Orbitulata*), grasshoppers (Orthoptera: Gomphocerus), and earthworms (Haplotaxida: Hormogastridae). In addition, we detected other predator taxa: DNA of another ground beetle (Coleoptera: *Bembidion*) was found in four plots, a predatory mite (Trombidiformes: Trombiculidae) in three plots, an earwig (Dermaptera: Forficulidae), and two spider taxa (jumping spiders; Araneae: Salticidae, and a huntsman spider; Sparassidae, likely *Micrommata virescens*).

In addition to feeding interactions, we detected organisms that likely interacted negatively (parasites) or neutrally (commensalism, neutralism) with *P. melanarius* (Table 1). Two organisms that were presumably parasites of *P. melanarius* were present in samples from five plots: an entomopathogenic fungus (Ascomycota: Hypocreales: Isaria sp.) known to infect carabid beetles (Draganova et al. 2010), and a group of parasitic protists (Apicomplexa: Eugregarinida) that frequently infects *P. melanarius* (Sienkiewicz & Lipa 2009). DNA of phoretic mites was found in regurgitates from 11 plots, with the family Histiostomatidae (Acariformes) represented eight times and the family Acaridae, genus *Histiogaster* sp. (Acariformes), found three times. None of the plots contained both families together. Most OTUs (N = 61) detected in the regurgitates of P. melanarius represented neutral interaction partners with no specific relation to the beetle (passive consumption, environmental DNA). Most of these organisms were fungi (N = 45), and protists (Amoebozoa, and SAR, N = 13), but we also detected terrestrial algae (N = 3).

3.3.2 Effects of plant biodiversity and vegetation cover on species interactions

Plant diversity affected the total number of feeding interactions and the taxon richness in all food groups including plant-derived food, animal total prey and intraguild prey (Table 1; Fig 2A–2D): the total number of feeding interactions was significantly positively affected by plant species richness and positively but not significantly by functional diversity and vegetation cover. The number of plant taxa detected in the regurgitates increased with the number of sown plant species in the plot. The total number of total prey species increased with plant species richness and vegetation cover, intraguild predation was only affected by vegetation cover. The occurrence of parasitic and phoretic interactions was not significantly related to any of the explanatory variables (Table 2). The richness of neutral interactions was not affected by plant species richness or functional diversity but increased with percentage vegetation cover (Table 2; Fig 2E). In weighted models, all effects from unweighted models remained significant. Additionally, marginal effects became significant.

Since the identity of OTUs was ignored in the aggregated data analysis, we show in Fig 3 how abundant individual families from the three kingdoms Animalia, Plantae, and Fungi respond to plant species richness and plant functional diversity.

Table 1. Organisms detected with NGS in regurgitates of *P. melanarius*, sorted by their most likely type of interaction with the beetle.

Interaction type	Kingdom	Phylum	Class	Order	Family	Genus	Nutrition, metabolism	N
Trophic (feeding, +/-)	Plantae	Tracheophyta	Magnoliopsida	Asterales	Asteraceae	*Artemisia*	autotrophic	1
	Plantae	Tracheophyta	Magnoliopsida	Caryophyllales	Polygonaceae	*Rumex*	autotrophic	4
	Plantae	Tracheophyta	Magnoliopsida	Dipsacales	Caprifoliaceae	*Triplostegia*	autotrophic	3
	Plantae	Tracheophyta	Magnoliopsida	Fabales	Fabaceae	n/a	autotrophic	3
	Plantae	Tracheophyta	Magnoliopsida	Gentianales	Rubiaceae*	*Guettarda**	autotrophic	1
	Plantae	Tracheophyta	Magnoliopsida	Lamiales	Plantaginaceae	*Plantago*	autotrophic	2
	Plantae	Tracheophyta	Magnoliopsida	Poales	Poaceae	*Triticum*	autotrophic	3
	Plantae	Tracheophyta	Magnoliopsida	Poales	Restionaceae	n/a	autotrophic	1
	Plantae	Tracheophyta	Magnoliopsida	Ranunculales	Ranunculaceae	*Ranunculus*	autotrophic	1
	Plantae	Tracheophyta	Magnoliopsida	Rosales	Rosaceae	*Prunus*	autotrophic	3
	Plantae	Tracheophyta	Magnoliopsida	Rosales	Urticaceae	*Urtica*	autotrophic	8
	Plantae	Tracheophyta	Magnoliopsida	Rosales	n/a	n/a	autotrophic	4
	Animalia	Annelida	Clitellata	Haplotaxida	Hormogastridae*	*Hormogaster**	detrivorous	1
	Animalia	Arthropoda	Arachnida	Araneae	Salticidae	*Goleba**	predatory	1
	Animalia	Arthropoda	Arachnida	Araneae	Sparassidae	*Micrommata*[+]	predatory	1
	Animalia	Arthropoda	Arachnida	Sarcoptiformes	Glycyphagidae	*Alabidopus**	fungivorous	1
	Animalia	Arthropoda	Arachnida	Sarcoptiformes	Oribatulidae	*Oribatula*	detrivorous	1
	Animalia	Arthropoda	Arachnida	Trombidiformes	Microtrombidiidae	*Microtrombidium*	parasitic on vertebrates	1
	Animalia	Arthropoda	Arachnida	Trombidiformes	Trombiculidae	n/a	predatory	3
	Animalia	Arthropoda	Insecta	Coleoptera	Carabidae	*Bembidion*	predatory	4
	Animalia	Arthropoda	Insecta	Dermaptera	Forficulidae	n/a	detrivorous	1
	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	*Gomphocerus*	herbivorous	1
	Animalia	Mollusca	Gastropoda	Stylommatophora	Agriolimacidae	*Deroceras*	herbivorous	2
	Animalia	Mollusca	Gastropoda	Stylommatophora	Hygromiidae	*Helicella*	herbivorous	1

Interaction type	Kingdom	Phylum	Class	Order	Family	Genus	Nutrition, metabolism	N
Parasitism (-/+)	Chromista	Miozoa	Conoidasida	Eugregarinorida	n/a	n/a	parasitic on insects	2
	Fungi	Ascomycota	Sordariomycetes	Hypocreales	Cordycipitaceae	*Isaria*	entomopathogenic	3
Phoresy (0/+)	Animalia	Arthropoda	Arachnida	Sarcoptiformes	Acaridae	*Histiogaster*	bacterivorous	3
	Animalia	Arthropoda	Arachnida	Sarcoptiformes	Histiostomatidae	*Anoetus*	bacterivorous	1
	Animalia	Arthropoda	Arachnida	Sarcoptiformes	Histiostomatidae	n/a	bacterivorous	7
Neutralism (0/0)	Chromista	Cercozoa	Gromiidea	Reticulosida	Gymnophryidae	*Gymnophrys*	omnivorous	2
	Chromista	Cercozoa	Sarcomonadea	Cercomonadida	Heteromitidae	*Heteromita*	bacterivorous	4
	Chromista	Cercozoa	Sarcomonadea	Cercomonadida	n/a	*Cercomonas*	bacterivorous	3
	Chromista	Cercozoa	Sarcomonadea	Glissomonadida	Bodomorphidae	*Bodomorpha*	bacterivorous	1
	Chromista	Cercozoa	Sarcomonadea	Glissomonadida	n/a	n/a	bacterivorous	1
	Chromista	Cercozoa	Thecofilosa	Cryomonadida	Rhizaspididae	*Rhogostoma*	bacterivorous	3
	Chromista	Cercozoa	Vampyrellidea	Vampyrellida	Vampyrellidae	n/a	omnivorous	1
	Chromista	Ciliophora	Colpodea	Colpodida	Colpodidae	*Exocolpoda*	bacterivorous	1
	Chromista	Miozoa	Apicomonadea	Colpodellida	Colpodellidae	*Colpodella*	predatory on protists	1
	Chromista	Pseudofungi	Hyphochytrea	Hyphochytriida	n/a	n/a	phytopathogenic	1
	Chromista	Pseudofungi	Oomycetes	Pythiales	Pythiaceae	*Pythium*	phytopathogenic	1
	Fungi	Ascomycota	Dothideomycetes	Acrospermales	Acrospermaceae	*Acrospermum*	saprotrophic	1
	Fungi	Ascomycota	Dothideomycetes	Capnodiales	n/a	n/a	phytopathogenic	11
	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Didymellaceae	*Didymella*	phytopathogenic	1
	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Didymellaceae	*Phoma*	phytopathogenic	8
	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Phaeosphaeriaceae	*Parastagonospora*	phytopathogenic	3
	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	*Pyrenophora*	phytopathogenic	1
	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Tubeufiaceae	*Tubeufia*	saprotrophic	2
	Fungi	Ascomycota	Dothideomycetes	Pleosporales	n/a	n/a	saprotrophic	9
	Fungi	Ascomycota	Eurotiomycetes	Chaetothyriomycetidae	Herpotrichiellaceae	*Coniosporium*	n/a	1
	Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Penicillium*	saprotrophic	1
	Fungi	Ascomycota	Leotiomycetes	Helotiales	Helotiaceae	*Cudoniella*	saprotrophic	3

Interaction type	Kingdom	Phylum	Class	Order	Family	Genus	Nutrition, metabolism	N
	Fungi	Ascomycota	Leotiomycetes	Helotiales	Vibrisseaceae	*Phialocephala*	endophytic	1
	Fungi	Ascomycota	Leotiomycetes	Helotiales	n/a	n/a	n/a	2
	Fungi	Ascomycota	Pezizomycetes	Pezizales	n/a	n/a	saprotrophic	1
	Fungi	Ascomycota	Saccharomycetales	Saccharomycetales	Debaryomycetaceae	*Priceomyces*	n/a	5
	Fungi	Ascomycota	Saccharomycetales	Saccharomycetales	Dipodascaceae	*Yarrowia*	n/a	13
	Fungi	Ascomycota	Saccharomycetales	Saccharomycetales	Hanseniaspora	n/a	n/a	1
	Fungi	Ascomycota	Saccharomycetales	Saccharomycetales	Saccharomycetaceae	*Candida*	n/a	1
	Fungi	Ascomycota	Sordariomycetes	Hypocreales	Fusarium	*Fusarium*	phytopathogenic	2
	Fungi	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	*Acremonium*	phytopathogenic	1
	Fungi	Ascomycota	Sordariomycetes	Hypocreales	n/a	n/a	n/a	5
	Fungi	Ascomycota	Sordariomycetes	Sordariales	Chaetosphaeriaceae	*Chaetosphaeria*	phytopathogenic	1
	Fungi	Ascomycota	Sordariomycetes	Sordariales	Sordariaceae	*Neurospora*	saprotrophic	1
	Fungi	Ascomycota	Sordariomycetes	Xylariales	Hyponectriaceae	*Microdochium*	phytopathogenic	2
Neutralism (0/0)	Fungi	Basidiomycota	Agaricomycetes	Agaricales	Bolbitiaceae	*Conocybe*	saprotrophic	1
	Fungi	Basidiomycota	Agaricomycetes	Agaricales	Marasmiaceae	*Baeospora*	saprotrophic	1
	Fungi	Basidiomycota	Agaricomycetes	Agaricales	Physalacriaceae	*Hymenopellis*	saprotrophic	1
	Fungi	Basidiomycota	Agaricomycetes	Agaricales	Tricholomataceae	*Clitocybula*	saprotrophic	1
	Fungi	Basidiomycota	Agaricomycetes	Boletales	Hygrophoropsidaceae	*Leucogyrophana*	saprotrophic	1
	Fungi	Basidiomycota	Agaricomycetes	Hymenochaetales	Tubulicrinaceae	*Hyphodontia*	saprotrophic	1
	Fungi	Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae	*Tyromyces*	saprotrophic	2
	Fungi	Basidiomycota	Exobasidiomycetes	n/a	n/a	*Tilletiopsis*	phytopathogenic	3
	Fungi	Basidiomycota	Microbotryomycetes	Heterogastridiales	Heterogastridiaceae	*Colacogloea*	saprotrophic	3
	Fungi	Basidiomycota	Microbotryomycetes	Heterogastridiales	Heterogastridiaceae	*Heterogastridium*	saprotrophic	11
	Fungi	Basidiomycota	Microbotryomycetes	Sporidiobolales	Sporidiobolaceae	*Rhodotorula*	saprotrophic	3
	Fungi	Basidiomycota	Microbotryomycetes	Sporidiobolales	Sporidiobolaceae	*Sporobolomyces*	saprotrophic	5
	Fungi	Basidiomycota	Microbotryomycetes	Sporidiobolales	n/a	n/a	n/a	1
	Fungi	Basidiomycota	n/a	Malasseziales	Malasseziaceae	*Malassezia*	animal-pathogenic	4
	Fungi	Basidiomycota	Pucciniomycetes	Pucciniales	Malasseziaceae	n/a	phytopathogenic	3

Interaction type	Kingdom	Phylum	Class	Order	Family	Genus	Nutrition, metabolism	N
Neutralism (0/0)	Fungi	Basidiomycota	Tremellomycetes	Filobasidiales	Filobasidiaceae	n/a	n/a	1
	Fungi	Basidiomycota	Tremellomycetes	Tremellales	Tremellaceae	*Cryptococcus*	animal-pathogenic	3
	Fungi	Basidiomycota	Tremellomycetes	Tremellales	Tremellaceae	*Dioszegia*	parasitic on fungi	1
	Fungi	Basidiomycota	Tremellomycetes	Tremellales	n/a	n/a	n/a	4
	Fungi	Zygomycota	n/a	Mortierellales	n/a	n/a	saprotrophic	3
	Fungi	Zygomycota	n/a	Mucorales	Mucoraceae	*Mucor*	saprotrophic	4
	Plantae	Chlorophyta	Chlorophyceae	Chlamydomonadales	Dunaliellaceae	n/a	autotrophic	3
	Plantae	Chlorophyta	Chlorophyceae	Chlamydomonadales	Haematococcaceae	n/a	autotrophic	3
	Plantae	Chlorophyta	Trebouxiophyceae	Prasiolales	Prasiolaceae	*Stichococcus*	autotrophic	2
	Protozoa	Amoebozoa	Flabellinia	Vanellida	Vanellidae	*Vanella*	bacterivorous	1
	Protozoa	Amoebozoa	Myxogastrea	Physarida	Physaridae	*Physarum*	saprotrophic	1

Nutrition and metabolism indicate the most common source of energy uptake for the taxa, with predators and omnivores referred to as intraguild predation.

"N" indicates the detection frequency. Taxonomy follows a Linnaean classification as proposed by (Ruggiero et al. 2015).

* The closest match in the SILVA database is not endemic in Thuringia, Germany. In this case we consider the next higher taxonomic level as representative.

+ Since the spider family Sparassidae is represented only by the genus *Micrommata* in the sampling region, we added this information to the list of taxa.

Table 2. Summary of generalized linear models results on the effect of plant species richness, plant functional diversity and percent vegetation cover on the number of OTUs detected in each interaction group.

Interaction group	Parameter	Estimate	SE	*Z* value	*p* value
1a) Total feeding interactions	(Intercept)	0.275	0.25	1.1	0.295
	Plant species richness	0.195	0.048	4.114	**0.002**
	(Intercept)	0.254	0.457	0.555	0.590
	Plant functional diversity	0.289	0.145	1.996	0.071
	(Intercept)	-1.206	1.298	-0.929	0.373
	Vegetation cover [%]	0.028	0.016	1.797	0.100
1b) Feeding on plant taxa	(Intercept)	0.209	0.296	0.708	0.494
	Plant species richness	0.138	0.06	2.295	**0.042**
	(Intercept)	0.435	0.46	0.946	0.365
	Plant functional diversity	0.113	0.155	0.727	0.483
	(Intercept)	-0.102	1.201	-0.085	0.934
	Vegetation cover [%]	0.011	0.015	0.711	0.492
1c) Feeding on prey taxa	(Intercept)	-1.087	0.663	-1.639	0.129
	Plant species richness	0.245	0.119	2.067	0.063
	(Intercept)	-1.681	1.041	-1.616	0.134
	Plant functional diversity	0.549	0.306	1.796	0.100
	(Intercept)	-5.989	3.045	-1.967	0.075
	Vegetation cover [%]	0.071	0.035	2.04	0.066
1d) Intraguild predation	(Intercept)	-1.379	0.681	-2.023	0.068
	Plant species richness	0.222	0.125	1.769	0.105
	(Intercept)	-2.087	1.076	-1.939	0.079
	Plant functional diversity	0.549	0.316	1.736	0.110
	(Intercept)	-6.728	2.899	-2.32	0.041
	Vegetation cover [%]	0.075	0.033	2.266	**0.045**
1e) Parasitism	(Intercept)	-1.099	0.699	-1.571	0.144
	Plant species richness	0.041	0.163	0.252	0.806
	(Intercept)	-1.063	0.902	-1.178	0.264
	Plant functional diversity	0.042	0.315	0.133	0.897
	(Intercept)	-2.562	2.607	-0.983	0.347
	Vegetation cover [%]	0.02	0.032	0.636	0.538
1f) Phoretic interaction	(Intercept)	-0.153	0.233	-0.656	0.525
	Plant species richness	-0.004	0.058	-0.073	0.943
	(Intercept)	0.122	0.282	0.434	0.672
	Plant functional diversity	-0.118	0.106	-1.114	0.289
	(Intercept)	0.171	0.682	0.25	0.807
	Vegetation cover [%]	-0.004	0.009	-0.501	0.626
1g) Neutral interaction	(Intercept)	2.013	0.27	7.456	<0.001
	Plant species richness	0.059	0.065	0.911	0.362
	(Intercept)	2.008	0.355	5.65	<0.001
	Plant functional diversity	0.082	0.125	0.662	0.508
	(Intercept)	0.345	0.82	0.421	0.674
	Vegetation cover [%]	0.023	0.01	2.325	**0.020**
2a) Total feeding interactions (weighted)	(Intercept)	0.296	0.251	1.179	0.263
	Plant species richness	0.198	0.039	5.021	**<0.001**
	(Intercept)	-0.08	0.555	-0.145	0.888
	Plant functional diversity	0.44	0.16	2.745	**0.019**
	(Intercept)	-1.084	1.68	-0.645	0.532
	Vegetation cover [%]	0.028	0.019	1.458	0.173

Interaction group	Parameter	Estimate	SE	*Z* value	*p* value
2b) Feeding on plant taxa (weighted)	(Intercept)	0.166	0.254	0.653	0.527
	Plant species richness	0.176	0.043	4.134	**0.002**
	(Intercept)	0.015	0.552	0.028	0.978
	Plant functional diversity	0.33	0.168	1.969	*0.075*
	(Intercept)	0.227	1.875	0.121	0.906
	Vegetation cover [%]	0.009	0.022	0.424	0.679
2c) Feeding on prey taxa (weighted)	(Intercept)	0.242	0.2	1.212	0.251
	Plant species richness	0.104	0.031	3.348	**0.007**
	(Intercept)	-0.112	0.362	-0.311	0.762
	Plant functional diversity	0.276	0.101	2.731	**0.020**
	(Intercept)	-1.25	0.798	-1.566	0.146
	Vegetation cover [%]	0.023	0.009	2.642	**0.023**
2d) Intraguild predation (weighted)	(Intercept)	-0.076	0.27	-0.28	0.785
	Plant species richness	0.096	0.038	2.529	**0.028**
	(Intercept)	-1.3	0.558	-2.33	0.040
	Plant functional diversity	0.489	0.143	3.416	**0.006**
	(Intercept)	-3.218	1.407	-2.287	0.043
	Vegetation cover [%]	0.041	0.015	2.706	**0.020**
2e) Parasitism (weighted)	(Intercept)	-0.006	0.169	-0.034	0.973
	Plant species richness	-0.007	0.053	-0.123	0.905
	(Intercept)	-0.191	0.335	-0.57	0.580
	Plant functional diversity	0.057	0.114	0.5	0.627
	(Intercept)	-0.126	0.686	-0.183	0.858
	Vegetation cover [%]	0.001	0.009	0.146	0.886
2f) Phoretic interactions (weighted)	(Intercept)	0.001	0.033	0.026	0.980
	Plant species richness	-0.002	0.012	-0.142	0.890
	(Intercept)	0.011	0.04	0.274	0.789
	Plant functional diversity	-0.008	0.021	-0.391	0.703
	(Intercept)	0.027	0.139	0.198	0.847
	Vegetation cover [%]	0	0.002	-0.223	0.828
2g) Neutral interactions (weighted)	(Intercept)	2.466	0.04	61.912	<2e-16
	Plant species richness	0.011	0.009	1.178	0.239
	(Intercept)	2.478	0.055	45.236	<2e-16
	Plant functional diversity	0.01	0.019	0.523	0.601
	(Intercept)	0.753	0.128	5.888	<0.001
	Vegetation cover [%]	0.021	0.001	14.068	**<2e-16**

All OTUs were assigned to interaction groups (see methods). We tested the effects of three explanatory variables on all interaction groups and compared two types of models. Models 1a-g were based on counts of interactions per plot, while models 2a-g additionally included a weights argument for the number of sequences. All models used 2 degrees of freedom and had 11 residual degrees of freedom. A quasipoisson distribution was used for all models except neutral interactions, for which negative binomial models were fitted. SE = standard error. *p* values <0.05 are reported in bold numbers.

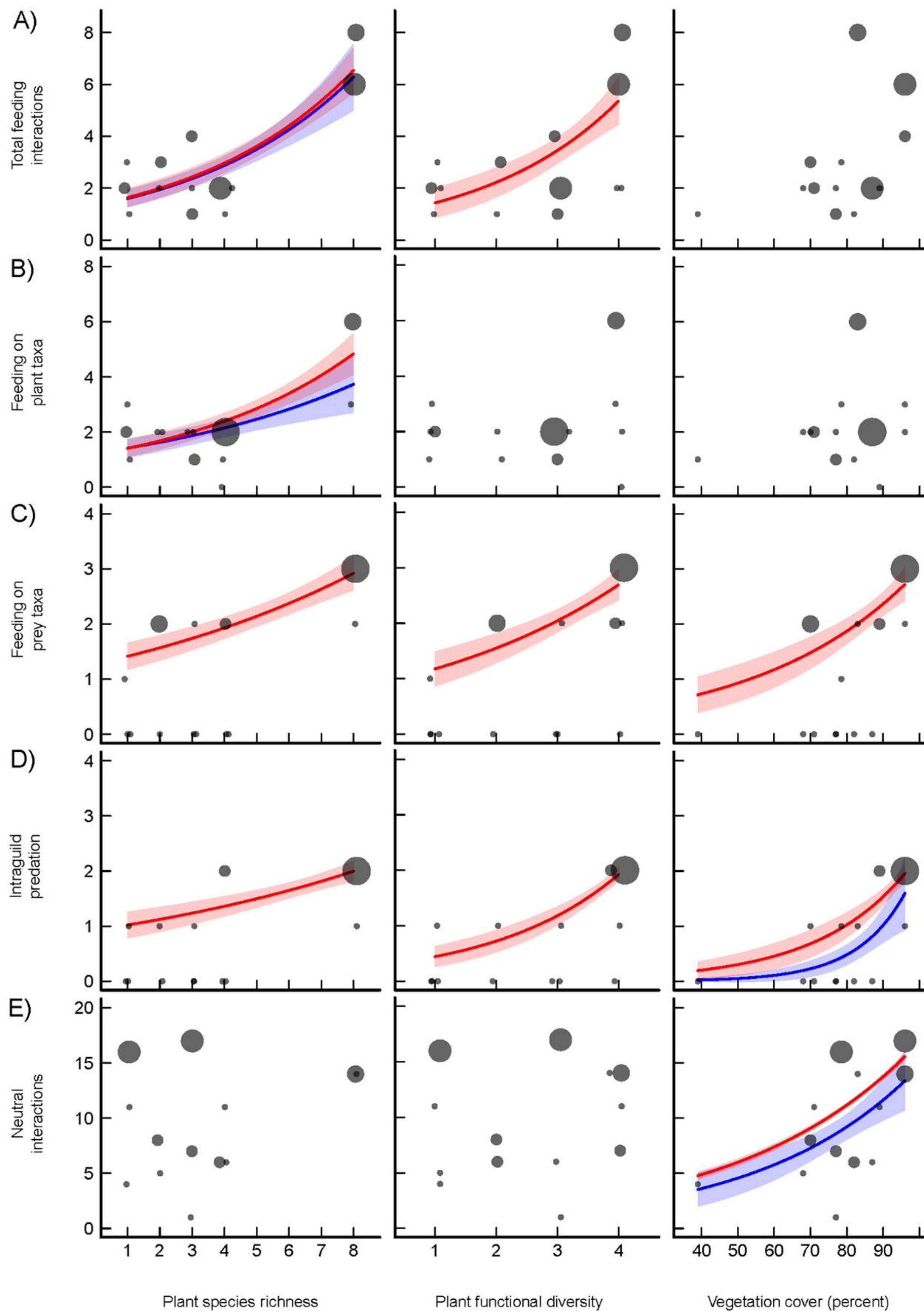

Fig 2. Effects of plant species richness, plant functional diversity, and percentage vegetation cover on feeding interactions and neutral interactions detected in regurgitates of *P. melanarius*. Points represent individual plots and are scaled based on the logarithm of the number of sequences, blue lines show GLM predictions, blue polygons show 95% confidence intervals for effects with $p<0.05$, red lines and red polygons refer to GLMs weighted by the number of sequences. A) Total number of feeding interactions including prey and plant taxa, B) feeding interactions involving plant taxa, C) feeding interactions involving total prey taxa, D) feeding interactions involving intraguild predation, and E) neutral interactions.

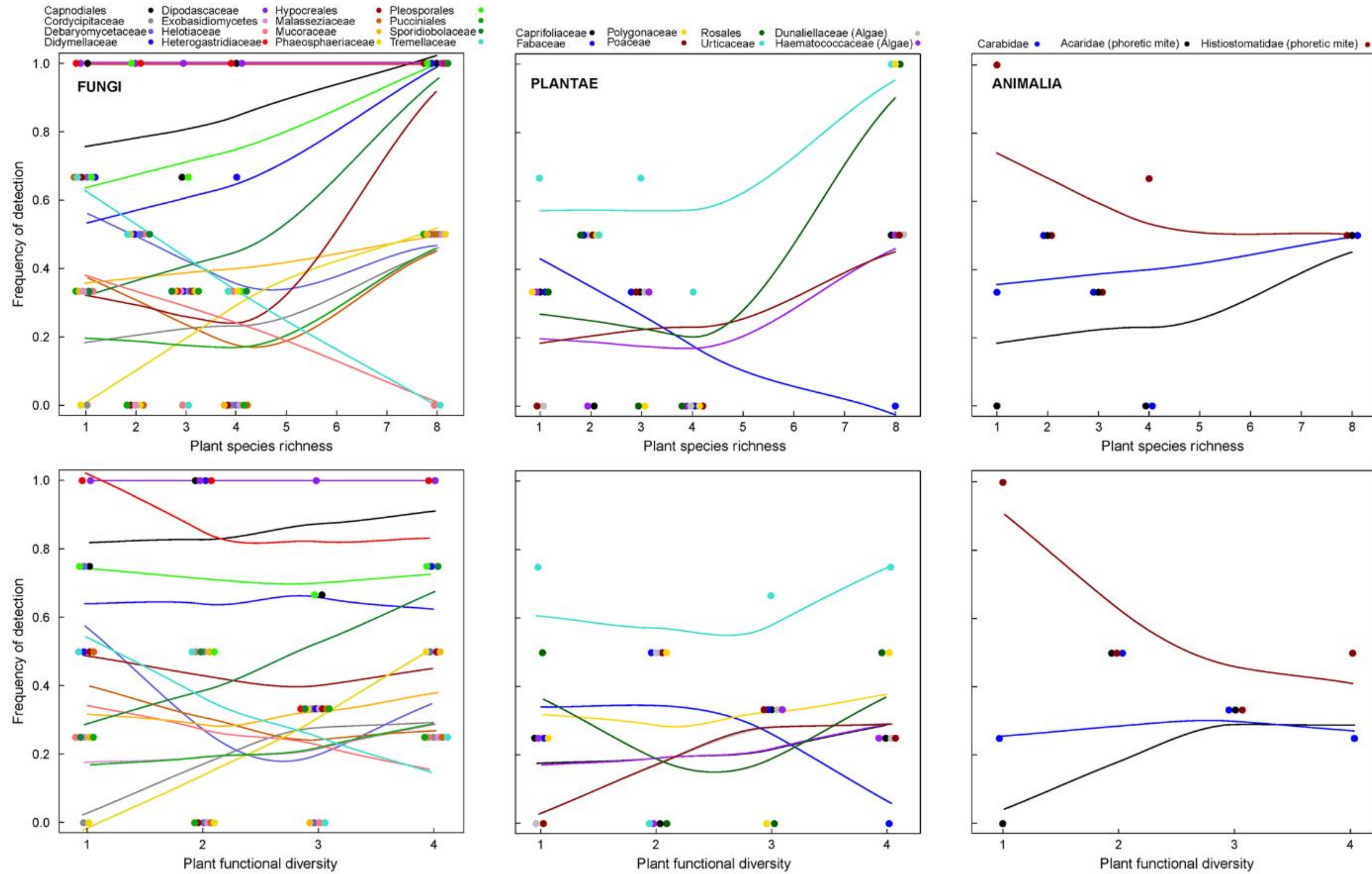

Fig 3. Effects of plant species richness and plant functional diversity on detection frequency of abundant OTUs detected in regurgitates of *P. melanarius*. The six panels show the three kingdoms (Plantae, Animalia, and Fungi). Points represent OTUs, aggregated at family level, that were detected in at least two levels of plant species richness. Lines (smoother span = 1.6) show least-squares fits for illustrative purposes only.

3.4 Discussion

3.4.1 Assessment and interpretation of trophic and non-trophic interactions

NGS of regurgitates of the omnivore *P. melanarius* with primers targeting a spectrum of organisms as broad as eukaryotes allowed us to directly assess trophic and non-trophic interactions involving a wide range of taxa. Any sequencing-based list of interactions will require further validation, as the quality of reference libraries or databases may affect assignment of sequences to taxa. As our study was performed within the framework of a larger biodiversity experiment, we had considerable knowledge on the presence of taxa in the study area, providing extensive species inventories that we used to validate the results. Additionally, for well-studied species such as *P. melanarius*, feeding interactions identified by NGS were compared to a broad body of literature on dietary range, feeding preferences, and behaviour. Literature research may also help to reveal which live stage of an animal or type of plant tissue has likely been consumed, as this information cannot be provided by DNA-based food detection. For example, seeds are a putative source of plant DNA since they are frequently consumed by *P. melanarius* (Petit et al. 2014) and more often found in guts of the carabid subfamily Harpalinae than pollen or other plant tissue (Hengeveld et al. 1979).

Many taxa we detected are well-known prey of *P. melanarius*, including slugs (Bohan et al. 2000; McKemey et al. 2001), earthworms (Symondson et al. 2000), spiders (Davey et al. 2013), and small beetles (Prasad et al. 2006; Davey et al. 2013). More surprising was the detection of grasshopper DNA. Grasshoppers were abundant at the field site during our study (see also Specht et al. 2008), and although it is unlikely that the beetle captured an adult grasshopper, predation on egg pods (Parker & Wakeland 1957) or scavenging on dead specimen can be considered a likely source of DNA in the gut (Foltan et al. 2005). Most surprising was the frequent detection of mite DNA, an observation that was supported by mite remains in dissected guts of *P. melanarius* specimens collected from the Jena Experiment (Fig 4A and 4B). Mites are within the food range of ground beetles (Thiele 1977) but their role in the diet of *P. melanarius* remains unclear. Generally, the broad dietary range of *P. melanarius* reported in the literature (Thiele 1977; Hengveld 1979) is well reflected by our NGS-based results on trophic interactions.

Among the non-trophic interactions revealed in the current study, parasitic interactions included an entomopathogenic fungus and a group of parasitic protists. Both could either have been parasites of *P. melanarius* or associated with its prey (Piñol et al. 2013). Despite this uncertainty, detecting parasite DNA in regurgitates of *P. melanarius* proves that the beetles were likely exposed to potential antagonists. Mite DNA detected using NGS may also indicate phoretic interactions, that is, mites may have used beetles as transporters between habitats (Fain et al. 1995). Mite DNA could either originate from mites or their remains, like exuviae, that were externally attached to prey or to *P. melanarius* itself and have fallen off during sampling. Additional observations showed that *P. melanarius* specimens are frequently infested by mites (Fig 4C).

Fig 4. Mites as prey and parasites of P. melanarius. (A) Predatory mite (Trombidiformes: Trombiculidae) in a plot of the Jena Experiment. (B) Mite isolated from a gut of *P. melanarius* (C). Phoretic mites (Mesostigmata: Parasitidae) on *P. melanarius*. Photographs by C. Scherber.

Most taxa we detected could not be assigned to a specific type of interaction with *P. melanarius* and were assumed to be neutral interactions with organisms that coexist with the beetles without affecting them in a particular way. By this simplification, we may have included organisms with a more specific but up to date unknown interaction with *P. melanarius*, e.g. yeasts that are beneficial to digestion processes, since the microbiome of ground beetles is largely unexplored (Lundgren et al. 2007, Lundgren& Lehman 2010). Most of the organisms classified as neutral interaction partners could, however, be identified as phytopathogens or saprotrophs for which an effect on *P. melanarius* is unlikely. Vice versa, the beetle could have contributed to the dispersal of spores (Lillekov & Bruns 2005) but information on the taxon-specific survival through the gut passage is required for assumptions on more specific interactions. It is likely that carabid beetles accidentally ingest all kinds of organisms during feeding or simply by dwelling in their environment, because even non-nutritional material, such as sand, has commonly been reported in their gut contents (Hengveld 1979). Boyer et al. (2015) suggest the use of faeces as 'biodiversity capsules' for species inventories of the foraging area. Similarly, species composition in regurgitates may provide information on species diversity and ecosystem processes in the beetles' habitat.

Further studies are essential to supplement the list of interaction partners by expanding the analysis to bacteria. Facultative bacterial symbionts have an impact on seed consumption by the omnivorous ground beetle *Harpalus rufipes* (Lundgren & Lehman 2010) and may also alter the food choice of field populations of ground beetles.

3.4.2 Regurgitates as source material for NGS

Our study is among the first to use NGS for the analysis of regurgitates to assess species interactions. Even without the use of blocking primers, only two samples yielded consumer DNA sequences and in one of them there was too much consumer DNA so that the sample had to be removed from the analysis. These results demonstrate that regurgitates contain only little consumer tissue and are asuitable source material for diet analysis of omnivorous or predatory insects because they can be analysed without blocking primers, avoiding drawbacks related to this approach (Piñol et al. 2013; 2014). In addition, the DNA recovered from food remains regurgitated from the foregut is likely more intact than from posterior gut sections or faeces. This allowed us to use primers that target a relative large DNA fragment of about 600 bp, which is beyond the recommended size of DNA fragments for molecular gut content analysis (but see Waldner et al. 2013), and to assign most sequences to genus or family level. Targeting long DNA sequences may also reduce the chance to detect degraded DNA from prey guts (secondary predation; (Sheppard et al. 2005)), or environmental sources. In the present study, we further avoided an overestimation of feeding events by discarding OTUs with low reads.

Defensive regurgitation is not only common in Carabidae (Forsythe 1982; Raso et al. 2014) but also in other coleopteran families commonly occurring in a wide range of ecosystems, for example, Chrysomelidae (Gross et al. 1998; Olckers 2000), Staphylinidae (personal observation) and Silphidae (Robertson 1992), but also in Orthoptera (Lymbery & Bailey 1980) as well as the larval stages of some Lepidoptera (Grant 2006). As regurgitate-sampling is non-invasive it could even be used to analyse the diet of endangered species or gut content samples of an individual at multiple time points. Using regurgitates for NGS based analysis represents a straightforward method to assess trophic and non-trophic interactions. Over all, our results demonstrate that regurgitates are a suitable source material for diet analysis of omnivorous or predatory insects with NGS.

3.4.3 Effects of plant biodiversity and vegetation cover on species interactions

We conducted our study within the framework of a biodiversity experiment, where aspects of plant taxonomic and functional diversity are experimentally manipulated (Ebeling et al. 20149 to allow testing for the effects of plant diversity *per se* on trophic and non-trophic interactions, as opposed to observational studies (Tilman 1999; Hector et al. 2007). So far, research on plant diversity effects on higher trophic levels rarely goes beyond measuring species richness and abundance. Although our findings are limited by the small sample size, our study provides insights into how plant diversity affects how well species in a community are connected with each other.

Our results indicate that experimentally manipulated plant diversity may indeed affect interactions between a generalist consumer and its potential food. Both the number of plant and prey taxa detected in regurgitates increased with the number of sown plant species. Plots with high plant species richness support a more diverse consumer community in relation to species poor plots (Scherber et al. 2010) and may provide more potential food items for the omnivorous beetles, thereby facilitating a mixed diet.

Prey detection and intraguild predation also increased with vegetation cover. Large carabid beetles, as *P. melanarius* (body size 12–18 mm), prefer structural complexity over open plots because it lowers their vulnerability to predation (Brose 2003) and may facilitates extensive foraging. The abundance of predators relative to herbivores has been reported to increase with plant diversity (Haddad et al. 2009), potentially increasing the chances that *P. melanarius* captures other predators. Hunter suggests that omnivorous consumers preferentially feed on other higher order consumers because they are rich in nitrogen (2009).

In regurgitates of beetles from plots with dense vegetation, we detected more neutral interactions with passively consumed organisms. High vegetation cover may provide a more humid microclimate that facilitates fungi and protists (Toberman et al. 2008; Stefan et al. 2014) and therefore increases the likelihood of encounters with ground-dwelling beetles.

It should be made clear, however, that more replicates and a greater range of consumer taxa will be needed to further elucidate the trends reported here. Nevertheless, our findings agree well with a large body of empirical work (Cardinale et al. 2006; Scherber et al. 2010; Lefcheck et al. 2015) showing a facilitating effect of plant diversity on trophic interactions. Thus, our study presents the intriguing possibility that our understanding of multitrophic food webs can be considerably advanced using molecular tools such as NGS.

NGS-based gut content analysis was so far mainly used to describe the dietary spectrum of species (Piñol et al. 2013; Vesterinen et al. 2013; Clare et al. 2014) but is underexploited in research on biodiversity and ecosystem functioning and has rarely been applied in plant diversity experiments. Expanding the spectrum of applications of NGS to address questions and to empirically test theories in biodiversity research is the way forward. With profound knowledge of the species pool and the often extensive data on ecological parameters available in biodiversity experiments, NGS-based gut content analysis can contribute too a mechanistic understanding of diversity effects. Applying very general primers allows assessing trophic interactions on various food types and non-trophic interactions simultaneously in one approach. By using regurgitates as source material, blocking primers for consumer DNA are no longer required and NGS becomes easily applicable even for predators or omnivores.

3.5 Acknowledgements

We especially acknowledge Christiane Roscher for providing data on vegetation cover, Jan-Hendrik Dudenhöffer for his support during fieldwork, Sigrid Dassen, Sarah Zieger, and Stefan Geisen for information on taxa. We thank the gardeners, technical staff, and student helpers for maintaining the experimental plots. We also thank two anonymous reviewers of PLoS ONE for useful comments and suggestions. The authors acknowledge support by Deutsche Forschungsgemeinschaft and Open Access Publication Funds of Georg-August University Goettingen.

3.6 Data accessibility

DNA sequences were deposited in the Sequence Read Archive (SRA) of the National Center for Biotechnology Information under accession SRA282133. Data used for statistical analyses and an R script are provided as Supporting Information files.

3.7 Supporting Information

The following supporting information is available for this article online http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0148781#sec017

S1 Data. OTU table for analysis with S1 R-script. (TXT)

S2 Data. Plot information for analysis with S1 R-script. (TXT)

S1 R-Script. R script used for statistical analyses. (R)

S1 Table. List of removed OTUs. (XLSX)

CHAPTER 4

Plant diversity effects on multitrophic interactions analyzed by gut content metabarcoding

Julia Tiede, Bernd Wemheuer, Michael Traugott, Rolf Daniel, Teja Tscharntke, Anne Ebeling, Christoph Scherber

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Abstract

Plant diversity has been shown to affect species richness and abundance of associated consumer communities. Species in diverse communities are likely connected by interspecific interactions. Omnivores, in particular, may link multiple trophic levels (e.g., plant and prey) and multiple resource channels (e.g., herbivore and detritivore resources) in interaction webs. However, it has remained unclear if and how changing plant diversity affects trophic interactions of omnivores, because appropriate methods were lacking. Moreover, the effects of plant and prey diversity on the gut microbiome of omnivores are largely unexplored. A promising approach is the DNA-based analysis of gut contents using next generation sequencing (NGS) technologies in combination with universal primers, allowing the detection of interactions from a wide range of taxa.

Here, we use the framework of a grassland biodiversity experiment (The Jena Experiment) where plant taxonomic and functional diversity were manipulated to analyze how three species of surface-active invertebrates with varying degree of omnivory respond to plant biodiversity. We released and recaptured ground beetles (*Pterostichus melanarius*, and *Harpalus rufipes*) and sampled field slugs (*Deroceras* sp.) in 138 fenced plots with experimental plant communities. Beetle regurgitates and slug feces were collected and DNA from these dietary samples was sequenced (Illumina MiSeq). We analyzed trophic and non-trophic interactions using general primers for plants, animals, fungi, and bacteria.

Our results show that omnivores in our study system choose their food independently of plant species richness (1 to 8 species). Plant diversity had only weak direct effects on the dietary and microbial diversity in gut content and seems to act more indirectly via the bottom-up food web. We detected strong differences in gut contents among the three omnivores that reflect their different food preferences. Overall, metabarcoding is a promising novel approach to study multitrophic interactions in biodiversity experiments and ecology in general.

4.1 Introduction

Species interactions in multitrophic communities are the hidden drivers of ecosystem processes and determinants of ecosystem functioning (Scherber et al. 2010; Lefcheck et al. 2015; Brose & Hillebrand 2016). However, the knowledge on how changing environmental conditions affect interactions between species is currently limited. Many studies concerning the influence of declining plant diversity in terrestrial ecosystems, have measured the numerical response of species at higher trophic levels or measured ecosystem process rates. These studies have shown, that producer diversity has cascading effects on the abundance and richness of consumer species (Knops et al. 1999; Koricheva et al. 2000; Borer et al. 2012), induces shifts in the functional structure and diversity across trophic levels (Haddad et al. 2009; Ebeling et al. 2017), and affects consumer-mediated ecosystem processes (Scherber et al. 2010; Allan et al. 2015; Hertzog et al. 2016b; Meyer et al. 2017).

Despite the importance of multitrophic interactions for a mechanistic understanding of dynamics and processes within ecological communities, few studies have empirically measured plant diversity effects on species interactions. Direct measurements are often limited to easily

observable interactions between plants and pollinators (Venjakob et al. 2016) or aboveground herbivores (Meyer et al. 2017), while resolving feeding interactions at higher trophic levels has remained challenging. Non-trophic interactions especially with microbial interaction partners like, e.g., gut endosymbionts are usually ignored completely in species interaction webs. Recent advances in DNA sequencing offer new possibilities to examine trophic and non-trophic interactions empirically (Traugott et al. 2013; Clare 2014; Vacher et al. 2016; Kamenova et al. 2017) Next generation sequencing (NGS) technologies combined with barcoding primers for broad taxonomic groups allows for the detection of multi-level species interactions from a wide range of potential interaction partners in complex and species-rich ecosystems (Pompanon & Samadi 2015; Tiede et al. 2016) and offers new insights into how the flow of energy and nutrients from basal resources translates to ecosystem processes in multitrophic communities.

Omnivores, are particularly interesting for studying interspecific interactions, as they link multiple trophic levels (e.g., plant and prey) and multiple resource channels (e.g., herbivore and detritivore resources) (Thompson et al. 2007; Wolkovich et al. 2014). Moreover, they may weaken top-down effects via intraguild predation, or strengthen population dynamics in food webs by adaptive feeding on abundant resources (McCann & Hastings 1997; Finke & Denno 2004; Kratina et al. 2012). Previous studies have shown, that plant diversity has no strong impact on species richness and abundance of omnivores (Scherber et al. 2010) but increases the functional diversity within omnivore communities and shifts the communities towards more carnivorous species (Ebeling et al. 2017). However, little is known about how omnivores respond trophically to changes in biodiversity. In particular, the adaption of feeding behavior within a species or even within individuals are rarely considered even though omnivores and other polyphagous feeders are known for their dietary plasticity (Hunter et al. 2009, Wolkovich et al. 2014). In a pilot study (n=13 replications), we found that the diet of an omnivorous ground beetle became more divers with increasing plant diversity (Tiede et al. 2016), which could indicate opportunistic foraging. In this study, we aim to elucidate, if this pattern can be generalized for a broader range of omnivores.

We included interactions between omnivores and bacterial and fungal microbes in our analysis. Previous studies have shown, that facultative bacterial symbionts can affect the diet of omnivores and vice versa (Lundgren & Lehman 2010), and that gut bacteria of arthropods at higher trophic levels respond to local and landscape diversity (Schmid et al. 2015; Tiede et al. 2017). The guts of invertebrates contain besides host associated microbes also passengers from environmental sources that are co-sequenced with the gut community in metabarcoding studies. Such environmental DNA (eDNA) can provide considerable information about the diversity of microbes and ecological processes in the source environments. Previous studies have shown that plant diversity increased the microbial activity and shifted the soil community towards more fugal compared to bacterial biomass (Markus Lange 2015; Eisenhauer et al. 2017). Further, the diversity of co-occurring fungi and protists in beetle guts where indirectly affected by plant diversity via vegetation cover (Tiede et al, 2016). Microbial richness has in turn positive effects on the multifunctionality of ecosystems (Wagg et al. 2014). Invertebrates might affect the soil microbial community via increasing herbivory rates along the plant diversity gradient that can speed up nutrient cycling (Meyer et al. 2017) and by distributing species that survive the gut passage (Vega & Blackwell 2005). In turn, soil bacteria and fungi are connected to aboveground invertebrate omnivores through the detrital food web (Scheu

2002). Systematic studies on the response of microbe-invertebrate interactions to changing diversity of basal resources are, however, lacking so far.

Here, we empirically assessed the multitude of trophic and microbial interactions of surface-active invertebrates with omnivorous feeding habits in response to biodiversity manipulations within a grassland biodiversity experiment (The Jena Experiment; Roscher et al. 2004; Ebeling et al. 2014). In particular, we identified DNA in gut contents and feces of three model consumer species with varying degree of omnivory, the ground beetles *Pterostichus melanarius* Illiger, and *Harpalus rufipes* DeGeer, and field slugs of the genus *Deroceras* Rafinesque (Pulmonata: Agriolimacidae) by high throughput sequencing (Illumina MiSeq) of PCR products from common barcoding regions for plants, animals, fungi, and bacteria (Pompanon *et al.* 2012; Traugott *et al.* 2013; Tiede *et al.* 2016).

We specifically asked the following questions:

1. Trophic interactions: Will omnivores from a more diverse local environment have a more diverse diet? We hypothesize that the direct impact of plant diversity is proportional to the amount of plant material in the diet (*D.* sp. > *H. rufipes* > *P. melanarius*). Indirect plant diversity effects via the trophic chain will become more important with increasing proportion of prey food in the diet (*D.* sp. < *H. rufipes* < *P. melanarius*).

2. Host associated microbes: Does the ratio of beneficial to harmful microbes change along the plant diversity gradient? We assume that an increase in plant diversity results in a higher proportion of beneficial organism in consumer guts.

3. Microbial eDNA: Does plant diversity affect the richness of microbes without direct relation to the consumers (e.g., plant pathogens, saprobionts)? Based on previous findings, we assume that the taxonomic richness of microbes will increase with plant diversity. Further, we hypothesize that microbial communities are more dissimilar in plant monocultures and will become more similar with increasing plant diversity.

4.2 Methods

4.2.1 Experimental field sites

We conducted this study within the framework of a grassland biodiversity experiment (The Jena Experiment, Germany, 50°95′ N, 11°63′ E, 130 m above sea level; Roscher et al. 2004) in experimental plots of the Trait-Based Diversity Experiment (TBE; Ebeling et al. 2014). The TBE was established in 2010, to study the effects of taxonomic and functional diversity of grassland communities on ecosystem functions. The plant communities in the TBE were assembled from a total of 20 Central European grass and non-legume herbaceous species. The plant species were assigned to three (partially overlapping) species pools with eight species in each pool based on six continuous plant functional traits (e.g., plant height, and rooting depth) that are known to be important for resource use along a spatial (light, water, nutrients) and temporal (phenology) gradient. Pool 1 was comprised of plant species that differ in spatial resource use. Plants in pool 2 covered a gradient of temporal resource use, and pool 3 combined both gradients but with plants on the extreme ends of the spatial and temporal resource use

gradients. On 138 plots (3.5 m x 3.5 m) in three spatial blocks that account for differences in soil texture, plant species richness (1, 2, 3, 4, and 8 sown species) and plant functional diversity (1, 2, 3, and 4) were manipulated. Plant functional diversity represented the levels from low (1) to high (4) trait complementarity in the plant community (Ebeling et al. 2014). The experimental plots were maintained by biannual mowing and weeded three times per year to remove unwanted species and maintain the sown communities.

On each plot, we installed an enclosure (3.5 m x 1 m, Fig. 1 A) to prevent inter-plot movement of consumers for the time of the experiment (July till September 2014). For the enclosures, transparent construction foil (PE, 20 µ, Rajapack, Ettlingen) was wrapped around four corner poles up to a height of 40 cm and sunken into the soil about 15 cm depth using plastic panels. Additional to the 138 experimental plots (3.5 m x 3.5 m), we installed one control plot in each of the three blocks within the plant matrix between the plots. The matrix consists of a mix of various plants from the total species pool of the Jena Experiment, including legumes, and further common grassland species and is mown frequently.

4.2.2 Plant and invertebrate community measures

The percentage of the total vegetation cover within each enclosure was visually estimated at the end of August 2014. Plant aboveground biomass was measured at the beginning of September 2014. Therefore, plant material was cut at two random locations within the plots (20 x 50 cm) but outside the enclosed area, dried, and weighted to the nearest 0.1 g. Mean values per plot were extrapolated to g per m^2.

The invertebrate community was sampled by pitfall trapping and suction sampling in each plot outside our experimental enclosures. One pitfall trap containing 3% formaldehyde was centrally installed on each plot (outside the enclosed area) and emptied biweekly. For our analysis, we used data from four sampling dates within the timeframe of gut content sampling from end of July till mid of September 2014. Suction sampling was conducted using a modified vacuum cleaner (A2500, Kärcher GmbH, Winnenden, Germany) to sample arthropods from caged vegetation (75 x 75 cm). All samples were stored in 70 % ethanol and identified to species level for Araneae, Chilopoda, Coleoptera, Dermaptera, Diplopoda, Diptera, Hemiptera, Heteroptera, Hymenoptera, Isopoda, Julida, Lithobiomorpha, Orthoptera, and Polydesmida. Acari, Clitellata, Diptera, Gastropoda, and Thysanoptera were not further identified. Based on literature research, we assigned the identified taxa to trophic levels and trophic positions (TP), into herbivores, mycetophages, saprophages (TP 1), omnivores (TP 1.5), carnivores, parasites, and parasitoids (TP2). Additionally, we estimated the most likely trophic interaction with the consumer species that we used for gut content analysis (e.g., consumption, secondary consumption, parasitation). We calculated the mean richness and abundance of invertebrates per plot and sampling method for total invertebrates, on class and order level, and on trophic level over the different sampling dates for trap and suction catches. For 10 plots no suction samples existed, here we used the mean values per plant species richness level.

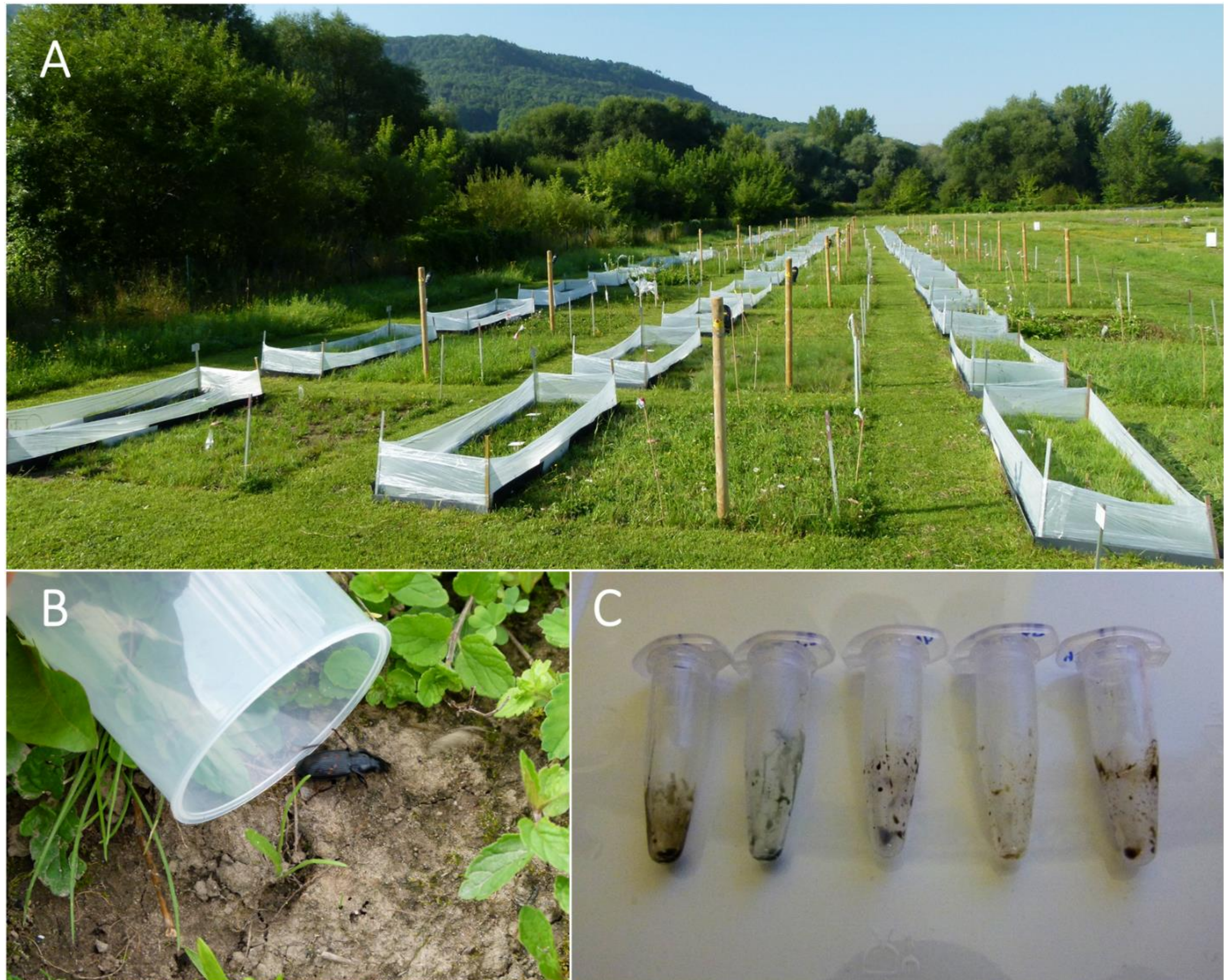


Figure 1. A) Experimental plots with enclosures; B) Release of marked *P. melanarius*; C) Beetle regurgitates

4.2.3 Gut content sampling of omnivores

As model organism for consumers we used three locally abundant, geographically wide spread and ecological relevant invertebrate species. All are polyphagous feeders on a wide range of food types but include varying degrees of plant matter in their diet (Thiele 1977; Kerney *et al.* 1983). The two ground beetles *Pterostichus melanarius* Illiger and *Harpalus* (Pseudoophonus) *rufipes* De Geer (Coleoptera; Carabidae) are dominant species in many natural and agricultural ecosystems. *Pterostichus melanarius* is primarily carnivorous but its diet also includes plant material. *H. rufipes* is an omnivore with a diet comprised half of plant-derived resources, mainly seeds (Thiele 1977; Hengeveld 1979; Lovei & Sunderland 1996; Jørgensen & Toft 1997). Field slugs (*Deroceras* sp. Rafinesque; Pulmonata: Agriolimacidae) forage predominantly above ground on living plant material and are pest in many crops and also abundant in temperate grasslands (Kerney *et al.* 1983; Barlow *et al.* 2013) but occasionally consumes small prey like aphids or death animal matter (Barker & Efford 2004).

Adult *P. melanarius* and *H. rufipes* were collected preceding the experiment using dry pitfall traps in the surrounding grass margins of the Jena Experiment in June and July 2014 and maintained in fauna boxes on a diet of cat food (K-Classic Adult, Kaufland AG, Germany) and seeds (*Trifolium pretense* L., *Rosa canina* L.*)*. The beetles were marked with an engraving tool (AGT Gravurset, Pearl.GmbH, Buggingen, Germany) with an individual pattern on the elytron

that allowed us to distinguish the beetles within a plot and between neighboring plots and released in the enclosures on July 27-28th (Fig. 1B). After allowing the released beetles to adapt to the plot conditions for 9 days, we repeatedly recaptured the marked beetles and additional ambient beetles during the period from August 6th to September 3rd, 2014 in three dry pitfall traps (6 cm diameter) per plot. The traps were filled with clay pebbles and emptied daily to minimize within-trap predation events. Beetles from traps including organism remains were excluded from analyzes.

To sample the beetles' gut contents, we placed them individually headfirst in 1.5 mL reaction tubes and exposed the tubes for a few seconds to hot water (~60°C) to induce regurgitation. Regurgitates were immediately frozen at -18°C and subsequently stored at -80°C (Fig. 1C). Afterwards, the beetles were released to the plot of origin but from August 25th onward kept for additional analysis.

Slugs were abundant on most plots and were collected by hand from August 27th to September 3rd, 2014. Slugs were individually placed in 2 mL microtubes. The tubes were checked for feces every couple of hours for up to 24 h. Feces were immediately frozen at -18°C and subsequently stored at -80°C. Slugs were kept for additional analyzes.

4.2.4 Sample processing

4.2.4.1 DNA extraction

Total DNA was extracted from regurgitates and feces (gut content hereafter) in a molecular diagnostic laboratory at the Institute of Ecology, University of Innsbruck, Austria on a BioSprint 96 robotic DNA extraction platform using the MagAttract DNA Blood M96 Kit (Qiagen, Hilden, Germany). Four negative extraction controls (DNA extraction blanks) were included to monitor for carry-over DNA contamination during the extraction process and subsequently tested in PCR reactions.

4.2.4.2 Amplification of marker gene sequences

Amplicon preparation and next generation sequencing of gut contents was conducted at the Department of Genomic and Applied Microbiology (University of Göttingen, Germany). We analyzed DNA remains in gut content samples of the three consumer species with four sets of primers targeting either the trnL intron of plant chloroplasts, the mitochondrial cytochrome c oxidase subunit I (COI) of animals, the fungal ITS sequence, or the bacterial 16S rRNA genes. Details on primers, PCR reaction mixes and thermal cycling schemes are listed in Table 1. For the amplification of fungal ITS sequences, we used a nested PCR approach to suppress co-amplification of non-fungal taxa. The amplicons from a first PCR with highly specific fungi primers were used as template in a second PCR that reduced the size of the amplicons to fit the read length of the MiSeq sequencer.

All PCR runs included one template-free control and one positive control. Samples that showed PCR products on agarose gel were purified with the NucleoMag 96 PCR Kit and in the case of COI amplicons with the NGS Clean-up Kit (Machery-Nagel, Dueren, Germany). DNA concentration was quantified using the QuantiFluor dsDNA System kit (Promega Corporation, Madison, USA) with a microplate reader (Synergy™ 2 Microplate Reader, Biotek Synergy HT, Winooski, VT, USA).

Table 1. Primer sequences and PCR conditions used for amplification of marker genes of animals, plants, fungi, and bacteria.

Target taxa	Locus	Length (bp)	Primer name	Sequence 5′-3′	Reference	PCR reaction mix	PCR conditions
Animals	COI, mtDNA	300	S878-S1-DG[1]	GGDRCWGGWTGAACWGTWTAYCCNCC	Modified after Leray et al. 2013	Total volume 35 µL: 7 µL DEPC water[a], 1.8 µL BSA (10 mg*ml^{-1}), 3.5 µL 10 µM primer mix, 17.5 µL 2x Master Mix[b], 5.3 µL template	Denaturation 98°C 30 sec; 35 cycles at 98°C 15 sec, 55°C 15 sec, 72°C 30 sec; Final elongation 72°C 1 min
			A867-jgHCO2198[2]	TANACYTCNGGRTGNCCRAARAAYCA	Geller et al. 2013		
Plants	trnL, cpDNA	170-230	trnL-c (A49325)[1]	CGAAATCGGTAGACGCTACG	Taberlet et al. 1991, 2007	Total volume 50 µL: 22.5 µL DEPC water[a], 10 µL HF buffer[c], 1.5 µL DSMO, 2 µL BSA (10 mg*ml^{-1}), 1 µL 10 µM dNTPs[c], 5 µL 10 µM primer mix, 0.5 µL 1U Phusion[c], 7.5 µL template	Denaturation 98°C 30 sec; 35 cycles at 98°C 15 sec, 55°C 30 sec, 72°C 30 sec; Final elongation 72°C 5 min
			trnL-h (B49466)[2]	CCATTGAGTCTCTGCACCTATC	Taberlet et al. 2007		
Bacteria	16S, rRNA	460	341F[1]	CCTACGGGNGGCWGCAG	Herlemann et al. 2011	Total volume 50 µL: 28 µL DEPC water[a], 10 µL HF buffer[c], 1.5 µL DSMO, 1 µL 10µM dNTPs[c], 4 µL 10 µM primer mix, 0.5 µL 1U Phusion[c], 5 µL template	Denaturation 98°C 30 sec; 32 cycles at 98°C 10 sec, 55°C 15 sec, 72°C 30 sec; Final elongation 72°C 5 min
			785R[2]	GACTACHVGGGTATCTAATCC	Herlemann et al. 2011		
Fungi	ITS, rDNA	~800	ITS1 KYO2	TAGAGGAAGTAAAAGTCGTAA	Toju et al. 2012	Total volume 50 µL: 30 µL DEPC water[a], 10 µL HF buffer[c], 1.5 µL DSMO, 1 µL 10µM dNTPs[c], 4 µL 10 µM primer mix, 0.5 µL 1U Phusion[c], 3 µL template	Denaturation 98°C 30 sec; 30 cycles at 98°C 15 sec, 50°C 15 sec, 72°C 30 sec; Final elongation 72°C 1 min
			ITS4	TCCTCCGCTTATTGATATGC	White et al. 1990		
		~350	ITS3 KYO2[1]	GATGAAGAACGYAGYRAA	Toju et al. 2012		
			ITS4[2]	TCCTCCGCTTATTGATATGC	White et al. 1990		

[1] primers included MiSeq adaptor sequence 5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-3′

[2] primers included MiSeq adaptor sequence 5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-3′

[a] Sterile-filtered and DEPC-treated water

[b] Qiagen Multiplex PCR Master Mix, Qiagen GmbH, Hilden, Germany

[c] FermentasTM 10 mM dNTP Mix; Phusion High-Fidelity DNA Polymerase; 5x HF reaction buffer, Thermo Fisher Scientific, Waltham, USA

4.2.4.3 Sequencing and sequence processing

In total 1821 samples were multiplexed using the Nextera XT index kit v2 and sequenced on a MiSeq sequencer with the MiSeq Reagent Kit v3 (Illumina, San Diego, CA, USA) using 2 × 300 bp paired-end reads and a sequencing depth of 20,000 per sample.

Sequences were processed separately for each primer set. Raw reads were truncated with Trimmomatic (version 0.32; Bolger et al. 2014) with a quality cut-off of 20 in a sliding window of 10 bp. Datasets were processed as described (Granzow et al. 2017; Wemheuer & Wemheuer 2017). In brief, paired-end reads were merged and low-quality reads (expected errors >1, and ambitious bases >1) and reads shorter than 200 bp were discarded. Processed sequences were clustered in operational taxonomic units (OTUs) at 97 % genetic similarity using the UPARSE algorithm in Usearch (version 8.0.1623; Edgar 2010). Clustering included a de novo chimera removal step. Remaining chimeric sequences were removed with Uchime in reference mode with the most recent RDP training set (version 15; Cole et al. 2009) for bacteria and the most recent UCHIME reference data (version 7.1, UNITE database; Kõljalg et al. 2013) for fungi. Processed sequences were mapped on OTU sequences to calculate the distribution and abundance of each OTU in every sample.

4.2.4.4 Taxonomic assignment

Bacterial OTU sequences were taxonomically classified using QIIME (Caporaso et al. 2010) by BLAST alignment against the SILVA database (SILVA SSURef 128 NR) and fungal OTUs by the QIIME release of the UNITE database (version 7.1; August 2016). Co-amplified non-bacterial or non-fungal taxa and hits with e-values > $1e^{-10}$ were removed from the respective dataset. In addition, we discarded OTUs that could not be classified to class level for fungi and to phylum level for bacteria. COI und plant-derived sequences were classified by BLAST against the most recent nucleotide database using a e-value cut-off of 1e-20 and a minimum alignment length of 200 bp for COI and 150 bp for plants (NCBI nt; January 2017). Information on the closest hit in the nt database were retrieved from NCBI using the NCBI e-utilities. Moreover, COI sequences were classified by BLAST alignment against the BOLD database (version 6.50). Information for each hit were retrieved from the BOLD database using the BOLD API. Obtained information was converted using customized PERL scripts. Only hits with at least 93 % identity were kept. If the bitscore of the second and third best match was > 30 compared to the best match, we kept only the best match. If it was lower, we kept up to three matches in the OTU assignment table.

We compared the assigned plant taxa to a list of the sown plant species and common weeds in the Jena Experiment and the additional plots in Göttingen. The assigned OTUs had to be at least in a subfamily from the local pool to be kept in the dataset. In the cases that multiple taxonomic assignments of the same OTU were left in the dataset, we kept the one that matched best with our local species pool and in case of equally good matches we classified the OTU to the lowest common taxonomic level. The database derived taxon matches for animals were compared to an extensive list of invertebrate taxa that were previously found in the Jena Experiment, in Germany and the neighboring countries. We kept only taxa within orders that were previously found in the Jena Experiment, families reported from Germany, and genera from the European neighbor countries. In the case that multiple assignments per OTU were left, we decided for the taxon with the most local reference. Sequences from each of the three

consumer taxa were excluded from all samples due to the high risk of cross contamination between samples.

All taxon matches were clustered at genus level or at higher taxonomic levels, depending on the taxonomic resolution. A detection threshold for taxa was set at 0.3 % of the total reads per sample (post quality filtering). Samples with a low number of total reads (< 1500), or high numbers of consumer sequences (> 96%) were excluded from the analysis. The remaining 1210 samples represented 47-110 plots depending on the combination of consumer and primer (see Table S1 for details). The information for samples for which technical replicates excited was combined for the further analysis.

4.2.4.5 Assignment of interaction types

Taxa were grouped based on literature information on their most likely interaction with the consumers in trophic interactions, interactions with consumer associated microbes, and environmental species with unknown relation to the consumer (eDNA).

Trophic interactions:

- Total food: plant or animal food
- Plant food: plant taxa from the pool of sown species and common weeds
- Predation: animal taxa from the orders Annelida, Arthropoda, Gastropoda
- Intraguild predation: prey with predatory or omnivorous nutrition

Microbial associations:

- Microbiome: microbes (bacteria and fungi) that are associated with arthropods or slugs (symbionts and taxa without known function, no pathogens)
- Symbionts: microbes that are reported as symbionts of arthropods or slugs
- Pathogens: microbes that are reported as pathogens of arthropods or slugs

Environmental microbes:

- Neutral: microbes without known interaction with the consumer that were likely passively consumed together with food (eDNA)

For the analysis of plant diversity effects on the richness of organisms in gut content, we calculated the sum of different interaction partners in group and sample. Note that the number of sequences per detected taxon cannot be used as a measure of consumed biomass as it is affected by the time since consumption and characteristics of the food, e.g., tissue density, or copy number of targeted DNA marker (O´Rorke et al. 2012, Deagle et al. 2010, Deagle et al. 2013, Piñol et al. 2014).

4.2.5 Statistical analysis

We used R 3.3.2 (R Development Core Team, 2016), and R Studio 1.0.153 (RStudio Team 2017) for the statistical analysis. Biological replicates were averaged per plot, consumer, and interaction type. We accounted for differences in the number per replicates (1 to 4) by giving more weight to samples with more replications in statistical models.

4.2.5.1 Richness of trophic and microbial interactions

First, we aimed to test if the results from the pilot study (Tiede et al. 2016) can be confirmed in an extended study that included all experimental plots from the TBE and three different omnivores. We used generalized linear models (GLM) with negative binomial or quasipoisson errors, to analyze if the taxa richness in the different interaction groups (total food, plant food, prey, IGP, microbiome, symbionts, pathogens, and neutral interactions) responds to plant species richness, plant functional diversity, or percent vegetation cover, as these were the variables we also used in the pilot study. We did not test these three variables in a single model as they were colinear.

In a first set of models we analyzed the data of all three consumers together. We included consumer identity as a factor and tested its interaction with either plant species richness, plant functional diversity or vegetation cover at a time. In this all-consumer models, samples from *D.* sp. were excluded from models that tested effects on IGP and on associated microbes.

Due to the strong effect of consumer identity, that might have masked weaker effects of plant species richness, plant functional diversity and vegetation cover, we used a second set of models in which the data of each consumer were analyzed individually.

4.2.5.2 Cascading effects of plant diversity on prey richness

We additionally used structural equation modeling (SEM; *lavaan*; Rosseel 2012) to test for direct and indirect effects of plant species richness via chances in the plant and arthropod community on the richness of prey taxa in gut content. In the model, we used the log-transformed detected prey richness in gut content as a response variable and included consumer identity as a grouping factor in the model. As an exogenous variable we included the design variable sown plant species richness. As plant species richness has been shown to affect plant biomass, vegetation cover and species richness and abundance of animals in the plot (Ebeling et al. 2017; Weisser et al. 2017) we included these as independent variables and modeled a path from plant species richness to each of these variables.

4.2.5.3 Average trophic position of food items

All detected plant and prey taxa were first assigned to trophic modes and trophic positions (autotrophs = 0; herbivores, saprophages, and mycetophages = 1, omnivores = 1.5, carnivores, and parasitoids = 2). We than calculated the mean trophic position of total food (plant and prey) and prey and tested the effects of plant species richness, plant functional diversity, percentage vegetation cover, and the richness and abundance of animals in the plot in separate GLMs. All models included an interaction with the consumer species (*D.* sp. was excluded from the prey models) and were fitted to a quasipoisson distribution.

4.2.5.4 Proportional composition of gut content

We analyzed the proportional composition of gut content and feces with GLMs with quasibinomial errors. The response variables were i) proportion of prey taxa in the diet (prey and plant food), ii) proportion of intraguild prey from total prey, iii) proportion of symbiotic microbes from the sum of symbionts and pathogens, and iv) proportion of fungi in the total microbial community. We tested the interaction between consumer identity (*D.* sp. was only included in model i) and iv)) and plant species richness, plant functional diversity, percentage vegetation cover, and richness and abundance of invertebrates in the plot.

4.2.5.5 Microbial community composition

We separately analyzed the bacterial and fungal community assemblage with the *vegan* package (Oksanen et al. 2017). The number of sequences per taxon was Hellinger transformed (*vegan: decostand;* Legendre & Gallagher 2001) and Bray–Curtis dissimilarities were calculated (*vegan: vegdist*). We tested the effects of consumer species on community composition with permutational multivariate analysis of variance (perMANOVA; *vegan*: *adonis*) and permutation tests for the between group homogeneity in multivariate dispersions (*vegan: betadisper*, *permutest*; Oksanen et al. 2017; Anderson 2006; McArdle & Anderson 2001) for all three omnivores and for the two beetles only. In a second set of models we tested the effect of plant species richness on the community composition and dispersion in separate models for each consumer species. Similarities between samples of all three consumers were visualized by NMDS (vegan: *metaMDS*).

4.3 Results

4.3.1 General composition of detected taxa

We detected a total number of 946 taxa in the regurgitates of beetles and feces of slugs. An overview is given in Table 2.

Table 2. Sample overview on the number (n) of detected taxa per consumer species and represented plots.

	total	*D.* sp.		*H. rufipes*		*P. melanarius*	
Kingdom	n taxa	n taxa	n plots	n taxa	n plots	n taxa	n plots
Plantae	45	29	55	31	80	31	56
Animalia	80	16	62	32	61	37	48
Fungi	226	121	67	134	100	101	108
Bacteria	595	121	63	462	104	367	112
total	946	287		659		536	

Kingdoms refer to the targeted group of primers used for DNA detection.

Figure 2 illustrates the most frequently detected taxa (details in Table S1, Supporting Information). The plant orders Asterales, Lamiales, and Fabales were most frequently found in gut contents of all three omnivores, whereas Rosales were frequently detected only in beetles. Although the sown species pool of the TBE does not contain Fabales, legumes are the most common weeds in the experiment. Of all animal orders, Hemitera (aphids) were most

frequently detected in *D.* sp. and *H. rufipes.* Additional morphological gut content analysis confirmed the presence of aphids remain in guts of the field slug. Earthworm (Haplotaxida) DNA was commonly found in slugs and *P. melanarius*. Hymenopteran taxa (ants and parasitoids) were detected in all three consumers. Spider DNA was only detected in beetles. The fungal class Saccharomycetes was found in all consumers but as Agaricomycetes more commonly in beetles, whereas Microbotryomycetes were more frequently found in slug samples. The bacterial phyla Proteobacteria, Bacteroidetes, Firmicutes, and Actinobacteria were present in almost all samples. In the beetles, Saccharibacteria, and Acidobacteria were additionally found in higher frequency.

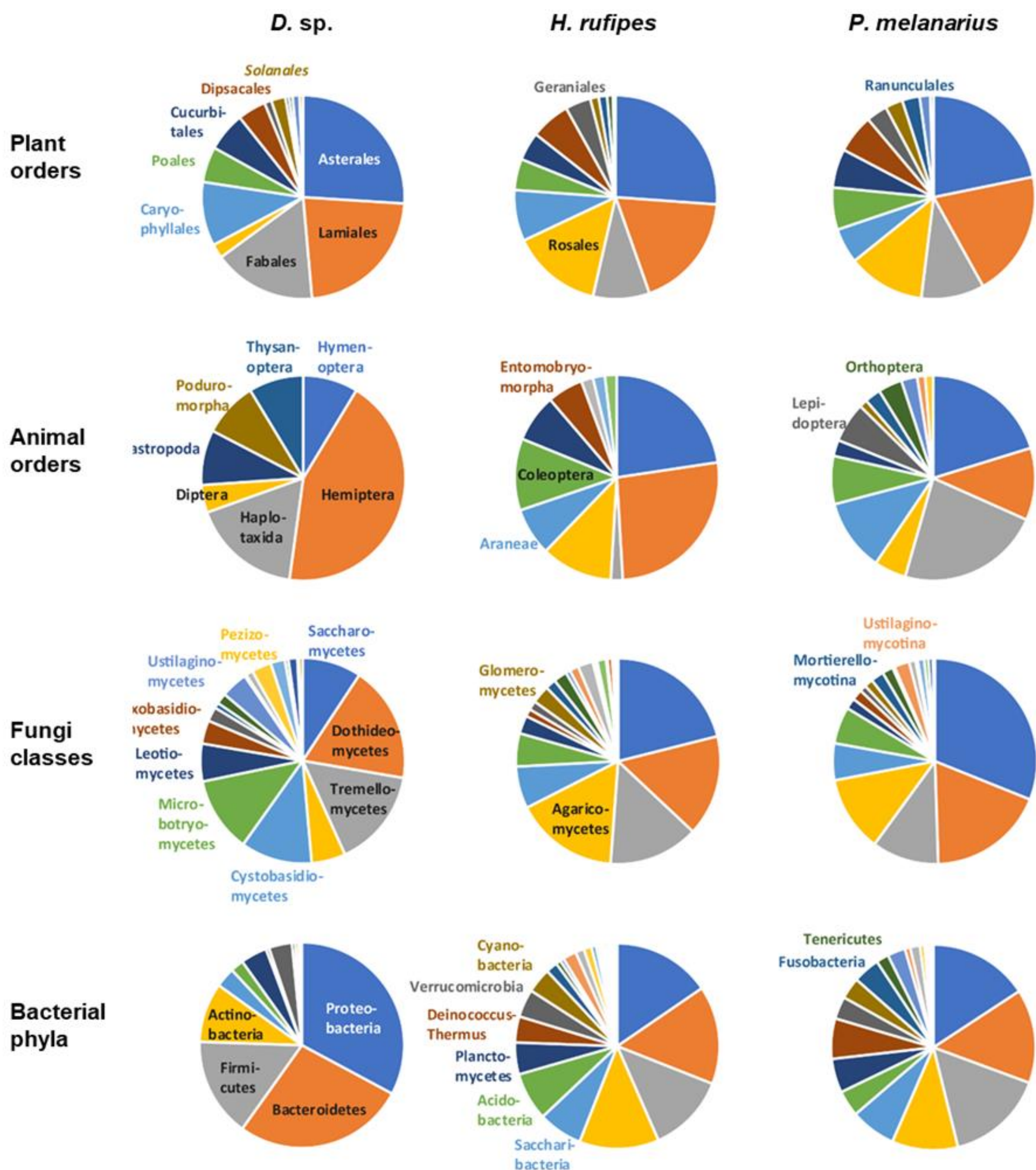


Figure 2. Most frequently detected taxa in slug feces and beetle regurgitates. See Table 2 for details on the number of samples and Table S1 for details on sample composition.

Figure 2 illustrates the most frequently detected taxa (details in Table S1, Supporting Information). The plant orders Asterales, Lamiales, and Fabales were most frequently found in gut contents of all three omnivores, whereas Rosales were frequently detected only in beetles. Although the sown species pool of the TBE does not contain Fabales, legumes are the most common weeds in the experiment. Of all animal orders, Hemitera (aphids) were most frequently detected in *D.* sp. and *H. rufipes.* Additional morphological gut content analysis confirmed the presence of aphids remain in guts of the field slug. Earthworm (Haplotaxida) DNA was commonly found in slugs and *P. melanarius*. Hymenopteran taxa (ants and parasitoids) were detected in all three consumers. Spider DNA was only detected in beetles. The fungal class Saccharomycetes was found in all consumers but as Agaricomycetes more commonly in beetles, whereas Microbotryomycetes were more frequently found in slug samples. The bacterial phyla Proteobacteria, Bacteroidetes, Firmicutes, and Actinobacteria were present in almost all samples. In the beetles, Saccharibacteria, and Acidobacteria were additionally found in higher frequency.

4.3.2 Richness of trophic and microbial interactions

We tested the effect of plant species richness, plant functional diversity and percentage vegetation cover on the taxonomic richness within the different types of trophic and microbial interactions. Consumer identity was included in a first set of models and explained most of the variation in taxon richness over all types of interaction groups. The richness of symbionts in regurgitates was significantly affected by an interaction between consumer identity and percentage vegetation cover; symbiont richness increased in *P. melanarius* with percentage vegetation cover but decreased in *H. rufipes* (Table S3, Fig. 3). Plant species richness and plant functional diversity had no effects on any interaction group.

A second set of separate models for each consumer (Table 3; Fig. 4-6) confirmed the negative effect of vegetation cover on symbiont richness in *H. rufipes*, although the effect was only marginally significant (Fig. 4). The response of symbiont richness in *P. melanarius* to vegetation cover was not confirmed (Fig. 5). Neutral taxa in feces of *D.* sp. was the only group for which significant effects were found. The richness of neutral interactions increased with all three plant measures (Fig. 6).

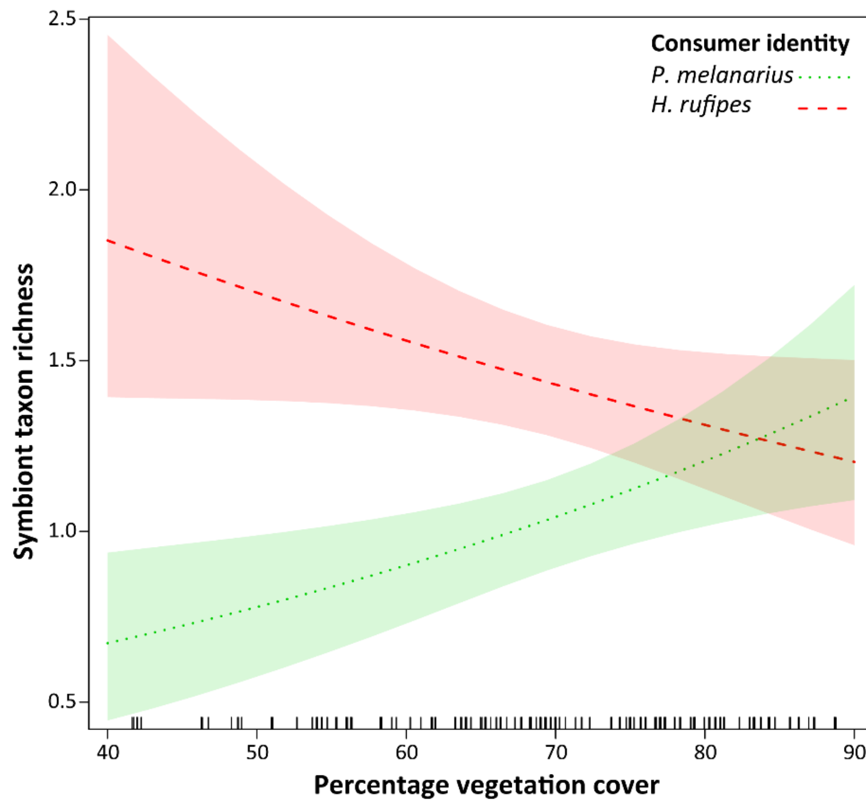


Figure 3. Symbiont richness in regurgitates of ground beetles responds species specific to vegetation cover. Effect of the interaction of beetle species and percentage vegetation cover on the richness of microbial taxa that are potential symbionts. Lines and shaded regions show response predictions and 95% confidence intervals from the generalized linear model.

Table 3. Effect of plant species richness (PSR), plant functional diversity (FD), and percent vegetation cover (% Cover) on the number of detected taxa in each interaction group.

Model	Parameter	*Pterostichus melanarius*					*Harpalus rufipes*					*Deroceras* sp.				
		denom. df	Estimate	*SE*	*t* value	*p* value	*denom. df*	Estimate	*SE*	*t* value	*p* value	*denom. df*	Estimate	*SE*	*t* value	*p* value
a) Total food	(Intercept)		1.300	0.191	6.817	<0.001		0.708	0.141	5.031	<0.001		1.093	0.236	4.634	<0.001
	PSR	74	-0.074	0.066	-1.125	0.264	94	0.066	0.045	1.467	0.146	68	-0.045	0.078	-0.580	0.564
	(Intercept)		1.269	0.216	5.866	<0.001		0.862	0.169	5.099	<0.001		1.098	0.268	4.099	<0.001
	FD	74	-0.068	0.083	-0.819	0.415	94	0.010	0.064	0.150	0.881	68	-0.054	0.105	-0.519	0.606
	(Intercept)		0.433	0.482	0.897	0.373		0.840	0.383	2.193	0.031		0.993	0.641	1.548	0.126
	% Cover	74	0.010	0.007	1.434	0.156	94	0.001	0.005	0.118	0.906	68	0.000	0.009	-0.037	0.971
b) Plants	(Intercept)		1.361	0.163	8.359	<0.001		0.809	0.109	7.433	<0.001		1.466	0.169	8.652	<0.001
	PSR	54	-0.047	0.056	-0.838	0.406	78	0.057	0.035	1.632	0.107	52	-0.037	0.055	-0.670	0.506
	(Intercept)		1.312	0.195	6.711	<0.001		0.859	0.133	6.466	<0.001		1.605	0.187	8.582	<0.001
	FD	54	-0.030	0.076	-0.397	0.693	78	0.042	0.050	0.840	0.403	52	-0.100	0.072	-1.385	0.172
	(Intercept)		1.078	0.460	2.341	0.023		0.922	0.286	3.230	0.002		1.467	0.481	3.051	0.004
	% Cover	54	0.002	0.006	0.360	0.721	78	0.001	0.004	0.137	0.891	52	-0.001	0.007	-0.216	0.830
c) Predation	(Intercept)		0.393	0.261	1.504	0.140		-0.310	0.276	-1.120	0.267		-0.562	0.500	-1.123	0.266
	PSR	45	0.011	0.088	0.125	0.901	59	0.078	0.088	0.886	0.379	58	-0.245	0.193	-1.269	0.210
	(Intercept)		0.453	0.275	1.651	0.106		-0.258	0.331	-0.781	0.438		-0.876	0.507	-1.730	0.089
	FD	45	-0.013	0.102	-0.123	0.903	59	0.065	0.123	0.530	0.598	58	-0.135	0.211	-0.637	0.526
	(Intercept)		-0.012	0.556	-0.022	0.982		-0.915	0.891	-1.027	0.308		-1.758	1.257	-1.399	0.167
	% Cover	45	0.007	0.008	0.803	0.426	59	0.011	0.012	0.934	0.354	58	0.008	0.017	0.472	0.639
d) IGP	(Intercept)		-0.342	0.422	-0.810	0.422		-1.525	0.410	-3.716	<0.001					
	PSR	45	-0.068	0.149	-0.458	0.649	59	0.145	0.123	1.174	0.245	45				
	(Intercept)		-0.509	0.447	-1.138	0.261		-1.827	0.564	-3.237	0.002					
	FD	45	-0.005	0.166	-0.032	0.975	59	0.274	0.195	1.410	0.164	45				
	(Intercept)		-0.181	0.832	-0.218	0.829		-2.592	1.461	-1.775	0.081					
	% Cover	45	-0.005	0.013	-0.416	0.679	59	0.020	0.020	1.029	0.308	45				

Model	Parameter	*Pterostichus melanarius*					*Harpalus rufipes*					*Deroceras* sp.				
		denom. df	Estimate	*SE*	*t* value	*p* value	*denom. df*	Estimate	*SE*	*t* value	*p* value	*denom. df*	Estimate	*SE*	*t* value	*p* value
e) Microbiome	(Intercept)		2.072	0.054	38.470	<2e-16		2.086	0.107	19.520	<2e-16		1.980	0.080	24.744	<2e-16
	PSR	112	0.012	0.017	0.710	0.478	104	0.015	0.035	0.440	0.661	60	0.001	0.025	0.039	0.969
	(Intercept)		2.130	0.062	34.512	<2e-16		2.163	0.121	17.935	<2e-16		1.920	0.092	20.970	<2e-16
	FD	112	-0.010	0.024	-0.422	0.673	104	-0.014	0.045	-0.318	0.751	60	0.027	0.036	0.757	0.452
	(Intercept)		2.016	0.145	13.864	<2e-16		2.289	0.276	8.291	<0.001		1.834	0.211	8.682	<0.001
	% Cover	112	0.001	0.002	0.629	0.530	104	-0.002	0.004	-0.591	0.556	60	0.002	0.003	0.713	0.479
f) Symbionts	(Intercept)		0.277	0.084	3.307	0.001		0.321	0.135	2.379	0.019					
	PSR	112	0.043	0.027	1.604	0.110	104	0.016	0.044	0.356	0.723	112				
	(Intercept)		0.386	0.098	3.949	<0.001		0.493	0.149	3.305	0.001					
	FD	112	0.003	0.037	0.089	0.929	104	-0.053	0.056	-0.931	0.354	112				
	(Intercept)		0.223	0.232	0.963	0.336		0.961	0.332	2.891	0.005					
	% Cover	112	0.002	0.003	0.752	0.453	104	-0.009	0.005	-1.809	*0.073*	112				
g) Pathogens	(Intercept)		-0.392	0.144	-2.712	0.007		-0.353	0.222	-1.594	0.114					
	PSR	112	-0.073	0.050	-1.456	0.146	104	-0.056	0.075	-0.738	0.462	112				
	(Intercept)		-0.364	0.156	-2.328	0.021		-0.260	0.236	-1.103	0.273					
	FD	112	-0.094	0.062	-1.512	0.132	104	-0.101	0.091	-1.105	0.272	112				
	(Intercept)		-1.012	0.392	-2.581	0.010		-0.241	0.554	-0.435	0.664					
	% Cover	112	0.006	0.005	1.116	0.265	104	-0.004	0.008	-0.479	0.633	112				
h) Neutral (eDNA)	(Intercept)		2.636	0.079	33.551	<2e-16		2.708	0.106	25.526	<2e-16		2.694	0.070	38.682	<2e-16
	PSR	112	-0.013	0.027	-0.467	0.641	104	0.016	0.035	0.472	0.637	63	0.049	0.021	2.335	**0.020**
	(Intercept)		2.698	0.088	30.830	<2e-16		2.901	0.119	24.415	<2e-16		2.632	0.083	31.708	<2e-16
	FD	112	-0.041	0.034	-1.204	0.229	104	-0.060	0.044	-1.359	0.174	63	0.087	0.032	2.687	**0.007**
	(Intercept)		2.453	0.205	11.982	<2e-16		2.424	0.276	8.777	<2e-16		2.339	0.204	11.451	<2e-16
	% Cover	112	0.002	0.003	0.745	0.457	104	0.005	0.004	1.206	0.228	63	0.007	0.003	2.463	**0.014**

All taxa were assigned to interaction groups (see methods). We tested the effects of three explanatory variables on taxon richness in interaction groups for all consumers (except intraguild predation (IGP), symbiotic, and pathogenic interactions for *D.* sp.). A quasipoisson distribution was used for all models except neutral interactions, for which negative binomial models were fitted. *p* values <.05 are reported in bold numbers and *p* <.10 in italics. *denom. df*, denominator degrees of freedom; *SE*, standard errors

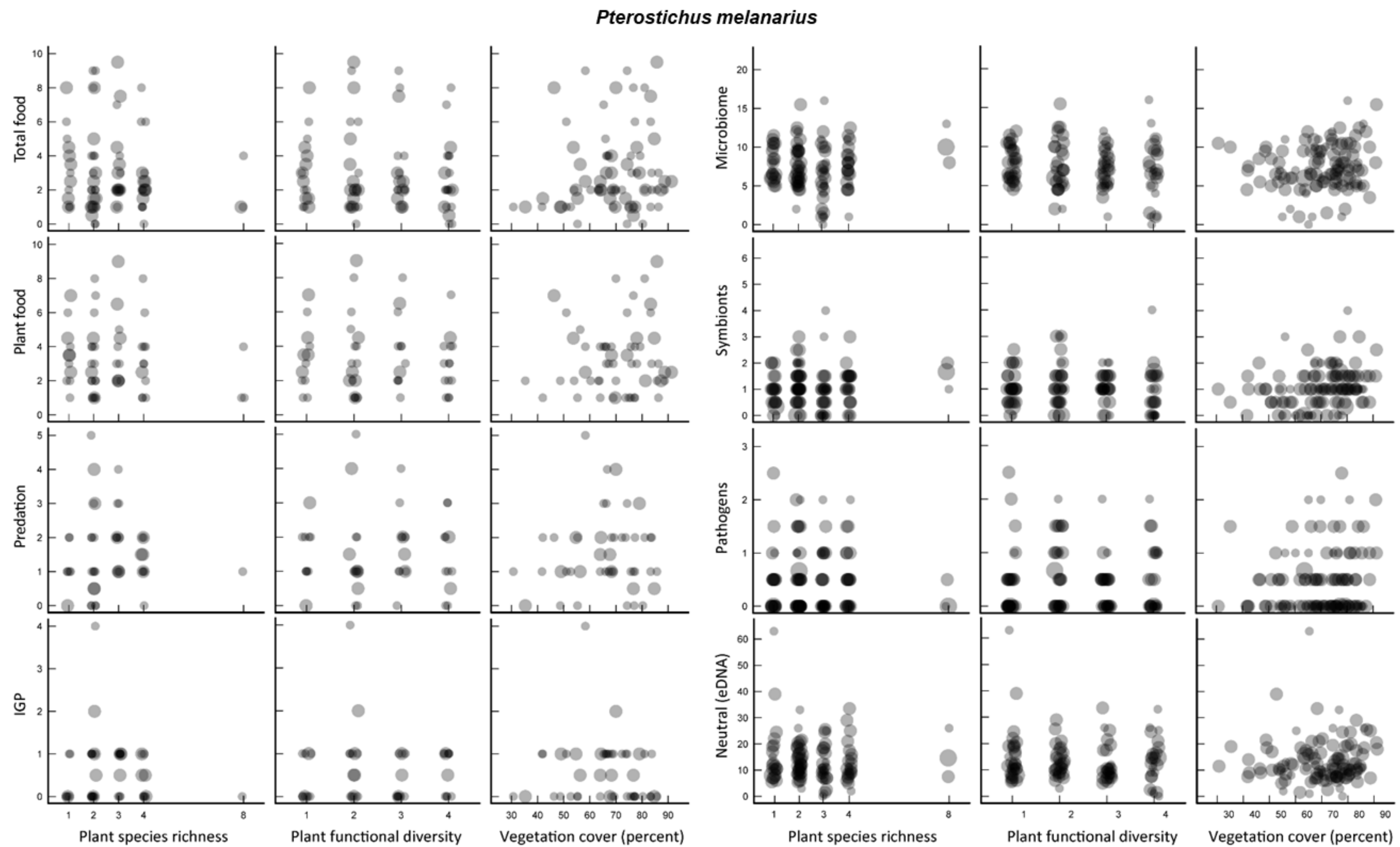


Figure 4. Effects of plant species richness, plant functional diversity, and percentage vegetation cover on trophic and microbial interactions detected in regurgitates of *P. melanarius.* Points represent individual plots and are scaled based on number of averaged biological replicates. None of the effects was significant.

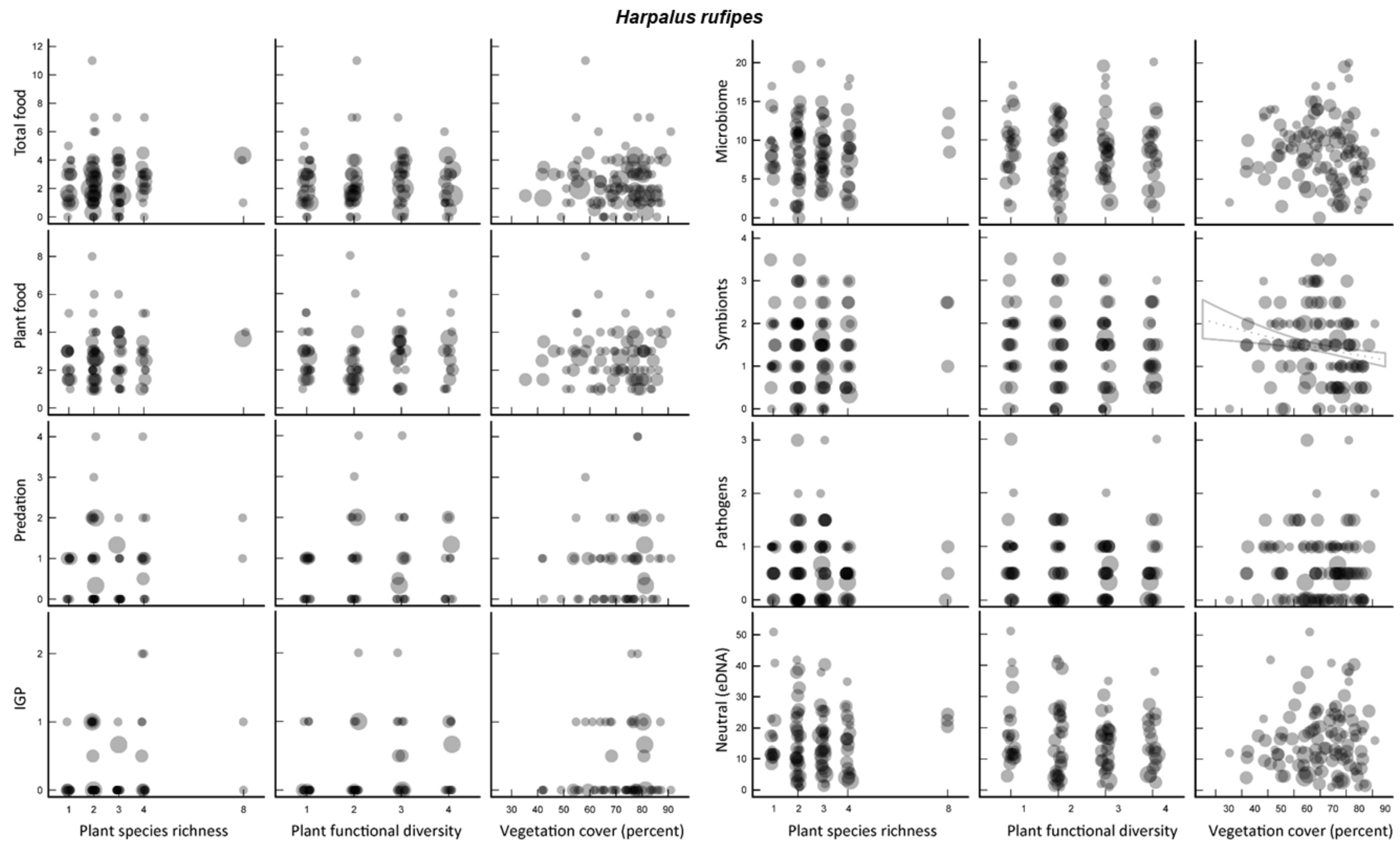


Figure 5. Effects of plant species richness, plant functional diversity, and percentage vegetation cover on trophic and microbial interactions detected in regurgitates of *H. rufipes*. Points represent individual plots and are scaled based on number of averaged biological replicates. Line shows model prediction, polygon shows 95% confidence intervals for effects with p<0.1.

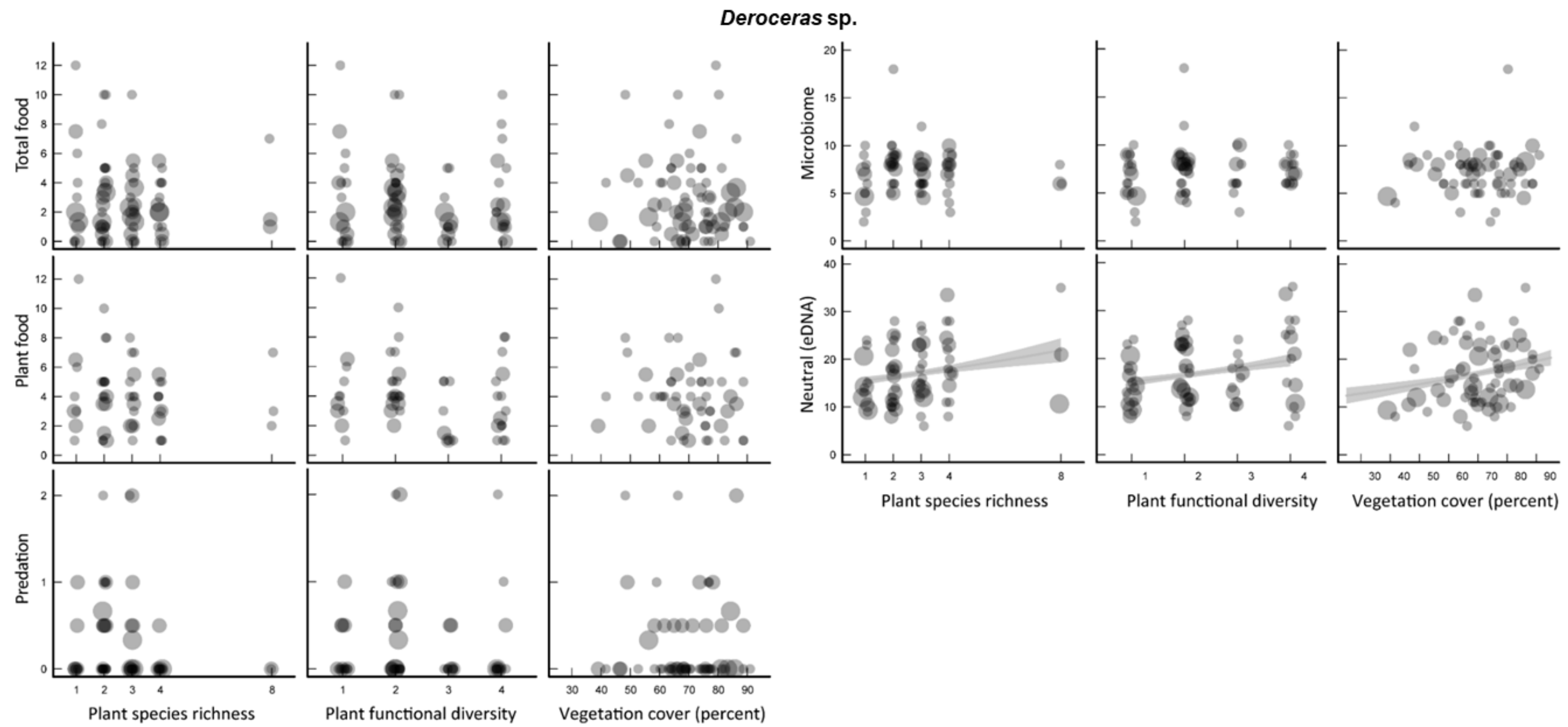


Figure 6. Effects of plant species richness, plant functional diversity, and percentage vegetation cover on trophic and microbial interactions detected in feces of *D.* sp. Points represent individual plots and are scaled based on number of averaged biological replicates. Lines show model predictions, polygons show 95% confidence intervals for effects with $p<0.05$.

4.3.3 Cascading effects of plant diversity on prey richness

We used a structural equation model to highlight pathways between manipulated plant species richness, the performance of the plant and animal (invertebrate) community and prey richness in gut contents of consumers as response variable (Fig. 7, Table S4). The results indicate that the three omnivores respond to a different set of variables. Plant species richness had a direct positive effect on prey richness in *D.* sp. and an indirect positive effect via the animal community in the plot. In contrast, plant species richness had direct negative effects on prey richness in samples of *H. rufipes* and no direct effect on *P. melanarius*. Animal richness and abundance were always the strongest predictors for the detected richness of prey species in gut contents of the three consumers. The effect of animal richness on prey richness was always negative, animal abundance had both, positive or negative effects. Differences between the impact of plant species richness on biomass and the arthropod community can be explained by a different set of plots for which we succeeded to sample gut contents for each consumer.

4.3.4 Average trophic position of food items

The average trophic position of food items (plant derived food and prey) was affected by an interaction between consumer identity and vegetation cover (Fig. 8, Table 4, Table S5). In *P. melanarius* the average trophic position of food decreased from 1.1 to 0.4 and increased in *H. rufipes* from 0.2 to 0.6 when vegetation cover increased from 40 to 90 percent. The field slug was feeding on an average trophic position of 0.2 irrespectively of cover. When only the two beetles and only prey as a response was analyzed, the effect stayed basically the same.; the average trophic position of *P. melanarius* dropped from 1.45 to 1.2 and increased in *H. rufipes* from 1.1 to 1.4.

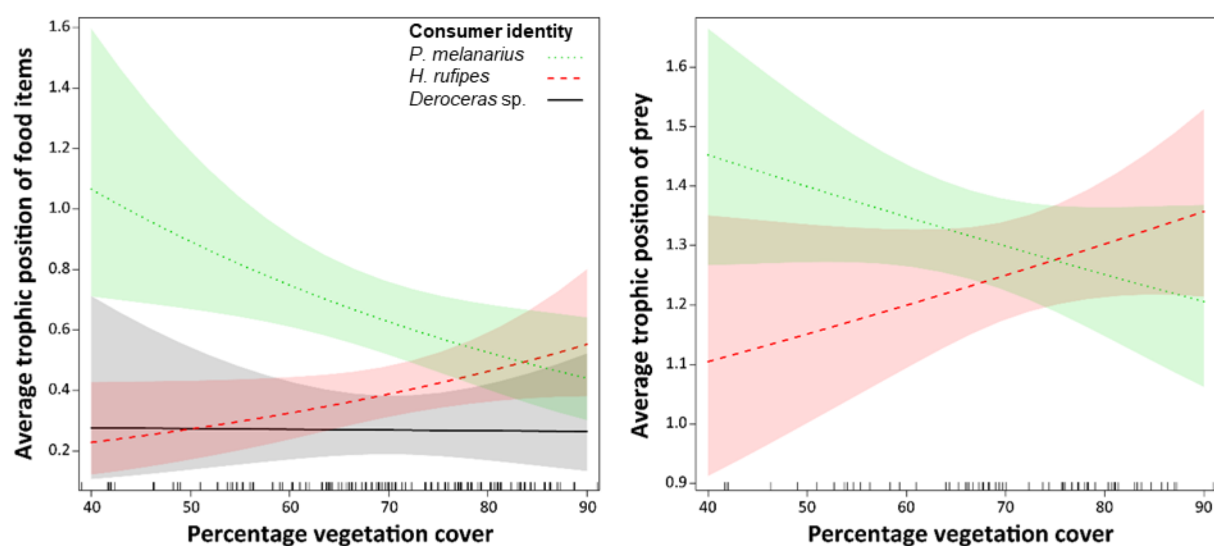


Figure 8. Vegetation cover has different effects on the average trophic position of total food items (plant and prey food) and prey in gut contents of omnivorous beetles. Lines and shaded regions show response predictions and 95% confidence intervals from the generalized linear models.

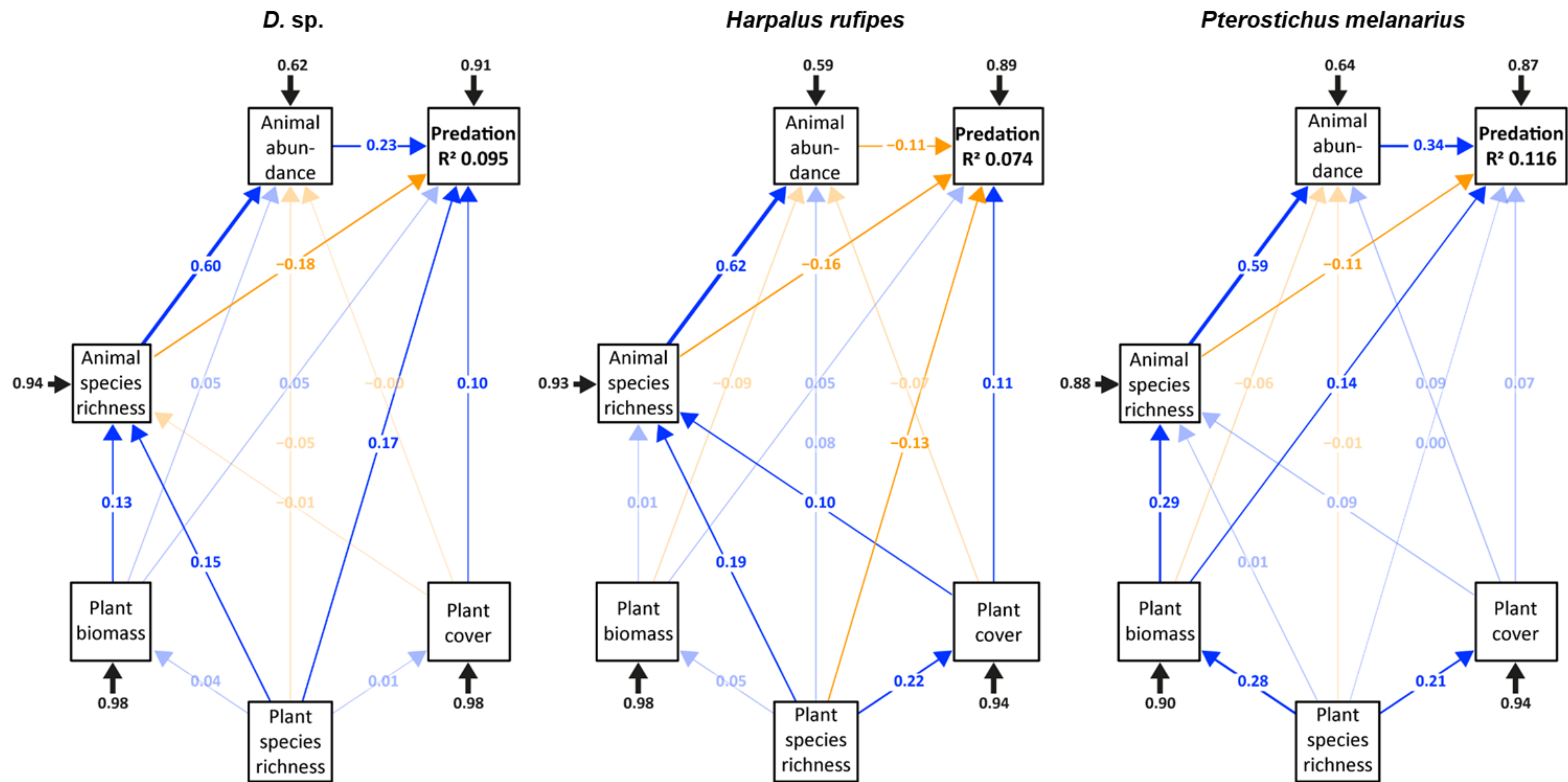


Figure 7. SEM showing the direct effects of plant species richness on plant community measures (plant biomass and plant cover) and the invertebrate community in the plot (animal abundance and animal richness) on the log-transformed detected richness of prey taxa in the gut of three omnivores ($\chi2$ = 2.2652; p = 0. 519, 3 degrees of freedom). Short black arrows are error terms, numbers standardized path coefficients, arrow width shows effect strength, blue color indicates a positive (+), and orange a negative (-) relationship. Arrows for effect sizes <0.1 were faded. R^2 values reported. See Supporting Information Table S4 for detailed results.

Table 4. Average trophic position of food items in gut content and feces of three omnivorous consumers.

Response	Term	*df*	*denom. df*	χ2	*p* value
a) Mean trophic position of total food	Plant species richness	1	216	0.003	0.957
	Consumer identity	2	214	24.843	**<0.001**
	Plant species richness x consumer identity	2	212	2.550	0.280
	Plant functional diversity	1	216	0.833	0.362
	Consumer identity	2	214	24.971	**<0.001**
	Plant functional diversity x consumer identity	2	212	2.459	0.292
	Vegetation cover	1	216	0.337	0.562
	Consumer identity	2	214	25.308	**<0.001**
	Vegetation cover x consumer identity	2	212	9.879	**0.007**
	Invertebrate species richness	1	216	0.025	0.873
	Consumer identity	2	214	25.393	**<0.001**
	Invertebrate species richness x consumer identity	2	212	7.332	0.026
	Invertebrate abundance	1	216	0.677	0.411
	Consumer identity	2	214	25.505	**<0.001**
	Invertebrate abundance x consumer identity	2	212	2.602	0.272
b) Mean trophic position of prey	Plant species richness	1	75	0.097	0.755
	Consumer identity	1	74	0.877	0.349
	Plant species richness x consumer identity	1	73	1.039	0.308
	Plant functional diversity	1	75	2.338	0.126
	Consumer identity	1	74	0.875	0.350
	Plant functional diversity x consumer identity	1	73	2.418	0.120
	Vegetation cover	1	75	0.052	0.820
	Consumer identity	1	74	0.771	0.380
	Vegetation cover x consumer identity	1	73	4.491	**0.034**
	Invertebrate species richness	1	75	1.062	0.303
	Consumer identity	1	74	0.685	0.408
	Invertebrate species richness x consumer identity	1	73	0.151	0.697
	Invertebrate abundance	1	75	0.001	0.976
	Consumer identity	1	74	0.857	0.355
	Invertebrate abundance x consumer identity	1	73	0.088	0.767

We tested the effects of consumer species identity and five explanatory variables on the mean trophic position total food (plant and prey) and prey items in separate GLMs using a quasipoisson distribution. (*D.* sp. was excluded from models for the mean trophic position of prey). *p* values <.05 are reported in bold numbers and *p* <.10 in italics. denom. *df*, denominator degrees of freedom; *SE*, standard errors

4.3.5 Proportional composition of gut content

The proportional composition of animal to prey food in gut contents was affected by species identity, with a higher proportion of animal taxa compared to plants in gut contents of beetles

(Fig. 9, Table 5, Table S6). The proportion of intraguild prey in total prey slightly increased in *H. rufipes* and decreased in *P. melanarius*, but the effect was not significant. The proportion of fungal taxa in the total microbial community (fungi and bacteria) decreased significantly with plant species richness and percentage vegetation cover in all three consumer species. Within the subset of microbes with for which beneficial or harmful relations are reported in the literature, the proportion of symbionts increased slightly in both beetles, but the effect was not significant.

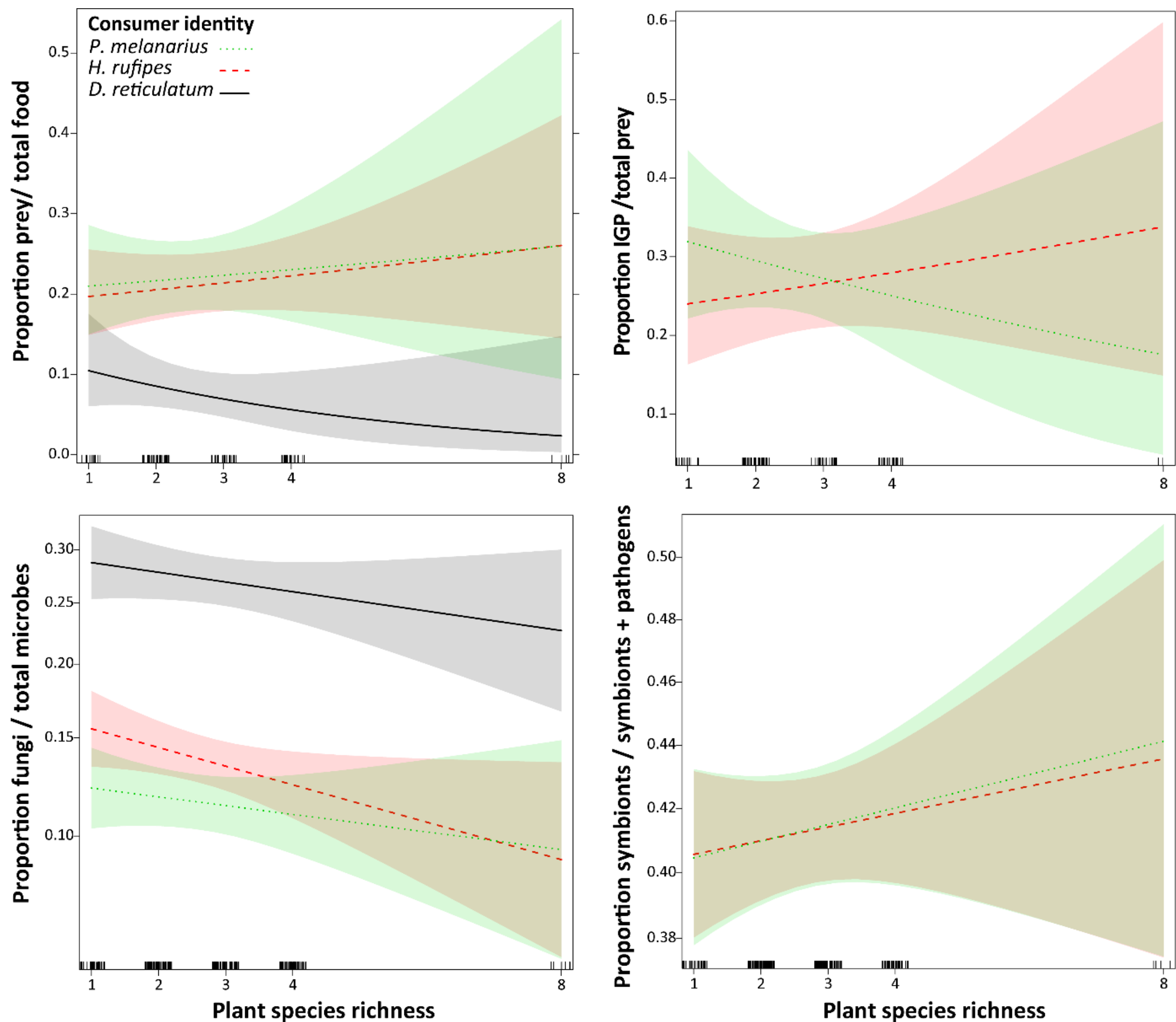


Figure 9. Response of the proportional composition of gut content to plant species richness. Lines and shaded regions show response predictions and 95% confidence intervals from the generalized linear models.

Table 5. Proportional composition of gut content and feces of three omnivorous consumers.

Response	Term	df	denom. df	χ2	p value
a) Proportion prey / total food	Plant species richness	1	114	0.083	0.773
	Consumer identity	2	112	44.568	**<0.001**
	Plant species richness x consumer identity	2	110	2.455	0.293
	Plant functional diversity	1	114	0.244	0.621
	Consumer identity	2	112	43.826	**<0.001**
	Plant functional diversity x consumer identity	2	110	0.110	0.947
	Vegetation cover	1	114	0.157	0.692
	Consumer identity	2	112	46.033	**<0.001**
	Vegetation cover x consumer identity	2	110	3.125	0.210
	Invertebrate species richness	1	114	0.188	0.665
	Consumer identity	2	112	47.660	**<0.001**
	Invertebrate species richness x consumer identity	2	110	1.472	0.479
	Invertebrate abundance	1	114	0.755	0.385
	Consumer identity	2	112	47.070	**<0.001**
	Invertebrate abundance x consumer identity	2	110	3.317	0.190
b) Proportion IGP / total prey	Plant species richness	1	75	0.000	0.992
	Consumer identity	1	74	0.168	0.682
	Plant species richness x consumer identity	1	73	1.206	0.272
	Plant functional diversity	1	75	0.998	0.318
	Consumer identity	1	74	0.190	0.663
	Plant functional diversity x consumer identity	1	73	1.292	0.256
	Vegetation cover	1	75	0.661	0.416
	Consumer identity	1	74	0.020	0.889
	Vegetation cover x consumer identity	1	73	2.004	0.157
	Invertebrate species richness	1	75	0.483	0.487
	Consumer identity	1	74	0.037	0.848
	Invertebrate species richness x consumer identity	1	73	1.087	0.297
	Invertebrate abundance	1	75	0.214	0.643
	Consumer identity	1	74	0.059	0.808
	Invertebrate abundance x consumer identity	1	73	0.500	0.479
c) Proportion symbionts / symbionts + pathogens	Plant species richness	1	204	0.819	0.366
	Consumer identity	1	203	0.089	0.765
	Plant species richness x consumer identity	1	202	0.000	0.988
	Plant functional diversity	1	204	0.046	0.831
	Consumer identity	1	203	0.000	0.984
	Plant functional diversity x consumer identity	1	202	0.158	0.691
	Vegetation cover	1	204	0.257	0.612
	Consumer identity	1	203	0.007	0.935
	Vegetation cover x consumer identity	1	202	0.069	0.792
	Invertebrate species richness	1	204	1.590	0.207
	Consumer identity	1	203	0.005	0.945
	Invertebrate species richness x consumer identity	1	202	0.108	0.742
	Invertebrate abundance	1	204	0.275	0.600
	Consumer identity	1	203	0.000	0.997
	Invertebrate abundance x consumer identity	1	202	0.166	0.683

Response	Term	df	denom. df	χ2	p value
d) Proportion fungi / total microbes	Plant species richness	1	258	6.301	0.012
	Consumer identity	2	256	174.782	**<0.001**
	Plant species richness x consumer identity	2	254	0.756	0.685
	Plant functional diversity	1	258	1.395	0.238
	Consumer identity	2	256	167.910	**<0.001**
	Plant functional diversity x consumer identity	2	254	0.048	0.976
	Vegetation cover	1	258	4.865	0.027
	Consumer identity	2	256	173.695	**<0.001**
	Vegetation cover x consumer identity	2	254	4.194	0.123
	Invertebrate species richness	1	258	1.220	0.269
	Consumer identity	2	256	159.913	**<0.001**
	Invertebrate species richness x consumer identity	2	254	0.613	0.736
	Invertebrate abundance	1	258	0.093	0.761
	Consumer identity	2	256	160.800	**<0.001**
	Invertebrate abundance x consumer identity	2	254	2.683	0.261

We tested the effects of consumer species identity and five explanatory variables on the proportional composition of gut content and feces in separate GLMs using a quasibinomial distribution. (*D.* sp. was excluded from models for intraguild prey (IGP), and proportion of symbionts). *p* values <.05 are reported in bold numbers and *p* <.10 in italics. *denom. df*, denominator degrees of freedom; *SE*, standard errors

4.3.6 Microbial community composition

We detected a significant effect of the identity of consumer species on the community composition and variances in sample dispersion of bacteria and fungi in the guts of three omnivores (perMANOVA; PERMDISP; Fig. 10, Table 6). The effect was weaker but significant when only the beetles were compared. No impact of plant species richness was detected in separate models for individual consumers.

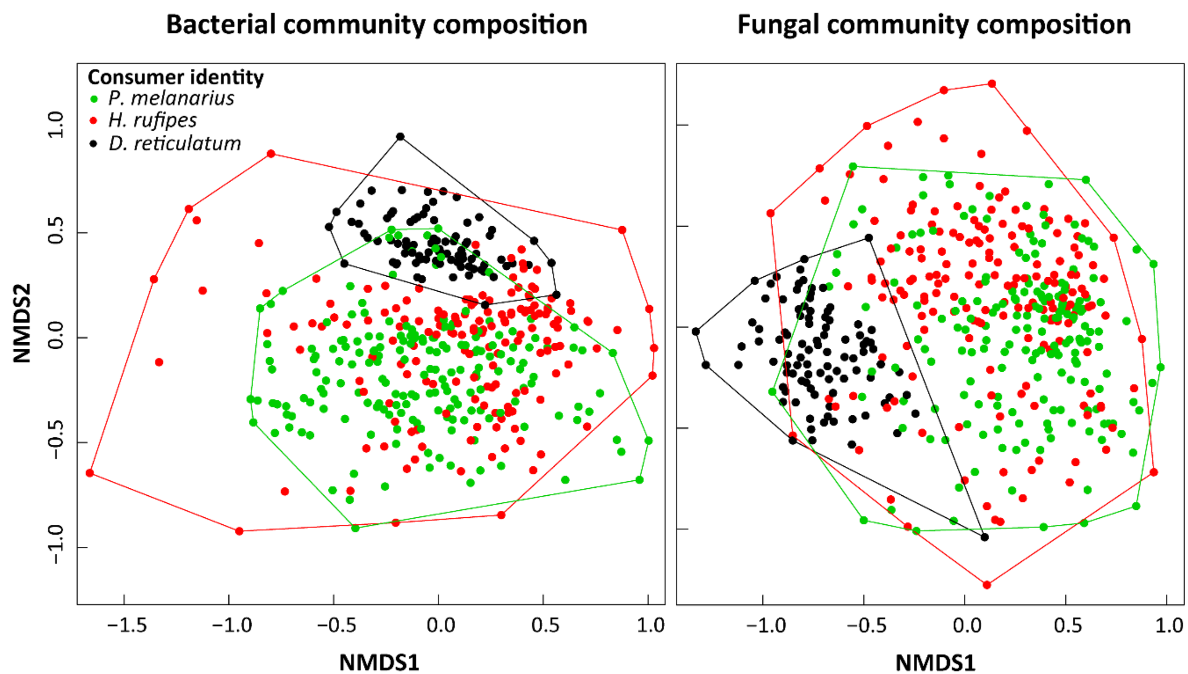


Figure 10. Community composition of bacteria and fungi in gut contents of three omnivores. NMDS plots (2D, stress = 0.23 for bacteria, and = 0.28 for fungi) are based on Bray–Curtis dissimilarities of the Hellinger-transformed taxon abundance (aggregated on genus level). Symbols represent individual samples; colors and enclosing polygons refer to consumer identity.

Table 6. Community composition of bacteria and fungi analyzed with perMANOVA and PERMDISP.

perMANOVA			*df*	SS	*F* value	*p* value
Bacteria	All consumers	Consumer identity	2	12.57	17.33	**0.001**
		Residuals	437	158.46	0.93	[R^2 = 0.07]
	P. melanarius and *H. rufipes*	Consumer identity (beetles)	1	3.09	8.15	**0.001**
		Residuals	351	133.29	0.98	[R^2 = 0.02]
	P. melanarius	Plant species richness	1	0.39	1.02	1
		Residuals	188	71.56	0.99	[R^2 = 0.01]
	H. rufipes	Plant species richness	1	0.35	0.91	1
		Residuals	161	62.06	0.99	[R^2 = 0.01]
	D. sp.	Plant species richness	1	0.48	1.48	1
		Residuals	85	27.53	0.98	[R^2 = 0.02]
Fungi	All consumers	Plant species richness	2	12.32	14.60	**0.001**
		Residuals	455	192.05	0.94	[R^2 = 0.06]
	P. melanarius and *H. rufipes*	Plant species richness	1	2.20	5.11	**0.001**
		Residuals	359	154.61	0.99	[R^2 = 0.01]
	P. melanarius	Plant species richness	1	0.40	0.92	1
		Residuals	185	79.92	1.00	[R^2 < 0.01]
	H. rufipes	Plant species richness	1	0.38	0.88	1
		Residuals	172	74.81	0.99	[R^2 = 0.01]
	D. sp.	Plant species richness	1	0.40	1.03	1
		Residuals	95	37.18	0.99	[R^2 = 0.01]
PERMDISP			*df*	SS	*F* value	*p* value
Bacteria	All consumers	Consumer identity	2	0.53	113.93	**0.001**
		Residuals	437	1.02		
	P. melanarius and *H. rufipes*	Consumer identity (beetles)	1	0.01	3.09	*0.071*
		Residuals	351	0.70		
	P. melanarius	Plant species richness	4	0.00	0.92	0.461
		Residuals	185	0.23		
	H. rufipes	Plant species richness	4	0.00	0.35	0.842
		Residuals	158	0.38		
	D. sp.	Plant species richness	4	0.02	1.41	0.248
		Residuals	82	0.23		
Fungi	All consumers	Consumer identity	2	0.04	4.53	**0.014**
		Residuals	455	1.94		
	P. melanarius and *H. rufipes*	Consumer identity (beetles)	1	0.02	3.69	**0.040**
		Residuals	359	1.60		
	P. melanarius	Plant species richness	4	0.04	1.88	0.126
		Residuals	184	0.95		
	H. rufipes	Plant species richness	4	0.01	0.71	0.592
		Residuals	169	0.49		
	D. sp.	Plant species richness	4	0.00	0.51	0.744
		Residuals	93	0.17		

PerMANOVA results on the effect of species identity and plant species richness on the community composition. PERMDISP results on homogeneity of multivariate sample dispersion. *p* values <.05 are reported in bold numbers. *df*, degrees of freedom; *SE*, standard errors; SS, sums of squares.

4.4 Discussion

Our study is the first to simultaneously examine the direct and indirect impact of plant diversity on the multitude of interactions involving omnivores in a controlled grassland biodiversity experiment. We found that dietary changes were mainly driven by indirect effects of plant diversity through differences in the performance of the plant community (percentage vegetation cover) or through bottom-up effects on the invertebrate community. Direct effects of plant species richness were only found for the microbial community. One of the strongest patterns observed in this study were differences in the response of the consumer species, indicating that each of the three omnivores interacts with a different subset of species in its local habitat.

4.4.1 General composition of detected taxa

All three omnivores had DNA of mostly the same plant orders in their gut content. This finding could be an indicator for non-selective feeding on plant material. Only taxa belonging to the Rosales were found in a bigger proportion in beetle samples than slug samples. To these belonged *Rubus* and *Urtica. Urtica* occasionally grows as a weed on the plots and *Rubus* is mainly found in the hedgerows surrounding the Jena Experiment. *Rubus* seeds were distributed by fruit feeding birds during the sampling period. The detection of *Rubus* DNA in Carabid gut contents could indicate that Carabids may prefer feeding on these relatively large seeds when they are available and hence limit the establishment of invasive *Rubus* species by seed predation (Erschbamer & Caccianiga 2016).

The three omnivores in our study were more selective in the consumption of animal prey than in the consumption of plant material. Many of the *D*. sp. specimen had consumed aphids and to a lower proportion also earthworms. Both are common prey types of the facultative carnivorous slugs (Barker & Efford 2004). Earthworms were also common prey for *P. melanarius*, confirming previous studies (Symondson et al. 2009). Ant predation was more common in beetles (Thiele et al. 1977) than in slugs. DNA of spiders and other beetles was exclusively found in beetles, both groups have been previously described as intraguild prey for carabids (Prasad & Snyder 2006; Davey et al. 2013). The detection of feeding links that are well described in the literature confirms that NGS of gut contents produces biologically meaningful results.

4.4.2 Direct and indirect effects of plant biodiversity on trophic interactions

In our pilot study, we found a general positive trend of plant taxonomic and functional diversity and vegetation cover on the richness of food types in gut content of *P. melanarius* (Tiede et al. 2016). This trend could not be confirmed with our extended study. In contrary, in plots with high plant species richness *P. melanarius*, fed on fewer food types, although this trend was not significant. The other two consumers showed no response to basal diversity either. One possible explanation for these differences might result from the low number of replicates in the pilot study (18 samples from 13 plots) produced no meaningful result. Considering the

general positive trends covering all analyzed interaction groups over different measures of the plant community this is unlikely. Alternatively, the different primer sets and nucleotide database that we used in the two studies may have detected different subsets of the total community of plants and animals (Alberdi et al. 2017). An additional ecological explanation is, that the invertebrate community that was present during the sampling campaign for the pilot study in August 2013 was not representative for other year. An extreme flood inundated the plots in the first weeks of June 2013 (Wright et al. 2015) and resulted in a high mortality of soil invertebrates (N. Eisenhauer, personal observation). Although some species, e.g. *Lasius niger* (Hertzog et al. 2016a) were unaffected, it is unlikely that the whole community had fully recovered just two months after the water retreated. In conclusion, the present study is more likely to have sampled the regular plot community.

In this study, we did not find a direct correlation between the richness of plant species in the plot and the richness of trophic interactions in gut contents of omnivores. This may not be too surprising for the two beetles, that are strongly linked to the animal community (Thiele et al. 1977). The mainly herbivorous field slugs (Barker & Efford 2004) did, unexpectedly, not respond to plant diversity either. The snap shot that gut contents provide might be too short to capture effects on the dietary diversity. Specimen can only handle a limited number of food items within a certain time range, which may limit the chances to detect increasing dietary diversity. Further studies could compare the beta diversity of multiple gut content samples per plot between levels of plant diversity to get a broader picture of the diet on a plot instead of an individuum level.

Structural equation modeling revealed that indirect effects of plant species richness were more important for prey richness in gut content than direct effects. Different variables were important for each consumer but overall, plant species richness increased the availability of basal resources, which positively affected the richness of invertebrates in the plot. Invertebrate richness strongly increased abundance and both were linked to prey richness in gut content, but the directional effects were different for different consumers. Animal abundance increased prey richness in gut contents of the more aggressive *P. melanarius* but decreased it in *H. rufipes*, maybe as a response of the later species to greater competition with other predators.

Vegetation cover mediated at which trophic level the two beetles were feeding. The more carnivorous *P. melanarius* fed at lower trophic levels when the vegetation cover was high. In contrast, the true omnivore *H. rufipes* included more food from higher trophic levels in densely covered plots. The proportion of prey taxa in the food was the same for both beetles and not changing with vegetation cover. Together these results indicate that intraguild predation plays a greater role for *P. melanarius* in low vegetation cover and vice versa in *H. rufipes*. Indeed, the proportion of intraguild prey increased in *H. rufipes* and decreased in *P. melanarius* although the effect was not significant.

4.4.3 Host associated microbes

The richness of microbes with potential beneficial effects for the consumer increased in dense vegetation in *P. melanarius* but decreased in *H. rufipes*, again highlighting the different

response of these two omnivores to vegetation cover. The ratio of potential symbionts to potential pathogens was the same in the two species and did not respond to vegetation cover or any other variable, not confirming our hypothesis that the ratio of beneficial to harmful microbes would change along the plant diversity gradient. Taxonomic richness of pathogens and infection rates do not necessarily correlate. Rottstock et al. (2014) found that the richness of plant pathogens increased with plant species richness, but the pathogen damage decreased.

A potential bias in the analysis of associated microbes is the limited knowledge on microbe-invertebrate interactions. Entomopathogens are well studied due to their use as biological control agents (e.g., Goettel et al. 2005; Parsa et al. 2013) but studies on symbiotic interactions are mostly limited to specialized herbivores, while the literature on the associations with consumer at higher trophic levels is generally sparse. Further, the role of the same microbial taxon can change depending on its co-occurring species, environment, and host conditions (Vega & Blackwell 2005).

4.4.4 Microbial eDNA

We hypothesized that plant diversity will directly or indirectly increase the richness of microbes without direct relation to the consumers (e.g., plant pathogens, saprobionts). This assumption was based on findings that the richness of plant pathogens, soil microbes, and the soil microbial activity increased with plant species richness (Scherber et al. 2010; Lange et al. 2015). Further, the richness of fungi and protists in guts of *P. melanarius* increased with vegetation cover (Tiede et al. 2017). Our findings did only partly confirm this pattern. Feces of *D*. sp. contained indeed a greater diversity of microbes when the slugs had fed in plots with high plant species richness or dense vegetation, whereas the microbial richness in beetle regurgitates was not affected. Interestingly the proportion of fungal taxa in the total microbial community in gut contents of all three omnivores decreased with increasing plant species richness and vegetation cover. This contrasts previous findings of an increasing richness of soil and plant pathogenic fungi and increasing ratio of fungal-to-bacterial biomass with plant diversity (Rottstock et al. 2014; Markus Lange 2015; Eisenhauer et al. 2017; Dassen et al. 2017).

Consumer identity strongly shaped the microbial community composition. Differences between the slug and beetle samples might originate from the use of different source material (feces, and regurgitates). The differences between the two beetle species might rather result from a different set of core microbes as it has been previously found in other omnivorous or carnivorous species (Anderson et al. 2012; Mrázek et al. 2008; Tiede et al. 2017). Diet is another major determinant for the gut microbiome of many invertebrates (Broderick et al. 2004; Lundgren & Lehman 2010; Mason & Raffa 2014; Wang et al. 2011). Differences in the feeding behavior between the beetles, that are indicated by the detection of different prey types, might further explain variation in the microbial community composition.

Our assumption that the microbial communities would become more similar with increasing plant species richness was not confirmed. In other studies, plant species richness was also only a weak predictor for the community composition of soil living bacteria, and fungi (Dassen et al. 2017).

4.5 Conclusions

Metabarcoding of gut contents allows new insights into the multitude of interactions between species in complex communities. Used in controlled biodiversity field experiments this approach can shed light on mechanisms that could not be observed before. In particular, the combined study of trophic and microbial interactions can increase our understanding of how plant diversity, directly or through cascading effects, shapes processes in the ecological communities and the communities themselves. Our results suggest that cascading effects of plant species richness via changes in the performance of the plant community and via the bottom-up food web are the main drivers for interactions involving omnivores. Overall, this study highlights the importance of including multiple trophic levels in empirical biodiversity studies to fully understand processes in complex communities.

4.6 Acknowledgements

We greatly acknowledge the great help of Camille Zwaan and Jan-Hendrick Dudenhöffer during the fieldwork in the Jena Experiment. We further thank all persons involved in the maintenance of the Jena Experiment. The authors thank Caroline Steffens, and Darja Andert for gut dissections and morphological prey analysis. We also thank Rebecca Meyer for DNA extractions at the University of Innsbruck, and Andrea Thürmer for sequencing our samples in the Göttingen Genomics Laboratory. The authors greatly acknowledge all persons who provides us species lists from the Jena Experiment and other biodiversity experiments. The Deutsche Forschungsgemeinschaft supported the Jena Experiment and this study (grants FOR 1451/2 and SCHE 1621/3-2).

4.7 Supporting Information

Table S1. Overview over the number of samples per plot, and primer set used for the analysis of plants (trnL), animals (COI), fungi (ITS), and bacteria (16S) in regurgitates of beetles or slug feces.

				P. melanarius				*H. rufipes*				*D.* sp.			
Experiment	Plant community	PSR	Plot ID	trnL	COI	ITS	16S	trnL	COI	ITS	16S	trnL	COI	ITS	16S
JE-TBE	monoculture	1	B0BA009	0	0	0	0	0	0	0	0	2	2	2	3
JE-TBE	monoculture	1	B0BA015	2	0	2	2	2	0	1	1	0	1	1	1
JE-TBE	monoculture	1	B0BA016	0	1	1	2	1	0	1	1	1	1	1	1
JE-TBE	monoculture	1	B0BA020	1	0	2	2	2	2	2	1	0	1	1	1
JE-TBE	monoculture	1	B0BA027	0	0	0	0	0	0	0	0	0	0	0	0
JE-TBE	monoculture	1	B0BA029	2	0	1	2	0	0	1	1	1	0	1	1
JE-TBE	monoculture	1	B0BA040	1	1	2	2	1	1	1	1	0	0	0	0
JE-TBE	monoculture	1	B0BA043	2	1	1	2	2	0	2	1	0	2	2	1
JE-TBE	monoculture	1	B0BB048	0	0	0	0	0	0	0	0	1	0	1	1
JE-TBE	monoculture	1	B0BB059	0	0	1	1	0	0	0	0	0	0	0	0
JE-TBE	monoculture	1	B0BB060	2	1	2	2	0	0	0	0	2	2	2	2
JE-TBE	monoculture	1	B0BB064	1	1	1	1	1	1	1	1	0	0	0	0
JE-TBE	monoculture	1	B0BB068	0	0	2	2	0	0	0	0	0	0	0	0
JE-TBE	monoculture	1	B0BB072	0	0	2	2	2	1	1	2	1	2	1	1
JE-TBE	monoculture	1	B0BB085	0	0	2	2	0	0	0	0	0	0	0	0
JE-TBE	monoculture	1	B0BB089	1	0	2	2	2	1	2	2	0	0	0	0
JE-TBE	monoculture	1	B0BC097	0	0	0	1	0	1	0	1	0	0	0	0
JE-TBE	monoculture	1	B0BC106	2	0	2	2	2	2	2	2	0	0	0	0
JE-TBE	monoculture	1	B0BC110	0	0	2	2	1	1	2	2	1	1	2	2
JE-TBE	monoculture	1	B0BC117	1	2	2	2	2	0	1	1	0	0	0	0
JE-TBE	monoculture	1	B0BC118	0	1	2	2	0	0	0	0	0	0	0	0
JE-TBE	monoculture	1	B0BC121	1	0	2	2	1	0	2	2	0	0	0	0
JE-TBE	monoculture	1	B0BC131	0	0	2	2	0	1	0	1	0	0	0	0
JE-TBE	monoculture	1	B0BC133	0	0	2	2	1	1	2	2	2	2	2	1
JE-TBE	mixed community	2	B0BA002	0	0	2	2	1	1	2	2	0	0	0	0
JE-TBE	mixed community	2	B0BA004	1	1	2	2	1	0	2	1	0	0	0	0
JE-TBE	mixed community	2	B0BA006	0	0	2	2	2	1	2	1	2	3	2	2
JE-TBE	mixed community	2	B0BA007	1	0	2	2	0	0	0	0	1	1	1	1
JE-TBE	mixed community	2	B0BA008	0	0	0	0	0	3	2	1	1	1	1	1
JE-TBE	mixed community	2	B0BA011	0	0	2	2	0	1	2	2	2	2	2	2
JE-TBE	mixed community	2	B0BA012	1	1	2	2	2	1	2	1	0	0	0	0
JE-TBE	mixed community	2	B0BA013	1	1	1	1	1	1	2	1	1	2	2	1
JE-TBE	mixed community	2	B0BA022	1	1	2	2	1	0	2	1	0	0	0	0
JE-TBE	mixed community	2	B0BA026	0	0	0	0	1	0	1	1	0	0	0	0
JE-TBE	mixed community	2	B0BA028	2	0	2	2	0	0	0	0	0	0	0	0
JE-TBE	mixed community	2	B0BA038	0	0	0	2	2	1	2	2	0	0	0	0
JE-TBE	mixed community	2	B0BA041	0	2	1	1	2	1	2	1	0	0	0	0
JE-TBE	mixed community	2	B0BA042	0	0	0	0	1	1	1	1	1	1	2	2
JE-TBE	mixed community	2	B0BA044	1	1	2	2	0	0	0	0	0	0	0	0
JE-TBE	mixed community	2	B0BA046	0	0	0	0	1	1	2	0	0	2	0	0
JE-TBE	mixed community	2	B0BB050	1	0	0	2	1	0	1	1	1	1	1	1
JE-TBE	mixed community	2	B0BB054	0	0	2	2	1	1	1	1	0	1	1	1
JE-TBE	mixed community	2	B0BB061	1	1	2	2	2	0	1	2	0	0	0	0
JE-TBE	mixed community	2	B0BB063	0	0	0	0	0	0	0	0	0	0	0	0
JE-TBE	mixed community	2	B0BB069	1	0	2	2	2	0	2	2	0	0	0	0
JE-TBE	mixed community	2	B0BB070	1	0	1	2	1	1	2	2	0	0	0	0
JE-TBE	mixed community	2	B0BB071	0	0	2	2	3	0	1	2	0	0	0	0
JE-TBE	mixed community	2	B0BB074	1	0	1	1	0	0	0	0	2	0	2	1
JE-TBE	mixed community	2	B0BB080	2	2	2	2	0	0	1	0	0	0	0	0

				P. melanarius				*H. rufipes*				*D.* sp.			
Experiment	Plant community	PSR	Plot ID	trnL	COI	ITS	16S	trnL	COI	ITS	16S	trnL	COI	ITS	16S
JE-TBE	mixed community	2	B0BB081	0	2	2	2	0	0	2	1	0	0	0	0
JE-TBE	mixed community	2	B0BB083	1	2	1	1	2	0	1	2	0	0	0	0
JE-TBE	mixed community	2	B0BB087	0	1	1	2	1	0	1	1	0	0	0	0
JE-TBE	mixed community	2	B0BB088	2	0	2	2	1	1	2	0	0	0	0	0
JE-TBE	mixed community	2	B0BB090	0	0	2	2	1	0	0	0	0	2	1	1
JE-TBE	mixed community	2	B0BB091	0	0	1	2	3	1	0	1	0	0	0	0
JE-TBE	mixed community	2	B0BB092	1	1	0	1	2	1	1	1	1	1	1	1
JE-TBE	mixed community	2	B0BC093	0	0	0	1	0	1	0	1	1	0	1	1
JE-TBE	mixed community	2	B0BC099	0	0	1	1	0	1	2	2	2	2	2	2
JE-TBE	mixed community	2	B0BC100	1	1	1	2	0	0	1	1	0	1	0	0
JE-TBE	mixed community	2	B0BC102	0	0	0	0	2	0	2	2	0	1	1	0
JE-TBE	mixed community	2	B0BC105	0	0	1	1	0	1	2	2	0	0	0	0
JE-TBE	mixed community	2	B0BC108	0	1	2	2	0	0	0	0	1	1	0	0
JE-TBE	mixed community	2	B0BC114	0	0	1	1	0	0	0	0	0	0	0	0
JE-TBE	mixed community	2	B0BC120	1	1	2	2	2	2	1	1	1	1	1	1
JE-TBE	mixed community	2	B0BC123	0	0	0	0	1	1	2	2	1	1	1	1
JE-TBE	mixed community	2	B0BC125	0	0	0	0	1	1	1	1	2	1	2	2
JE-TBE	mixed community	2	B0BC126	1	0	2	2	2	0	2	2	0	0	0	0
JE-TBE	mixed community	2	B0BC127	0	1	2	2	1	1	2	2	0	0	0	0
JE-TBE	mixed community	2	B0BC132	0	1	2	1	2	3	2	2	1	0	2	1
JE-TBE	mixed community	2	B0BC134	0	0	2	1	1	2	2	2	1	1	1	1
JE-TBE	mixed community	2	B0BC135	0	0	2	1	0	0	0	0	0	2	2	2
JE-TBE	mixed community	2	B0BC137	0	0	2	1	0	0	0	0	0	1	0	0
JE-TBE	mixed community	3	B0BA001	0	1	2	2	1	3	1	1	2	3	2	2
JE-TBE	mixed community	3	B0BA014	0	0	0	0	0	0	1	1	1	1	1	1
JE-TBE	mixed community	3	B0BA017	0	0	2	2	0	0	0	0	0	1	0	0
JE-TBE	mixed community	3	B0BA019	0	1	0	1	1	0	2	1	0	0	0	0
JE-TBE	mixed community	3	B0BA021	1	0	2	2	0	1	2	1	0	0	0	0
JE-TBE	mixed community	3	B0BA024	2	0	2	2	0	1	2	1	0	0	0	0
JE-TBE	mixed community	3	B0BA025	1	0	2	1	2	0	2	1	1	1	1	1
JE-TBE	mixed community	3	B0BA031	1	1	1	2	0	0	0	0	0	2	1	1
JE-TBE	mixed community	3	B0BA033	1	0	1	1	1	0	2	2	1	1	1	1
JE-TBE	mixed community	3	B0BA034	2	0	2	2	2	0	2	2	0	2	2	1
JE-TBE	mixed community	3	B0BA037	0	0	0	0	1	0	1	1	2	2	2	3
JE-TBE	mixed community	3	B0BA039	0	0	0	0	2	0	2	1	1	0	1	1
JE-TBE	mixed community	3	B0BB047	2	1	1	2	1	0	2	1	0	0	0	0
JE-TBE	mixed community	3	B0BB053	0	2	1	2	0	1	2	2	1	2	2	2
JE-TBE	mixed community	3	B0BB055	0	0	1	2	2	1	2	3	0	1	1	1
JE-TBE	mixed community	3	B0BB058	2	0	1	0	0	0	0	0	0	0	0	0
JE-TBE	mixed community	3	B0BB062	0	0	1	2	1	1	2	2	0	0	0	0
JE-TBE	mixed community	3	B0BB065	0	0	0	0	2	1	1	1	0	0	0	0
JE-TBE	mixed community	3	B0BB077	2	1	1	1	0	1	1	2	1	3	2	2
JE-TBE	mixed community	3	B0BB078	0	0	2	2	2	1	2	2	0	0	0	0
JE-TBE	mixed community	3	B0BB079	0	0	1	1	0	0	0	0	0	0	0	0
JE-TBE	mixed community	3	B0BB082	0	2	1	1	1	0	2	2	0	0	0	0
JE-TBE	mixed community	3	B0BB084	0	1	1	1	1	0	0	1	0	0	0	0
JE-TBE	mixed community	3	B0BB086	0	0	1	1	1	0	0	1	0	0	0	0
JE-TBE	mixed community	3	B0BC094	1	2	1	1	0	0	0	2	2	3	2	2
JE-TBE	mixed community	3	B0BC095	1	1	1	0	0	1	2	1	0	0	0	0
JE-TBE	mixed community	3	B0BC096	0	0	0	0	0	0	0	0	0	0	0	0
JE-TBE	mixed community	3	B0BC101	0	0	0	2	0	0	1	2	1	1	1	0
JE-TBE	mixed community	3	B0BC107	0	0	0	0	0	0	0	0	2	1	2	2
JE-TBE	mixed community	3	B0BC113	0	0	0	0	0	0	1	1	1	1	1	1

				P. melanarius				*H. rufipes*				*D.* sp.			
Experiment	Plant community	PSR	Plot ID	trnL	COI	ITS	16S	trnL	COI	ITS	16S	trnL	COI	ITS	16S
JE-TBE	mixed community	3	B0BC116	0	0	1	1	1	0	2	2	0	0	0	0
JE-TBE	mixed community	3	B0BC124	0	0	1	1	2	1	2	2	0	0	0	0
JE-TBE	mixed community	3	B0BC128	0	0	0	0	0	1	1	2	1	2	1	1
JE-TBE	mixed community	3	B0BC129	0	1	2	1	2	0	2	2	0	0	0	0
JE-TBE	mixed community	3	B0BC130	0	0	2	1	1	1	2	2	1	2	1	1
JE-TBE	mixed community	3	B0BC138	0	1	1	0	1	0	2	2	0	0	0	0
JE-TBE	mixed community	4	B0BA003	1	0	2	2	0	0	0	0	2	3	2	2
JE-TBE	mixed community	4	B0BA005	1	2	2	2	0	1	2	1	0	2	1	0
JE-TBE	mixed community	4	B0BA010	0	0	0	0	2	2	2	1	0	0	0	0
JE-TBE	mixed community	4	B0BA023	1	0	2	2	2	0	2	1	0	0	0	0
JE-TBE	mixed community	4	B0BA030	1	0	2	2	1	1	1	1	0	0	0	0
JE-TBE	mixed community	4	B0BA032	0	2	2	2	0	0	0	0	0	0	0	0
JE-TBE	mixed community	4	B0BA035	1	1	2	2	0	0	2	2	0	1	1	1
JE-TBE	mixed community	4	B0BA036	1	1	2	2	2	0	2	1	1	2	1	1
JE-TBE	mixed community	4	B0BA045	1	1	2	2	1	2	2	1	1	1	1	1
JE-TBE	mixed community	4	B0BB049	0	0	0	0	0	0	0	0	1	0	1	1
JE-TBE	mixed community	4	B0BB051	0	0	2	1	0	0	0	0	1	1	2	2
JE-TBE	mixed community	4	B0BB052	1	0	2	2	1	1	2	1	1	1	1	1
JE-TBE	mixed community	4	B0BB056	0	0	0	0	0	0	0	0	0	0	0	0
JE-TBE	mixed community	4	B0BB057	0	1	1	0	1	1	2	1	2	0	2	2
JE-TBE	mixed community	4	B0BB066	0	0	1	2	1	0	2	2	1	1	1	1
JE-TBE	mixed community	4	B0BB067	2	0	2	2	0	0	0	0	1	2	2	2
JE-TBE	mixed community	4	B0BB075	1	0	1	1	0	0	1	1	0	0	0	0
JE-TBE	mixed community	4	B0BB076	0	1	2	2	2	1	2	2	1	1	1	1
JE-TBE	mixed community	4	B0BC103	1	0	0	1	0	1	2	2	2	0	2	2
JE-TBE	mixed community	4	B0BC104	0	0	2	2	2	2	2	2	0	0	0	0
JE-TBE	mixed community	4	B0BC109	0	1	2	2	1	0	1	1	0	0	0	0
JE-TBE	mixed community	4	B0BC111	0	0	0	0	0	0	0	0	0	0	0	0
JE-TBE	mixed community	4	B0BC112	1	2	2	1	1	1	2	2	1	1	1	1
JE-TBE	mixed community	4	B0BC115	0	2	2	2	1	1	2	2	0	0	0	0
JE-TBE	mixed community	4	B0BC119	0	0	2	2	2	1	2	1	0	0	0	0
JE-TBE	mixed community	4	B0BC122	0	0	0	0	0	0	1	1	0	0	0	0
JE-TBE	mixed community	4	B0BC136	0	0	2	1	0	0	0	0	0	0	0	0
JE-TBE	mixed community	8	B0BA018	1	0	1	1	1	0	2	2	1	0	1	1
JE-TBE	mixed community	8	B0BB073	1	1	3	3	3	1	2	2	1	1	3	2
JE-TBE	mixed community	8	B0BC098	1	0	2	2	0	1	2	2	1	2	2	1
JE-control	control matrix	16	B0BA-CA	0	0	0	0	0	0	1	1	0	2	2	0
JE-control	control matrix	16	B0BB-CB	0	1	3	3	0	0	0	0	0	0	0	0
JE-control	control matrix	16	B0BC-CC	0	0	1	1	0	0	0	0	1	1	3	2

PSR = Plant species richness; JE-TBE = Jena Experiment, Trait-Based Experiment

Table S2. Overview on the detection frequency of taxa per consumer and kingdom (primer) in absolute numbers (n) and percent of samples (%).

		total		*D.* sp.		*H. rufipes*		*P. melanarius*	
Kingdom	Phylum/Class/Order	n	%	n	%	n	%	n	%
	Asterales (TBE)	148	74.7	46.0	83.6	58.0	72.5	39.0	69.6
	Lamiales (TBE)	122	61.6	40.0	72.7	41.0	51.3	36.0	64.3
	Fabales	71	35.9	29.0	52.7	20.0	25.0	18.0	32.1
	Rosales (TBE)	62	31.3	4.0	7.3	32.0	40.0	22.0	39.3
	Caryophyllales (TBE)	48	24.2	18.0	32.7	18.0	22.5	10.0	17.9
	Poales (TBE)	38	19.2	10.0	18.2	11.0	13.8	12.0	21.4
Plantae	Cucurbitales	34	17.2	11.0	20.0	10.0	12.5	11.0	19.6
	Dipsacales (TBE)	34	17.2	8.0	14.5	14.0	17.5	11.0	19.6
	Geraniales (TBE)	17	8.6	2.0	3.6	9.0	11.3	6.0	10.7
	Solanales	14	7.1	4.0	7.3	3.0	3.8	5.0	8.9
	Ranunculales (TBE)	12	6.1	1.0	1.8	3.0	3.8	5.0	8.9
	Brassicales	6	3.0	1.0	1.8	2.0	2.5		
	Apiales (TBE)	5	2.5	2.0	3.6			3.0	5.4
	Gentianales (TBE)	2	1.0	1.0	1.8	1.0	1.3		
	Hymenoptera	41	22.3	2.0	3.2	12.0	19.7	16.0	33.3
	Hemiptera	38	20.7	10.0	16.1	14.0	23.0	9.0	18.8
	Haplotaxida	23	12.5	4.0	6.5	1.0	1.6	18.0	37.5
	Diptera	18	9.8	1.0	1.6	6.0	9.8	4.0	8.3
	Araneae	13	7.1			4.0	6.6	9.0	18.8
	Coleoptera	13	7.1			6.0	9.8	6.0	12.5
	Stylommatophora	12	6.5	2.0	3.2	4.0	6.6	2.0	4.2
Animaila	Entomobryomorpha	7	3.8			3.0	4.9		
	Lepidoptera	5	2.7					5.0	10.4
	Sarcoptiformes	5	2.7						
	Poduromorpha	4	2.2	2.0	3.2			1.0	2.1
	Thysanoptera	4	2.2	2.0	3.2			2.0	4.2
	Orthoptera	3	1.6					3.0	6.3
	Julida	2	1.1						
	Mesostigmata	2	1.1					2.0	4.2
	Saccharomycetes	223	76.4	33.0	49.3	89.0	89.0	91.0	84.3
	Dothideomycetes	199	68.2	65.0	97.0	67.0	67.0	54.0	50.0
	Tremellomycetes	160	54.8	55.0	82.1	60.0	60.0	31.0	28.7
	Agaricomycetes	138	47.3	19.0	28.4	69.0	69.0	35.0	32.4
	Sordariomycetes	95	32.5	40.0	59.7	28.0	28.0	17.0	15.7
	Microbotryomycetes	89	30.5	42.0	62.7	22.0	22.0	17.0	15.7
	Leotiomycetes	43	14.7	21.0	31.3	12.0	12.0	5.0	4.6
	Exobasidiomycetes	27	9.2	13.0	19.4	5.0	5.0	5.0	4.6
	Eurotiomycetes	23	7.9	8.0	11.9	6.0	6.0	3.0	2.8
	Glomeromycetes	21	7.2			12.0	12.0	4.0	3.7
	Mortierellomycotina	21	7.2	3.0	4.5	7.0	7.0	6.0	5.6
Fungi	Agaricostilbomycetes	20	6.8	6.0	9.0	9.0	9.0	5.0	4.6
	Cystobasidiomycetes	19	6.5	15.0	22.4	3.0	3.0	1.0	0.9
	Ustilaginomycotina	19	6.5	1.0	1.5	6.0	6.0	7.0	6.5
	Mucoromycotina	18	6.2	4.0	6.0	10.0	10.0	3.0	2.8
	Pezizomycetes	15	5.1	11.0	16.4	2.0	2.0	1.0	0.9
	Ustilaginomycetes	12	4.1	8.0	11.9	1.0	1.0	3.0	2.8
	Pucciniomycetes	11	3.8	2.0	3.0	6.0	6.0	2.0	1.9
	Chytridiomycetes	10	3.4	5.0	7.5	1.0	1.0	2.0	1.9
	Wallemiomycetes	6	2.1						
	Lecanoromycetes	3	1.0			3.0	3.0		
	Rozellomycota	3	1.0	1.0	1.5			1.0	0.9
	Taphrinomycetes	3	1.0	2.0	3.0	1.0	1.0		

Kingdom	Phylum/Class/Order	total		*D.* sp.		*H. rufipes*		*P. melanarius*	
		n	%	n	%	n	%	n	%
Bacteria	Proteobacteria	291	99.3	63.0	100.0	102.0	98.1	112.0	100.0
	Bacteroidetes	274	93.5	52.0	82.5	102.0	98.1	105.0	93.8
	Firmicutes	234	79.9	30.0	47.6	82.0	78.8	110.0	98.2
	Actinobacteria	189	64.5	18.0	28.6	83.0	79.8	74.0	66.1
	Saccharibacteria	112	38.2	6.0	9.5	46.0	44.2	51.0	45.5
	Acidobacteria	89	30.4	4.0	6.3	50.0	48.1	29.0	25.9
	Planctomycetes	85	29.0	8.0	12.7	33.0	31.7	38.0	33.9
	Deinococcus-Thermus	74	25.3	1.0	1.6	27.0	26.0	45.0	40.2
	Verrucomicrobia	70	23.9	7.0	11.1	29.0	27.9	25.0	22.3
	Cyanobacteria	55	18.8			26.0	25.0	24.0	21.4
	Fusobacteria	45	15.4			12.0	11.5	30.0	26.8
	Tenericutes	26	8.9	1.0	1.6	5.0	4.8	14.0	12.5
	CKC4	25	8.5			4.0	3.8	20.0	17.9
	Armatimonadetes	22	7.5	1.0	1.6	14.0	13.5	6.0	5.4
	Chlamydiae	21	7.2			9.0	8.7	11.0	9.8
	Chloroflexi	14	4.8			8.0	7.7	5.0	4.5
	Nitrospirae	9	3.1			5.0	4.8	3.0	2.7
	Chlorobi	4	1.4			3.0	2.9	1.0	0.9
	TM6	4	1.4			1.0	1.0	2.0	1.8
	Elusimicrobia	3	1.0			2.0	1.9	1.0	0.9
	Spirochaetae	3	1.0	1.0	1.6	2.0	1.9		
	WCHB1-60	3	1.0			3.0	2.9		

Only taxa with an frequency of detection of ≥1% over all samples are shown. Red coloration of cells marks frequently detected taxa. TBE = sown species pool of the Trait-Based Experiment.

Table S3. Effect of the interaction between consumer identity and plant species richness, plant functional diversity, or percent vegetation cover on the number of detected taxa in each interaction group.

Model	Term	*df*	*denom. df*	χ2	*p* value
a) Total food	Plant species richness	1	240	0.121	0.728
	Consumer identity	2	238	3.423	0.181
	Plant species richness x consumer identity	2	236	3.114	0.211
	Plant functional diversity	1	240	0.547	0.460
	Consumer identity	2	238	3.383	0.184
	Plant functional diversity x consumer identity	2	236	0.542	0.763
	Vegetation cover	1	240	0.840	0.359
	Consumer identity	2	238	3.473	0.176
	Vegetation cover x consumer identity	2	236	1.304	0.521
b) Plants	Plant species richness	1	188	0.040	0.842
	Consumer identity	2	186	19.514	**<0.001**
	Plant species richness x consumer identity	2	184	3.108	0.211
	Plant functional diversity	1	188	0.000	0.983
	Consumer identity	2	186	58.713	**<0.001**
	Plant functional diversity x consumer identity	2	184	0.745	0.689
	Vegetation cover	1	188	1.632	0.201
	Consumer identity	2	186	61.228	**<0.001**
	Vegetation cover x consumer identity	2	184	0.100	0.951
c) Predation	Plant species richness	1	166	0.009	0.927
	Consumer identity	2	164	59.366	**<0.001**
	Plant species richness x consumer identity	2	162	2.724	0.256
	Plant functional diversity	1	166	0.559	0.455
	Consumer identity	2	164	19.626	**<0.001**
	Plant functional diversity x consumer identity	2	162	2.577	0.276
	Vegetation cover	1	166	0.026	0.873
	Consumer identity	2	164	19.085	**<0.001**
	Vegetation cover x consumer identity	2	162	0.203	0.903
d) IGP	Plant species richness	1	106	0.178	0.674
	Consumer identity	1	105	5.396	**0.020**
	Plant species richness x consumer identity	1	104	1.220	0.269
	Plant functional diversity	1	106	0.755	0.385
	Consumer identity	1	105	5.057	**0.025**
	Plant functional diversity x consumer identity	1	104	1.201	0.273
	Vegetation cover	1	106	0.052	0.820
	Consumer identity	1	105	5.311	**0.021**
	Vegetation cover x consumer identity	1	104	1.218	0.270
e) Microbiome	Plant species richness	1	218	0.338	0.561
	Consumer identity	1	217	3.681	0.055
	Plant species richness x consumer identity	1	216	0.016	0.898
	Plant functional diversity	1	218	1.316	0.251
	Consumer identity	1	217	4.075	**0.044**
	Plant functional diversity x consumer identity	1	216	0.375	0.540
	Vegetation cover	1	218	0.089	0.766
	Consumer identity	1	217	3.836	*0.050*
	Vegetation cover x consumer identity	1	216	1.464	0.226

Model	Term	*df*	*denom. df*	χ2	*p* value
f) Symbionts	Plant species richness	1	218	1.127	0.289
	Consumer identity	1	217	12.290	**<0.001**
	Plant species richness x consumer identity	1	216	0.355	0.551
	Plant functional diversity	1	218	1.348	0.246
	Consumer identity	1	217	13.197	**<0.001**
	Plant functional diversity x consumer identity	1	216	0.024	0.877
	Vegetation cover	1	218	0.330	0.566
	Consumer identity	1	217	93.152	**<0.001**
	Vegetation cover x consumer identity	1	216	11.585	**<0.001**
g) Pathogens	Plant species richness	1	218	0.579	0.447
	Consumer identity	1	217	13.194	**0.038**
	Plant species richness x consumer identity	1	216	11.156	0.830
	Plant functional diversity	1	218	0.808	0.369
	Consumer identity	1	217	4.323	**0.038**
	Plant functional diversity x consumer identity	1	216	0.298	0.585
	Vegetation cover	1	218	0.780	0.377
	Consumer identity	1	217	4.147	**0.042**
	Vegetation cover x consumer identity	1	216	2.574	0.109
h) Neutral	Plant species richness	1	218	0.008	0.931
	Consumer identity	1	217	6.469	0.011
	Plant species richness x consumer identity	1	216	0.494	**0.482**
	Plant functional diversity	1	218	3.382	*0.066*
	Consumer identity	1	217	7.046	**0.008**
	Plant functional diversity x consumer identity	1	216	0.130	0.719
	Vegetation cover	1	218	1.830	0.176
	Consumer identity	1	217	6.483	**0.011**
	Vegetation cover x consumer identity	1	216	0.283	0.595

All taxa were assigned to interaction groups (see methods). We tested the interaction effects of consumer identity and three explanatory variables for all interaction groups (*D*. sp. was included in a) total food, b) plant food, c) predation, and h) neutral)). A quasipoisson distribution was used for all models except neutral interactions, for which negative binomial models were fitted. *p* values <.05 are reported in bold numbers and *p* <.10 in italics. *denom. df*, denominator degrees of freedom

Table S4. Summary table for structural equation models for the direct and cascading effects of plant species richness on the log-transformed detected richness of prey taxa (Predation) in the guts of three omnivores.

left-hand-side	operator	right-hand-side	Estimate	*SE*	*z* value	*p* value
Regressions (*H. rufipes*):						
log(Predation)	~	Plant species richness	0.174	0.117	1.488	0.137
log(Predation)	~	Plant cover	0.101	0.108	0.933	0.351
log(Predation)	~	Plant biomass	0.051	0.125	0.408	0.683
log(Predation)	~	Animal abundance	0.230	0.152	1.514	0.130
log(Predation)	~	Animal species richness	-0.177	0.153	-1.155	0.248
Plant cover	~	Plant species richness	0.006	0.096	0.064	0.949
Plant biomass	~	Plant species richness	0.045	0.129	0.347	0.729
Animal species richness	~	Plant species richness	0.149	0.109	1.367	0.172
Animal species richness	~	Plant cover	-0.006	0.133	-0.045	0.964
Animal species richness	~	Plant biomass	0.135	0.081	1.668	*0.095*
Animal abundance	~	Plant species richness	-0.050	0.096	-0.519	0.603
Animal abundance	~	Plant cover	-0.002	0.080	-0.029	0.977
Animal abundance	~	Plant biomass	0.053	0.083	0.634	0.526
Animal abundance	~	Animal species richness	0.602	0.114	5.303	**0.000**
Intercepts (*H. rufipes*):						
log(Predation)	~1		0.000	0.125	0.000	1.000
Plant cover	~1		0.000	0.128	0.000	1.000
Plant biomass	~1		0.000	0.128	0.000	1.000
Animal species richness	~1		0.000	0.127	0.000	1.000
Animal abundance	~1		0.000	0.102	0.000	1.000
Variances (*H. rufipes*):						
log(Predation)			0.912	0.136	6.697	**0.000**
Plant cover			0.984	0.180	5.463	**0.000**
Plant biomass			0.982	0.279	3.524	**0.000**
Animal species richness			0.942	0.194	4.857	**0.000**
Animal abundance			0.622	0.164	3.803	**0.000**
Regressions (*D.* sp.):						
log(Predation)	~	Plant species richness	-0.134	0.098	-1.373	0.170
log(Predation)	~	Plant cover	0.106	0.147	0.724	0.469
log(Predation)	~	Plant biomass	0.050	0.085	0.589	0.556
log(Predation)	~	Animal abundance	-0.110	0.106	-1.036	0.300
log(Predation)	~	Animal species richness	-0.163	0.152	-1.067	0.286
Plant cover	~	Plant species richness	0.216	0.114	1.889	*0.059*
Plant biomass	~	Plant species richness	0.052	0.136	0.383	0.702
Animal species richness	~	Plant species richness	0.191	0.110	1.736	*0.083*
Animal species richness	~	Plant cover	0.103	0.128	0.807	0.420
Animal species richness	~	Plant biomass	0.013	0.118	0.107	0.914
Animal abundance	~	Plant species richness	0.082	0.095	0.864	0.388
Animal abundance	~	Plant cover	-0.067	0.141	-0.473	0.636
Animal abundance	~	Plant biomass	-0.087	0.067	-1.307	0.191
Animal abundance	~	Animal species richness	0.617	0.103	5.965	**0.000**
Intercepts (*D.* sp.):						
log(Predation)	~1		0.000	0.124	0.000	1.000
Plant cover	~1		0.000	0.129	0.000	1.000
Plant biomass	~1		0.000	0.129	0.000	1.000
Animal species richness	~1		0.000	0.128	0.000	1.000
Animal abundance	~1		0.000	0.100	0.000	1.000
Variances (*D.* sp.):						
log(Predation)			0.891	0.199	4.483	**0.000**
Plant cover			0.938	0.173	5.412	**0.000**
Plant biomass			0.981	0.272	3.603	**0.000**
Animal species richness			0.928	0.214	4.344	**0.000**
Animal abundance			0.587	0.151	3.877	**0.000**

left-hand-side	operator	right-hand-side	Estimate	*SE*	*z* value	*p* value
Regressions (*P. melanarius*):						
log(Predation)	~	Plant species richness	0.002	0.109	0.014	0.988
log(Predation)	~	Plant cover	0.067	0.146	0.458	0.647
log(Predation)	~	Plant biomass	0.140	0.132	1.066	0.287
log(Predation)	~	Animal abundance	0.339	0.177	1.908	*0.056*
log(Predation)	~	Animal species richness	-0.112	0.184	-0.607	0.544
Plant cover	~	Plant species richness	0.210	0.150	1.398	0.162
Plant biomass	~	Plant species richness	0.279	0.131	2.132	**0.033**
Animal species richness	~	Plant species richness	0.013	0.124	0.102	0.919
Animal species richness	~	Plant cover	0.094	0.124	0.757	0.449
Animal species richness	~	Plant biomass	0.292	0.128	2.276	**0.023**
Animal abundance	~	Plant species richness	-0.008	0.114	-0.072	0.943
Animal abundance	~	Plant cover	0.090	0.127	0.711	0.477
Animal abundance	~	Plant biomass	-0.063	0.118	-0.532	0.595
Animal abundance	~	Animal species richness	0.587	0.149	3.950	**0.000**
Intercepts (*P. melanarius*):						
log(Predation)	~l		0.000	0.137	0.000	1.000
Plant cover	~l		0.000	0.146	0.000	1.000
Plant biomass	~l		0.000	0.146	0.000	1.000
Animal species richness	~l		0.000	0.139	0.000	1.000
Animal abundance	~l		0.000	0.118	0.000	1.000
Variances (*P. melanarius*):						
log(Predation)			0.867	0.168	5.145	**0.000**
Plant cover			0.936	0.170	5.497	**0.000**
Plant biomass			0.903	0.224	4.036	**0.000**
Animal species richness			0.883	0.286	3.089	**0.002**
Animal abundance			0.642	0.194	3.304	**0.001**

p values <.05 are reported in bold numbers and *p* <.10 in italics. *SE* = Standard errors

Table S5. Average trophic position of food items in gut contents and feces of three omnivorous consumers.

Response	Term	Estimate	SE	*t* value	*p* value
a) Mean trophic position of total food	(Intercept)	-0.751	0.432	-1.738	*0.084*
	Plant species richness	-0.223	0.166	-1.342	0.181
	Consumer-*H rufipes*	-0.301	0.487	-0.619	0.537
	Consumer-*P. melanarius*	0.300	0.480	0.624	0.533
	Plant species richness x consumer-*H rufipes*	0.273	0.181	1.509	0.133
	Plant species richness x consumer-*P. melanarius*	0.232	0.180	1.293	0.197
	(Intercept)	-0.903	0.428	-2.112	**0.036**
	Plant functional diversity	-0.178	0.176	-1.009	0.314
	Consumer-*H rufipes*	-0.356	0.513	-0.693	0.489
	Consumer-*P. melanarius*	0.332	0.490	0.676	0.500
	Plant functional diversity x consumer-*H rufipes*	0.315	0.204	1.545	0.124
	Plant functional diversity x consumer-*P. melanarius*	0.237	0.197	1.202	0.231
	(Intercept)	-1.251	1.054	-1.187	0.237
	Vegetation cover	-0.001	0.015	-0.059	0.953
	Consumer-*H rufipes*	-0.934	1.250	-0.747	0.456
	Consumer-*P. melanarius*	2.021	1.154	1.751	*0.082*
	Vegetation cover x consumer-H rufipes	0.019	0.017	1.066	0.287
	Vegetation cover x consumer-P. melanarius	-0.017	0.016	-1.025	0.307
	(Intercept)	0.352	0.849	0.414	0.679
	Invertebrate richness	-0.108	0.056	-1.931	*0.055*
	Consumer-*H rufipes*	-0.887	0.982	-0.903	0.367
	Consumer-*P. melanarius*	-1.432	0.937	-1.528	0.128
	Invertebrate richness x consumer-*H rufipes*	0.085	0.063	1.345	0.180
	Invertebrate richness x consumer-*P. melanarius*	0.147	0.060	2.436	**0.016**
	(Intercept)	-0.048	0.744	-0.065	0.948
	Invertebrate abundance	-0.026	0.016	-1.680	*0.094*
	Consumer-*H rufipes*	-0.732	0.847	-0.864	0.389
	Consumer-*P. melanarius*	-0.370	0.827	-0.447	0.656
	Invertebrate abundance x consumer-*H rufipes*	0.024	0.017	1.370	0.172
	Invertebrate abundance x consumer-*P. melanarius*	0.026	0.017	1.542	0.125
b) Mean trophic position of total food	(Intercept)	0.206	0.071	2.919	**0.005**
	Plant species richness	0.010	0.023	0.451	0.654
	Consumer-*P. melanarius*	0.137	0.104	1.319	0.191
	Plant species richness x consumer-*P. melanarius*	-0.035	0.034	-1.020	0.311
	(Intercept)	0.059	0.088	0.677	0.501
	Plant functional diversity	0.069	0.032	2.175	**0.033**
	Consumer-*P. melanarius*	0.204	0.114	1.789	*0.078*
	Plant functional diversity x consumer-*P. melanarius*	-0.065	0.042	-1.555	0.124
	(Intercept)	-0.065	0.210	-0.310	0.757
	Vegetation cover	0.004	0.003	1.449	0.152
	Consumer-*P. melanarius*	0.587	0.264	2.225	**0.029**
	Vegetation cover x consumer-P. melanarius	-0.008	0.004	-2.114	**0.038**
	(Intercept)	0.192	0.185	1.043	0.301
	Invertebrate abundance	0.003	0.011	0.229	0.820
	Consumer-*P. melanarius*	-0.049	0.223	-0.218	0.828
	Invertebrate abundance x consumer-*P. melanarius*	0.005	0.013	0.389	0.698
	(Intercept)	0.258	0.130	1.987	*0.051*
	Invertebrate richness	0.000	0.002	-0.192	0.848
	Consumer-*P. melanarius*	-0.011	0.181	-0.062	0.951
	Invertebrate richness x consumer-*P. melanarius*	0.001	0.003	0.296	0.768

Parameter estimates and standard errors (*SE*) from GLMs (corresponding to Table 5) on the effect of consumer identity and measures of the plant and animal community in the plot on the mean trophic position of a) food items and b) prey (*D*. sp. excluded). *p* values <.05 are reported in bold numbers and *p* <.10 in *italics*.

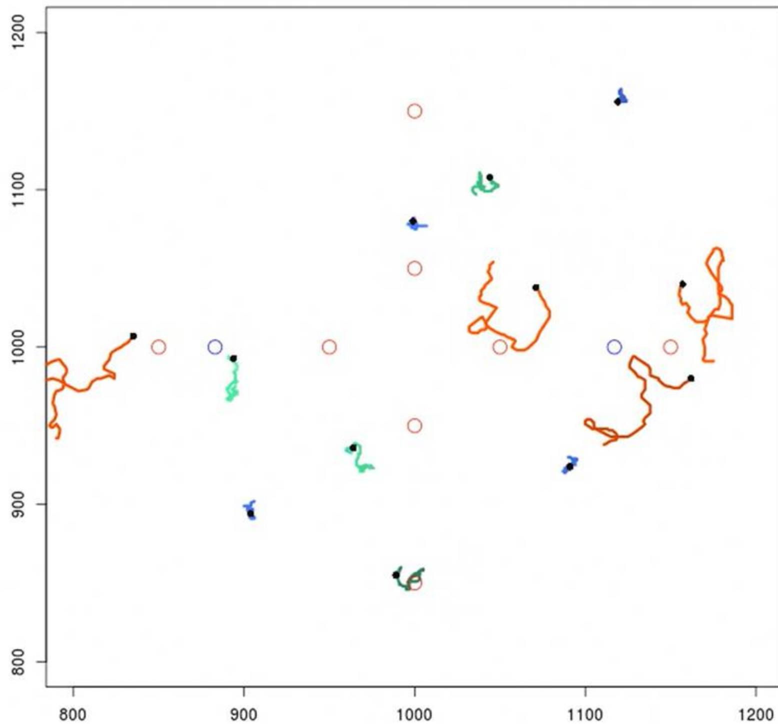
Table S6. Proportional composition of gut contents and feces of three omnivorous consumers.

Response	Term	Estimate	SE	*t* value	*p* value
a) Proportion prey / total food	(Intercept)	-1.921	0.454	-4.236	**<0.001**
	Plant species richness	-0.225	0.174	-1.292	0.199
	Consumer-*H rufipes*	0.464	0.507	0.917	0.361
	Consumer-*P. melanarius*	0.556	0.544	1.022	0.309
	Plant species richness x consumer-*H rufipes*	0.277	0.187	1.478	0.142
	Plant species richness x consumer-*P. melanarius*	0.265	0.206	1.288	0.200
	(Intercept)	-2.458	0.442	-5.557	**<0.001**
	Plant functional diversity	-0.012	0.179	-0.066	0.948
	Consumer-*H rufipes*	1.009	0.528	1.909	*0.059*
	Consumer-*P. melanarius*	1.109	0.554	2.002	**0.048**
	Plant functional diversity x consumer-*H rufipes*	0.069	0.209	0.330	0.742
	Plant functional diversity x consumer-*P. melanarius*	0.047	0.222	0.213	0.831
	(Intercept)	-2.975	1.023	-2.909	**0.004**
	Vegetation cover	0.007	0.014	0.489	0.626
	Consumer-*H rufipes*	0.812	1.226	0.663	0.509
	Consumer-*P. melanarius*	2.499	1.232	2.029	**0.045**
	Vegetation cover x consumer-H rufipes	0.005	0.017	0.290	0.772
	Vegetation cover x consumer-P. melanarius	-0.019	0.017	-1.069	0.287
	(Intercept)	-1.575	0.909	-1.732	*0.086*
	Invertebrate richness	-0.064	0.058	-1.098	0.275
	Consumer-*H rufipes*	-0.034	1.060	-0.032	0.975
	Consumer-*P. melanarius*	0.577	1.022	0.565	0.574
	Invertebrate richness x consumer-*H rufipes*	0.079	0.067	1.183	0.239
	Invertebrate richness x consumer-*P. melanarius*	0.051	0.064	0.796	0.428
	(Intercept)	-1.563	0.773	-2.021	**0.046**
	Invertebrate abundance	-0.021	0.016	-1.304	0.195
	Consumer-*H rufipes*	-0.256	0.871	-0.295	0.769
	Consumer-*P. melanarius*	-0.024	0.899	-0.027	0.979
	Invertebrate abundance x consumer-*H rufipes*	0.029	0.018	1.669	*0.098*
	Invertebrate abundance x consumer-*P. melanarius*	0.028	0.018	1.556	0.123
b) Proportion IGP / total prey	(Intercept)	-1.221	0.327	-3.740	**<0.001**
	Plant species richness	0.069	0.100	0.684	0.496
	Consumer-*P. melanarius*	0.576	0.494	1.167	0.247
	Plant species richness x consumer-*P. melanarius*	-0.181	0.166	-1.094	0.278
	(Intercept)	-1.644	0.447	-3.676	**<0.001**
	Plant functional diversity	0.238	0.158	1.504	0.137
	Consumer-*P. melanarius*	0.681	0.563	1.209	0.231
	Plant functional diversity x consumer-*P. melanarius*	-0.231	0.203	-1.134	0.261
	(Intercept)	-1.729	1.149	-1.505	0.137
	Vegetation cover	0.010	0.015	0.619	0.538
	Consumer-*P. melanarius*	1.933	1.381	1.400	0.166
	Vegetation cover x consumer-P. melanarius	-0.027	0.019	-1.401	0.165
	(Intercept)	-1.456	0.551	-2.645	**0.010**
	Invertebrate abundance	0.008	0.009	0.823	0.413
	Consumer-*P. melanarius*	0.602	0.808	0.745	0.459
	Invertebrate abundance x consumer-*P. melanarius*	-0.010	0.014	-0.706	0.482
	(Intercept)	-2.091	0.879	-2.378	**0.020**
	Invertebrate richness	0.063	0.051	1.242	0.218
	Consumer-*P. melanarius*	1.090	1.037	1.051	0.297
	Invertebrate richness x consumer-*P. melanarius*	-0.062	0.060	-1.037	0.303

Response	Term	Estimate	SE	*t* value	*p* value
c) Proportion symbionts / symbionts + pathogens	(Intercept)	-0.411	0.077	-5.328	**<0.001**
	Plant species richness	0.017	0.025	0.665	0.507
	Consumer-*P. melanarius*	0.015	0.115	0.126	0.900
	Plant species richness x consumer-*P. melanarius*	0.001	0.038	0.015	0.988
	(Intercept)	-0.384	0.086	-4.477	**<0.001**
	Plant functional diversity	0.014	0.033	0.425	0.671
	Consumer-*P. melanarius*	0.047	0.126	0.371	0.711
	Plant functional diversity x consumer-*P. melanarius*	-0.019	0.049	-0.398	0.691
	(Intercept)	-0.238	0.209	-1.139	0.256
	Vegetation cover	-0.002	0.003	-0.546	0.586
	Consumer-*P. melanarius*	-0.075	0.306	-0.246	0.806
	Vegetation cover x consumer-P. melanarius	0.001	0.004	0.263	0.793
	(Intercept)	-0.522	0.154	-3.398	**0.001**
	Invertebrate richness	0.010	0.009	1.145	0.254
	Consumer-P. melanarius	0.075	0.222	0.337	0.737
	Invertebrate richness x consumer-P. melanarius	-0.004	0.013	-0.329	0.742
	(Intercept)	-0.358	0.133	-2.694	**0.008**
	Invertebrate abundance	0.000	0.002	0.059	0.953
	Consumer-*P. melanarius*	-0.072	0.183	-0.391	0.697
	Invertebrate abundance x consumer-*P. melanarius*	0.001	0.003	0.408	0.684
d) Proportion fungi / total microbes	(Intercept)	-0.862	0.116	-7.408	**<0.001**
	Plant species richness	-0.046	0.036	-1.277	0.203
	Consumer-*H rufipes*	-0.741	0.172	-4.310	**<0.001**
	Consumer-*P. melanarius*	-1.067	0.178	-6.008	**<0.001**
	Plant species richness x consumer-*H rufipes*	-0.042	0.056	-0.762	0.447
	(Intercept)	-0.913	0.136	-6.701	**<0.001**
	Plant functional diversity	-0.034	0.053	-0.642	0.521
	Consumer-*H rufipes*	-0.849	0.197	-4.309	**<0.001**
	Consumer-*P. melanarius*	-1.011	0.204	-4.954	**<0.001**
	Plant functional diversity x consumer-*H rufipes*	0.000	0.075	0.004	0.997
	Plant functional diversity x consumer-*P. melanarius*	-0.016	0.081	-0.193	0.847
	(Intercept)	-1.108	0.336	-3.296	0.001
	Vegetation cover	0.002	0.005	0.348	0.728
	Consumer-*H rufipes*	0.050	0.467	0.108	0.914
	Consumer-*P. melanarius*	-0.355	0.470	-0.755	0.451
	Vegetation cover x consumer-H rufipes	-0.013	0.007	-1.964	0.051
	Vegetation cover x consumer-P. melanarius	-0.010	0.007	-1.487	0.138
	(Intercept)	-0.870	0.244	-3.574	**<0.001**
	Invertebrate richness	-0.007	0.014	-0.513	0.608
	Consumer-*H rufipes*	-0.666	0.344	-1.934	*0.054*
	Consumer-*P. melanarius*	-1.156	0.393	-2.939	**0.004**
	Invertebrate richness x consumer-*H rufipes*	-0.010	0.020	-0.516	0.607
	Invertebrate richness x consumer-*P. melanarius*	0.007	0.023	0.305	0.760
	(Intercept)	-0.963	0.192	-5.018	**<0.001**
	Invertebrate abundance	-0.001	0.004	-0.158	0.875
	Consumer-*H rufipes*	-0.613	0.283	-2.168	**0.031**
	Consumer-*P. melanarius*	-1.301	0.302	-4.309	**<0.001**
	Invertebrate abundance x consumer-*H rufipes*	-0.004	0.005	-0.828	0.408
	Invertebrate abundance x consumer-*P. melanarius*	0.005	0.005	0.913	0.362

Parameter estimates and standard errors (*SE*) from GLMs (corresponding to Table 6) on the effect of consumer species identity and measures of the plant and animal community in the plot on the proportional composition of gut content and feces (*D.* sp. excluded from b) and c)). *p* values <.05 are reported in bold numbers and *p* <.10 in *italics*.

CHAPTER 5



Pitfall trap sampling bias depends on body mass, temperature, and trap number: insights from an individual-based model

Jan Engel, Lionel Hertzog, **Julia Tiede**, Cameron Wagg, Anne Ebeling, Heiko Briesen, Wolfgang W Weisser

Published in Ecosphere 8(4): e01790. doi:10.1002/ecs2.1790 © 2017 Engel et al.

Abstract

The diversity and community composition of ground arthropods is routinely analyzed by pitfall trap sampling, which is a cost- and time-effective method to gather large numbers of replicates but also known to generate data that are biased by species-specific differences in locomotory activity. Previous studies have looked at factors that influence the sampling bias. These studies, however, were limited to one or few species and did rarely quantify how the species-specific sampling bias shapes community-level diversity metrics. In this study, we systematically quantify the species-specific and community-level sampling bias with an allometric individual-based model that simulates movement and pitfall sampling of 10 generic ground arthropod species differing in body mass. We perform multiple simulation experiments covering different scenarios of pitfall trap number, spatial trap arrangement, temperature, and population density. We show that the sampling bias decreased strongly with increasing body mass, temperature, and pitfall trap number, while population density had no effect and trap arrangement only had little effect. The average movement speed of a species in the field integrates body mass and temperature effects and could be used to derive reliable estimates of absolute species abundance. We demonstrate how unbiased relative species abundance can be derived using correction factors that need only information on species body mass. We find that community-level diversity metrics are sensitive to the particular community structure, namely the relation between body mass and relative abundance across species. Generally, pitfall trap sampling flattens the rank-abundance distribution and leads to overestimations of ground arthropod Shannon diversity. We conclude that the correction of the species-specific pitfall trap sampling bias is necessary for the reliability of conclusions drawn from ground arthropod field studies. We propose bias correction is a manageable task using either body mass to derive unbiased relative abundance or the average speed to derive reliable estimates of absolute abundance from pitfall trap sampling.

5.1 Introduction

Quantification of animal densities in the field is essential to understand impacts of climate and land-use change on community biodiversity (Iknayan et al. 2014). This is particularly true for the large group of ground-dwelling arthropods (here referred to as ground arthropods) as they are highly responsive to environmental changes and influence a large number of ecosystem functions such as predation and decomposition (Finke & Snyder 2010; Chaplin-Kramer et al. 2011; Prather et al. 2013). The passive sampling of ground arthropods with pitfall traps, that is, small containers buried to the ground, was used in many biodiversity and conservation studies across the recent decades and is still being used today (Greenslade 1964; Zhao et al. 2013; Brown & Matthews 2016). The benefits of pitfall trap sampling are its time efficiency and the high probability to detect rare and nocturnal species that other methods might miss (Spence & Niemelä 1994; Lang 2000; Cardoso et al. 2008). There are, however, a number of factors that produce biases in the species abundance estimated from pitfall trap sampling affecting the species-specific sampling efficiency. Sampling bias has been shown to depend on, for example, population density and factors that change locomotory activity such as body mass and ambient temperature (Halsall & Wratten 1988; Mommertz et al. 1996; Lang 2000; Perner & Schueler 2004; Woodcock 2005; Saska et al. 2013; Brown & Matthews 2016). Hence, the sampling bias

likely varies across species and environmental conditions hampering field experiments to get insight into how environmental changes affect arthropod communities.

Population density of ground arthropods varies across years and habitats; though, limited knowledge exists about how accurate the sampled density reflects variation in the real density (Collins et al. 2003; Hutchison 2007; Woodcock 2005). Variation in the ambient temperature can produce considerable bias in pitfall sampling because the locomotory activity of most ground arthropods varies with ambient temperature, and sampled densities are proportional to locomotory activity (Thomas et al. 1998; Woodcock 2005). The number of pitfall traps and their spatial arrangement have been shown to strongly influence the reliability of sampled densities for estimations of real densities (Scheller 1984; Parmenter & MacMahon 1989; Perner 2003; Zhao et al. 2013). Moreover, only limited knowledge exists about how the species-specific sampling bias may impact community-level metrics considering variation in community structure across ecosystems, such as the distribution of species body masses across the abundance ranks (Topping & Sunderland 1992).

Previous empirical studies that aimed at analyzing the species-specific pitfall sampling bias and providing recommendations for statistical corrections focused on only one or few of the confounding factors, mostly covering small ranges of parameter values (e.g., Greenslade 1964; Spence & Niemelä 1994; Thomas et al. 1998; Work et al. 2002). Today, there is only fragmented knowledge about how the pitfall trap sampling bias affects both sampled population densities and estimated metrics of community diversity across different combinations of, for example, trap number, trap arrangement, species body mass, community structure, and climatic conditions.

Computational simulations can reduce knowledge gaps by simulating ground arthropods movement and sampling across many factors, such as trap number, trap arrangement, and ambient temperature (Perner & Schueler 2004; Pyke 2015). Nevertheless, the simulation of large numbers of different ground arthropod species is limited by the great parameterization effort necessary to model realistic movement of many species. This parameterization effort, consequently, also proved to be a methodological frontier to analyses of how the species-specific sampling bias may affect community-level metrics, such as the species rank-abundance distribution (RAD) and Shannon diversity (McGill et al. 2007; Locey & White 2013). Recent simulation studies simulated the movement of one or two ground arthropod species specifically emphasizing the impact of the spatial arrangement of traps on efficiency and reliability of sampled densities (Crist & Wiens 1995; Perner & Schueler 2004; Ellis & Bedward 2014). These studies simulated the movement of virtual individuals in a homogeneous two-dimensional landscape. The virtually sampled population densities were compared to the simulated densities to validate the efficiency of trap arrangements and reveal the species-specific sampling bias. The basic idea behind these modeling exercises was to simulate data and observer models to mimic real species and their sampling, being in control of all conditions and aware of any sampling bias. This basic idea was formalized by Zurell et al. (2010) as evaluation framework for the assessment of sampling protocols and analysis in ecology, naming it the “virtual ecologist” approach. When applying the virtual ecologist approach to ground arthropod pitfall sampling, the effectiveness of sampling designs can be

rigorously tested against a simulated known truth, providing a strong basis for future field experiments and empirical validation.

Here, we apply the virtual ecologist approach to investigate the pitfall sampling bias at the species and the community level. We developed an individual-based model for simulating the movement and pitfall trap sampling across 10 “generic species” of actively hunting ground arthropods that differ in body mass ranging from 1 to 330 mg. We parameterized the simulated ground arthropod movement applying allometric relationships and empirical sampling data integrating knowledge about temperature and body mass effects on arthropod movement (e.g., Klazenga & Devries 1994; Thomas et al. 1998; Hurlbert et al. 2008). We conducted 840 simulation experiments to identify how (1) the species-specific sampling bias, (2) the observed RAD, and (3) the estimated community diversity metrics are affected by (i) trap number (1, 2, 4, 8, and 12), (ii) trap arrangement (Appendix S3: Fig. S1), (iii) body mass, (iv) body temperature (15–30°C), (v) population density (0.15–8 individuals/m2), and (vi) community structure (sequence of body masses across the abundance ranks). We aimed for simple ways to retrieve correction factors that would allow reasonable estimates of unbiased relative and absolute species densities from pitfall trap sampling.

5.2 Methods

The present study used an allometric individual-based model to simulate the movement of individuals across 10 generic species of actively hunting ground arthropods. The simulated individuals were “virtually” sampled applying different pitfall trap numbers and spatial arrangements. The 10 generic species (here referred to as species) differed only in body mass; that is, no specific real species were modeled. We applied an empirical relationship between movement speed and body mass plus body temperature to adequately simulate the species-specific movement speed (Hurlbert et al. 2008). Additionally, movement parameters across species were improved and validated using a different set of published empirical data (Klazenga & Devries 1994; Thomas et al. 1998; Byers 2001).

The model predicted the number of individuals per species sampled by pitfall traps across 14 simulation days. We defined the sampling bias as the species-specific proportion of simulated individuals that were not sampled. Multiple simulation experiments were conducted modeling all 10 species across various combinations of trap number, trap arrangement, population density, and body temperature.

The following sections describe the simulation model accordingly to the ODD protocol (overview, design concepts, and details; Grimm et al. 2010), the simulation experiments with the particular parameter values used, and the data analysis. The parameterization and validation of arthropod movement as well as the local sensitivity analysis are covered in Appendices S1 and S2. The model was implemented using the programming language C++.

5.2.1 Model description

5.2.1.1 Purpose

The purpose of the model is to predict the number of individuals per species that are sampled by pitfall traps during the simulation experiment. The simulation experiments enable reliable estimations of the pitfall trap sampling bias for various parameter combinations of body mass, population density, body temperature, trap number, and trap arrangement, for which no empirical data exist. The sampling of ground arthropod individuals is not imposed but emerges from the movement of individuals within the simulated area. The model simulates movement patterns of arthropod species that are actively hunting at the ground.

5.2.1.2 State variables and scales

Model entities were individuals resembling actively hunting ground arthropods of a distinct class of body mass. All state variables characterizing an individual are listed in Table 1. During one simulation experiment, the body mass and the body temperature were fixed, but individuals were different in their position in the simulated area, their direction of movement, and the mortality status. Species-specific activity periods were simplified such that all individuals across all species were active at the same time. Body temperature of each individual was assumed to exceed ambient temperature by 8°C simplifying effects from variation in microhabitat conditions (Casey 1976; Morgan 1985).

Table 1. State variables of simulated individuals

Variable name	Description	Possible values	Units
Body mass	Fixed species-specific body mass of species 1–10	1, 2, 4, 7, 13, 25, 48, 91, 173, 330[a]	mg
Body temperature	Fixed body temperature across the simulation	288, 291, 294, 297, 300, 303 (~15–30)	Kelvin (°C)
Position	x:y coordinate of the grid cell in the area	1–2000:1–2000	…
Previous direction	Direction of movement in the previous time step	0–360	degree
New direction	Direction of movement in the current time step	0–360	degree
Mortality status	Individual is alive or dead following a trapping event	Alive, dead	…
Activity	Whether an individual is active and moves	Yes, no	…
Start activity period	First time step of activity period; assumed to equal across individuals	3601	time step per day
End activity period	First time step where the individual is inactive following an activity period; assumed to equal across individuals	6481	time step per day

[a] Values are logarithmically spaced covering a proper subset of the body mass range of ground-dwelling arthropod species sampled in two Central European grasslands (Table S1 in Rzanny and Voigt 2012, Gossner et al. 2015); species smaller than 1 mg (≤3 mm body length) were not covered in our study.

The simulated, homogeneous, and featureless area spanned 20 × 20 m with a resolution of 1 × 1 cm, resulting in a grid of 2000 × 2000 cells. We assumed that the simulated area is enclosed in a very large field with a population density equal to the density within the simulated area (Perner & Schueler 2004). The boundaries of the area were simulated as permeable allowing individuals to leave and enter the simulated area. In the simulation experiments, an individual that left the area immediately re-entered the area at the opposite (i.e., the area was simulated as torus). One time step in the model was a discrete event corresponding to 10 s. Simulation experiments were run for 14 d, totaling 120,960 time steps, corresponding to the sampling period often used in empirical studies (e.g., Topping and Sunderland 1992; Diekotter et al. 2010). The pitfall traps had a diameter of 5 cm and were located according to the specific trap number and trap arrangement (Appendix S3: Figs. S1, S2).

5.2.1.3 Process overview and scheduling

At each time step, the processes presented in Fig. 1 were computed in the given order starting with "activity." Per time step the individuals were processed one by one using always the same sequence. Changes in state variables were updated immediately. All processes are briefly described below and in detail in the "submodels" section.

1. Activity: Whether an individual was active or not depended on the predefined activity period, which spanned eight consecutive hours and was assumed to be equal across species (e.g., Brunsting 1982).
2. Speed: The speed of an individual depended on body mass and body temperature (Morgan 1985; Hurlbert et al. 2008) and was calculated by applying the empirical relationship presented by Hurlbert et al. (2008).
3. Displacement: The displacement of an individual per time step depended on the speed of the individual and the directional persistence of movement during the particular time step.
4. Turning angle: The direction of movement of an individual was correlated across successive time steps, simulating a correlated random walk (Codling et al. 2008).
5. New position: The new position at the end of a time step was calculated from the previous position, the displacement, and the movement direction.
6. Trapping event: An individual died and was added to the number of sampled individuals if it was caught in a pitfall trap during the movement from the previous to the new position.

5.2.1.4 Design concepts

1. Basic principles: Animal movement is a continuous process of changes in speed and direction that is generally discretized to a sequence of steps in order to facilitate model simulations (Pyke 2015). The movement of ground arthropods at the scale of multiple body lengths has been identified as correlated random walk; that is, the movement direction at a time step depends on the direction at the previous step (directional persistence; Kareiva & Shigesada 1983; Bovet & Benhamou 1988; Wallin & Ekbom 1988; Codling et al. 2008; Pyke 2015). The specific assumption about the degree of directional persistence influences the simulated movement pattern of ground arthropods and, thus, affects the probability of a "trapping event" and the sampling bias, which concerns the purpose of our study (see empirical model parameterization in Appendix S1).
2. Emergence: A trapping event, and thus the number of sampled individuals, emerged purely from the movement of ground arthropod individuals across the simulated area with pitfall

traps integrated. To limit side-effects of the specific position of each individual at simulation start, each simulation experiment with a particular parameter set was repeated 50 times and results were averaged for model analysis.

3. Interaction: No interactions were considered among ground arthropod individuals or between ground arthropod individuals and pitfall traps, such as repelling or attraction due to preservative type in the trap (simplifying findings from Knapp & Ruzicka 2012 and Brown & Matthews 2016).
4. Stochasticity: Some key processes of ground arthropod movement were modeled by assuming they are random and follow a certain probability distribution including speed, displacement, and turning angle (see probability distributions within Fig. 1). Considering probability in model simulations was important to reflect differences in the specific movement path across individuals of one species.
5. Observation: During simulation experiments, individual and species-level factors were observed. This includes the trapping events across individuals and the proportion of sampled individuals per species.

5.2.1.4 Input data

The model does not use time-varying inputs, that is, input data representing time-varying processes in the model (Grimm et al. 2010).

5.2.1.5 Initialization

At the start of each simulation experiment, each individual was placed at a random cell of the 400-m^2 simulated area, where x and y coordinates of the cell were each randomly chosen from a uniform distribution. In case an individual was placed at a cell defined as pitfall trap, new x and y coordinates were drawn. The initial movement direction of each individual was randomly chosen from a uniform distribution between 0 and 359, where 0 would create movement along the y-axis with a constant x coordinate value. The body mass and body temperature of each individual were set at the start of a simulation experiment. Pitfall traps were installed across the whole simulation experiment at a position specific to one of the six trap arrangements as shown in Appendix S3: Fig. S1.

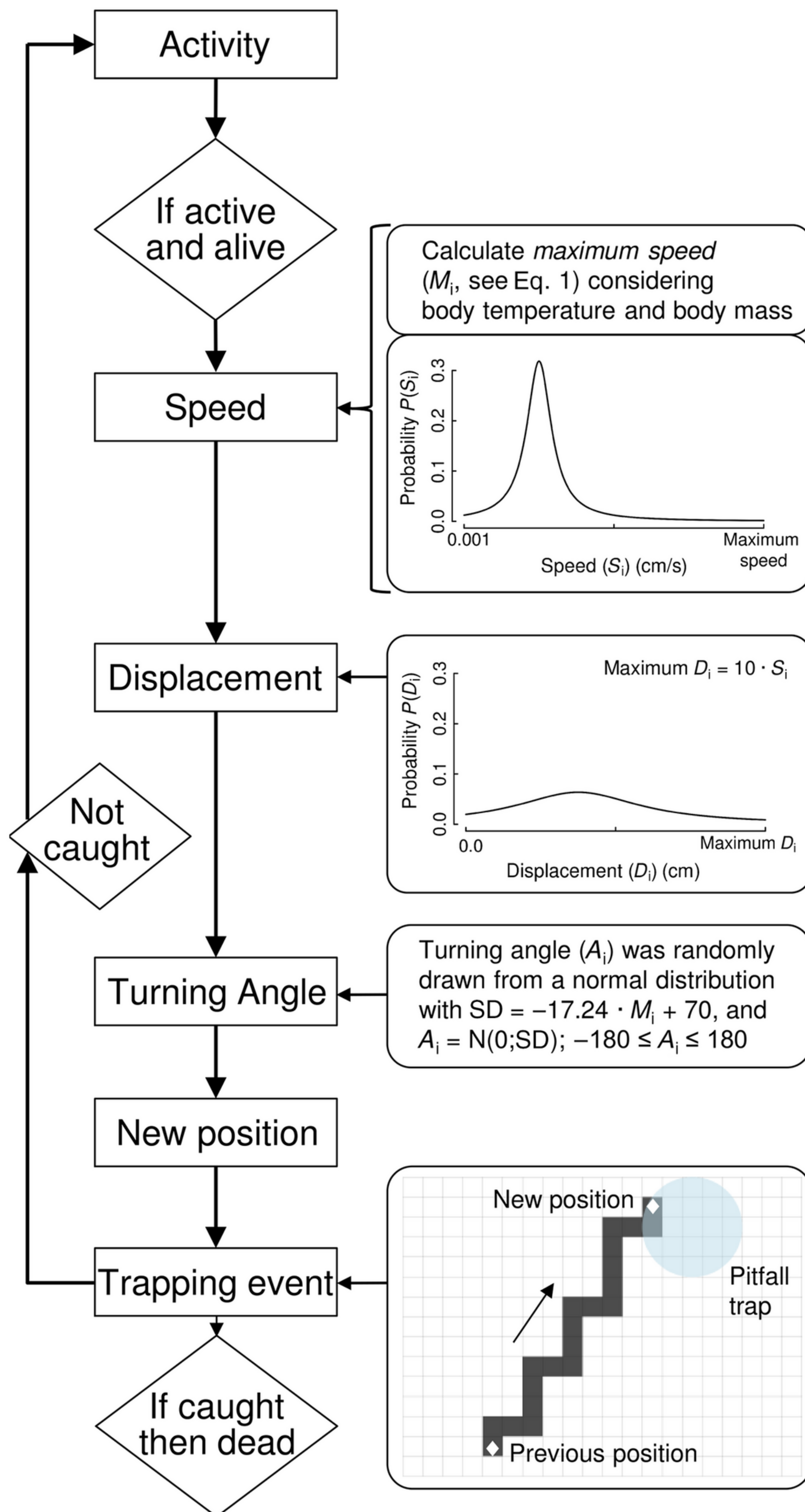


Figure 1. Overview of model processes during one model time step. The processes "speed" and "displacement" use a truncated Cauchy probability distribution. The shown Cauchy distributions represent actual distributions used in the model (see Appendix S1 for empirical parameterization).

5.2.1.6 Submodels

1. Activity: At the first time step of each full hour, it was checked across all individuals that are alive whether the activity period starts or finishes, applying the values of the state variables "start activity period" and "end activity period" (Table 1). The activity status of an individual may changed or changed not accordingly. During the activity period, resting behavior and intermittent movement were excluded; that is, speed was always larger than zero (simplifying findings of Firle et al. 1998 & Reynolds et al. 2015). An individual could be active only if it is alive (see process "trapping event").

2. Speed: The speed of a ground arthropod individual depends mostly on the body mass, the body temperature, and the behavioral mode (Morgan 1985, Hurlbert et al. 2008, Benhamou 2014). In our model, the actual speed S_i (cm/s) of an individual *i* per time step was derived by a two-step process.

First, the potential maximum speed M_i (cm/s) of the individual *i* was calculated considering both body temperature t_i (Kelvin) and body mass m_i (g) (Hurlbert et al. 2008):

$$M_i = 4.3 \times 10^{11} \times \left(m_i^{0.25}\right) \times e^{\left(\frac{-E}{k \times t_i}\right)} \tag{1}$$

where a general temperature effect on biological rates based on reaction kinetics is described by a Boltzmann factor $e^{(-E/kT)}$, with *T* being the temperature in Kelvin, *E* the average activation energy of reactions involved in metabolism (E = 0.65 eV), and *k* the Boltzmann's constant (8.62 × 10^{-5} eV·K^{-1}; Gillooly et al. 2001). This model provides an accurate relationship between metabolic rate and temperature over the range of most biological activity (0–40°C).

Second, S_i was drawn randomly from a Cauchy distribution ranging from 0.001 to M_i. To ensure a constant shape of the Cauchy distribution across different M_i, the Cauchy distribution was truncated to the range from 0.001 to 20 yielding a random value $S_{i,20}$. The actual speed S_i was subsequently scaled to have the maximum M_i by $S_i = S_{i,20}/20 \times M_i$.

The Cauchy distribution is characterized by a "fat tail" yielding a low number of high-speed values but a high number of low-speed values, which relates to empirical proportions of the movement and the search behavioral mode of animals in general (Benhamou 2004, 2014). The specific shape of the Cauchy distribution is defined by the two parameters scale (γ) and location (x_0; see Appendix S1 for empirical parameterization).

3. Displacement: The air-line displacement D_i of an individual *i* during one time step (10 s) does depend on both the speed S_i (cm/s) and the degree of directional persistence during this time step. The displacement D_i was drawn randomly from a Cauchy distribution truncated to the range from 0 to 10 × S_i. This can result in rare events of either no displacement (D_i = 0) or moving straight ($D_i = S_i \times 10$). Similar to the calculation of S_i, an initial value $D_{i,20}$ was drawn from a Cauchy distribution truncated to 0-20, and then, D_i was subsequently scaled to have the maximum 10 × S_i by $D_i = D_{i,20}/20 \times 10 \times S_i$ (see Appendix S1 for empirical parameterization).

4. Turning angle: At the beginning of each time step, the movement direction of an individual *i* during the previous time step may be changed by the turning angle A_i. The turning angle A_i was randomly chosen from a normal distribution with a certain standard deviation (SD). Values outside −180 and 180 degree were rejected and new values drawn, effectively creating a wrapped normal distribution. The mean of the normal distribution was set to

zero assuming an equal proportion of left and right turns. Individuals change the movement direction at scales related to their body length (Pyke 2015) and may increase directional persistence across time steps with increased speed. We calculated SD per individual *i* from a linear equation with a negative slope:

$$SD_i = a \times M_i + b \tag{2}$$

where M_i is the maximum speed at the particular time step and *a* and *b* are constants (see Appendix S1 for empirical parameterization). SD_i decreases with increasing maximum speed M_i. Thus, increasing body mass and temperature results in an increasing directional persistence across modeled time steps. The new direction of movement (i.e., an absolute angle) was calculated by adding the turning angle A_i to the previous direction of movement. To ensure $0 \leq A'_i \geq 360$ degree, 360 is either added to A'_i if $A'_i < 0$ or subtracted from A'_i if $A'_i > 360$.

5. New position: The new values for the x and y coordinates were calculated as follows:

$$X_{i,new} = X_{i,prew} + (D_i \times \cos A'_i) \tag{3a}$$

$$Y_{i,new} = Y_{i,prew} + (D_i \times \sin A'_i) \tag{3b}$$

Where A'_i was converted from degree to radian beforehand ($A'_{I,\ radian} = A'_{I,\ degree} \times \pi/180$). In case $X_{i,\ \mathrm{new}}$ or $Y_{i,\ \mathrm{new}}$ was lower than one or larger than 2000 (outside of the simulated area), the value 2000 was either added or subtracted. Positional *x* and *y* coordinates were rounded to integer values.

6. Trapping event: A trapping event occurred; namely, an individual was caught in a trap and died, if at least one cell of the movement path from the previous to the new position equals a cell of the simulated area designated as pitfall trap. The movement path during one time step was modeled explicitly, simplified to an almost straight path between the previous and the new position using Bresenham's line algorithm (Bresenham 1965). We represented a round pitfall trap by means of the quadratic cells assuming a certain catching probability per pitfall trap cell (Appendix S3: Fig. S2). The cell-specific catching probability equals the proportion of the cell covered by the pitfall trap. In case an individual moves at a cell that is defined as trap but covered by the trap <100%, the occurrence of a trapping event was drawn randomly from a uniform distribution between 0 and 100. The individual got caught if the random number drawn is lower than the proportion of the cell covered by the trap.

5.2.3 Simulation experiments

We conducted 840 simulation experiments systematically varying pitfall trap number, pitfall trap arrangement, body temperature, and population density to assess the species-specific sampling bias. In each simulation experiment, the movement and sampling of individuals across all 10 species were modeled, covering body masses between 1 and 330 mg (Table 1).

Five different numbers of pitfall traps were simulated (1, 2, 4, 8, and 12). Simulation experiments with pitfall trap numbers 4, 8, and 12 covered four different spatial arrangements of pitfall traps (nested cross, two circle, transect, and grid; Appendix S3: Fig. S1). These four trap arrangements were either frequently used in field studies or subject of model simulation studies aiming to improve the reliability of pitfall trapping (Crist & Wiens 1995; Perner & Schueler 2004; Zhao et al. 2013; Chenchouni et al. 2015).

Ten different population densities were simulated (0.15, 0.2, 0.3, 0.4, 0.6, 0.8, 1, 2, 4, and 8 individuals/m^2). This range of population densities covers empirical data of arthropod taxa obtained by true density measurements, such as mark–release–recapture experiments, across arable land and grasslands (Lovei & Sunderland 1996; Thomas et al. 1998; Elliott et al. 2006). The selected population densities followed a logarithmic curve.

The particular body temperature across all individuals ranged from 15° to 30°C (Table 1). This range fits well the ambient temperatures in places such as Central Europe and northeast China throughout the vegetation period (Appendix S4: Fig. S1), assuming that the body temperature exceeds the ambient temperature by 8°C (see section 'State variables and scales'). We modeled a constant temperature for each simulation experiment, simplifying variation between day and night as well as variation across the 14 d of sampling.

5.2.4 Animation of simulated arthropod movement

In addition to the theoretical description of how the model simulates the movement of ground arthropod species, an animation is provided showing in top view the simulated movement of three species (see Video S1; details described in Appendix S6, with Appendix S6: Fig. S1 providing a screenshot of the animation).

5.2.5 Data analysis

The data analysis covered (1) the simulated movement pattern across species and (2) the sampling bias. The latter consisted of two main parts: the species-specific sampling bias and correction factor, and the bias in community-level metrics. The R language version 3.2 together with the vegan package version 2.3-5 was used for data analysis (R Core Team 2015, Oksanen et al. 2016).

According to the virtual ecologist approach, we differentiate between "simulated," "sampled," "observed," and "estimated" values of certain parameters. "Simulated" corresponds to the model input parameters, "sampled" corresponds to the individuals that fell into a pitfall trap virtually (i.e., trapping event), "observed" corresponds to species-specific parameters that were directly derived from the number of sampled individuals, and "estimated" corresponds to community-level metrics derived from "observed" parameters. In a simulation experiment, for example, the "simulated" abundance of a species may be 400 and the number of "sampled" individuals 100, resulting in the "observed" sampling bias of 0.75 (species-specific proportion of simulated individuals not sampled). Subsequently, the RAD and the Shannon diversity could be "estimated" for a particular community of multiple species with certain "simulated" abundance and an "observed" sampling bias per member species.

5.2.5.1 Movement pattern

We used extra model simulations without pitfall trapping to analyze the species-specific movement pattern across 10 species with 1–330 mg body mass at 24°C body temperature. Across 8 h of movement, we recorded four key elements of animal movement for 1000 simulated individuals per species: the movement speed, the turning angle, the displacement at each time step, and the air-line displacement after 8 h.

5.2.5.2 Species-specific bias

For each of the simulation experiments, the number of sampled individuals was recorded per species and per day. For the data analysis, the averaged results of 50 repetitions per simulation experiment were used, essentially eliminating effects of the random start position of individuals. The information about the number of sampled individuals was used to calculate per simulation experiment the observed pitfall trap sampling bias after 14 d of simulated sampling.

Analyses covered individual and combined effects on the observed sampling bias from variation in pitfall trap arrangement, pitfall trap number, population density, and body temperature. Specifically, we analyzed (1) how the mean, the minimum, and the maximum sampling bias across species were affected by trap number and trap arrangement, each for three different body temperatures (15°C, the average across all body temperatures considered, and 30°C); (2) how the sampling bias of each species was affected by its median speed, considering each combination of trap number and trap arrangement separately; (3) how the sampling bias was affected by (i) body temperature, (ii) simulated population density, and (iii) body mass; (4) how species-specific correction factors, for deriving unbiased relative abundance, are related to species body mass.

The observed species-specific sampling bias of species *i* was defined as:

$$B_i = 1 - \frac{n_i}{N_i} \tag{4}$$

where N_i is the simulated abundance and n_i is the sampled abundance of the species *i*. Accordingly, the simulated abundance of species *i* (the unbiased *absolute* abundance in the field) can be calculated from the sampling bias and the sampled abundance of this species:

$$N_i = \frac{n_i}{1 - B_i} \tag{5}$$

We defined the species-specific correction factors for deriving unbiased relative species abundance as proportional to the inverse of the proportion of caught individuals:

$$\delta_i \sim \frac{N_i}{n_i} = \frac{n_i}{1 - B_i} \tag{6}$$

where *i* is the species index, δ is the correction factor, and *B* is the observed sampling bias (Eq. 4). Multiplying the sampled abundance n_i of species *i* by the correction factor δ_i gives the unbiased relative species abundance $N_{R,i}$ (i.e., relative to the simulated number of individuals or the unbiased absolute abundance in the field):

$$N_{R,i} = n_i \times \delta \tag{7}$$

We analyzed the relationship between the correction factor δ and species body mass *m*, which has a high relevance in community ecology and can be easily estimated for each species. This relationship we assumed to be of the form:

$$\delta_i \sim m_i^{ß} \tag{8}$$

where β is the strength of the body mass effect on the correction factor, or rather the slope of the relation between correction factors and body mass.

5.2.5.3 Community-level bias

Communities were not simulated in extra simulation experiments. Species-specific values of simulated and sampled abundance from the above-described simulation experiments were used to create virtual communities and calculate community-level metrics. Communities were

assembled from 10 differently sized species with 1–330 mg body mass (Table 1) and characterized by a specific RAD. In a community, the simulated population densities of the 10 member species followed a log curve. Species at ranks from 1 to 4 (with 3200, 1600, 800, and 400 individuals) were defined as dominant because their population abundance is larger than 10% of the most abundant species (sensu Grime 1998). We refer to the remaining species at ranks 5–10 as subordinate species. 50,000 random communities of ground arthropods were created by arranging the 10 species into different sequences along the abundance ranks. Of these communities, 378 were selected for analysis, using the ones with a realistic relationship between body mass and population abundance, that is, small species having a higher rank than larger species (for details, see Appendix S6). Additionally, we created two communities where the body mass across the abundance ranks strictly increases or decreases.

We calculated for all combinations of trap number and trap arrangement, averaged across all simulated body temperatures: (1) the simulated and the estimated relative abundance per species, displaying the RAD for the two “strict” communities and the average of the 378 random communities.

Further, we calculated for only the 378 random communities the following metrics across the combinations of all trap numbers, all trap arrangements, and three body temperatures (15°C, the average across all body temperatures considered, and 30°C): (2) the deviation of the estimated from the simulated species-specific abundance rank, (3) the proportion of simulated dominant species that were classified as subordinate species based on the estimated abundance ranks, and (4) the estimated and simulated Shannon diversity and Fisher's alpha.

5.3 Results

5.3.1 Body mass-related movement pattern

The relation between maximum speed and body mass was central to our simulations of pitfall trapping ground arthropod species (Eq. 1). Appendix S7: Fig. S1 presents the variation in four key elements of animal movement across the body masses used in our simulations. The median movement speed per time step increased with body mass from 0.2 for small species to 0.85 cm/s for large species. The median turning angle decreased from 37.9 to 8.4 degree. The median displacement during one time step and one day increased from 0.77 to 3.24 cm and 0.83 to 15.84 m, respectively. The simulated ground arthropod movement included rare events of extreme values in speed, displacement, and turning angle across all species, meeting an essential property of animal movement in general.

5.3 2 Species-specific sampling bias

5.3.2.1 Trap number and arrangement

Our model simulations highlight a strong decrease in the sampling bias with increasing pitfall trap number, consistently across trap arrangements (Fig. 2). When increasing the trap number from 1 to 2, 4, 8, and 12, the sampling bias decreased from 0.94 to 0.89, 0.82, 0.71, and 0.62 averaged across all species, temperatures, trap arrangements, and population densities (Fig.

2B). Importantly, the difference in the sampling bias between small and large-sized species increased with increasing pitfall trap number from 0.13 to 0.23, 0.36, 0.52, and 0.60, respectively. Consequently, the increase in trap numbers strongly affected two important metrics at the same time: lowering the mean sampling bias and increasing the variation in the sampling bias between small and large species.

We found considerable differences across the four trap arrangements nested cross, two circles, transect, and grid only when applying eight or 12 pitfall traps (Fig. 2; Appendix S6; animation in Video S2). Notably, eight traps arranged as grid or two circles produced about the same average sampling bias as 12 traps arranged as nested cross or diagonal transect. Body temperature had a strong impact on the absolute values of the sampling bias, but not on the relative differences in the sampling bias across pitfall trap number and arrangement.

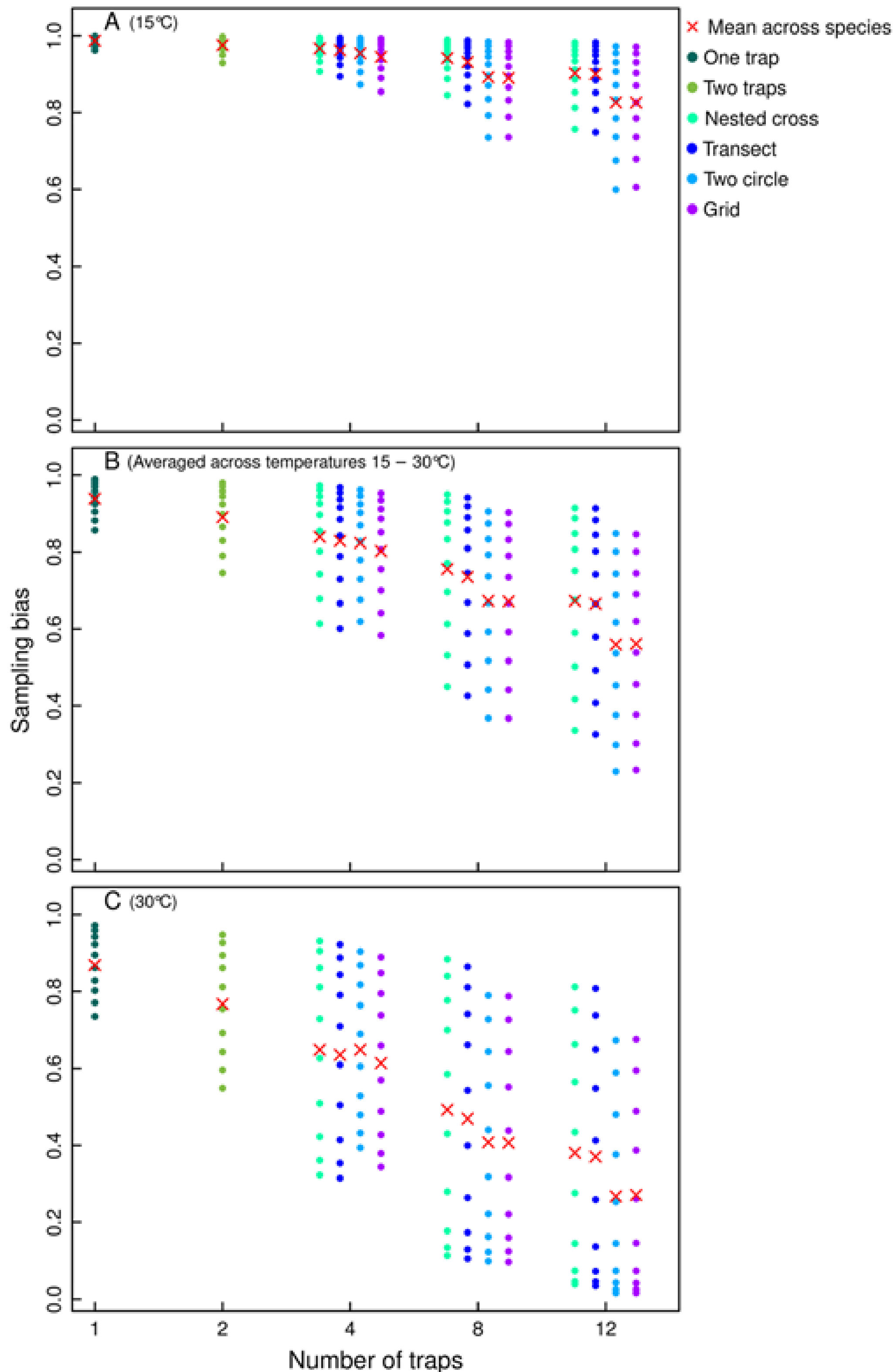


Figure 2. Species-specific sampling bias, that is, the proportion of simulated individuals not sampled, per pitfall trap number and trap arrangement (Appendix S3: Fig. S1) after simulated pitfall trap sampling of 14 d, with 8 h of activity per day. Values for 10 different species (1–330 mg body mass) and the mean across these species are shown. Values are averaged across simulation experiments with 10 different population densities. The pitfall trap arrangement is color-coded (see legend). The minimum and maximum values of the sampling bias per trap number and arrangement correspond with the largest and smallest species, respectively. Panels show results for (A) 15°C body temperature, (B) averages across six different body temperatures spanning 15–30°C, and (C) 30°C body temperature.

5.3.2.2 Movement speed

Our simulation experiments reveal a non-linear relationship between the sampling bias of a species and its median movement speed (Fig. 3). The shape of this relationship was considerably affected by the number of pitfall traps, but not so much by the specific trap arrangement. The sampling bias of very slow-moving species was high across all pitfall trap numbers. The sampling bias of fast-moving species varied strongly with changes in trap number.

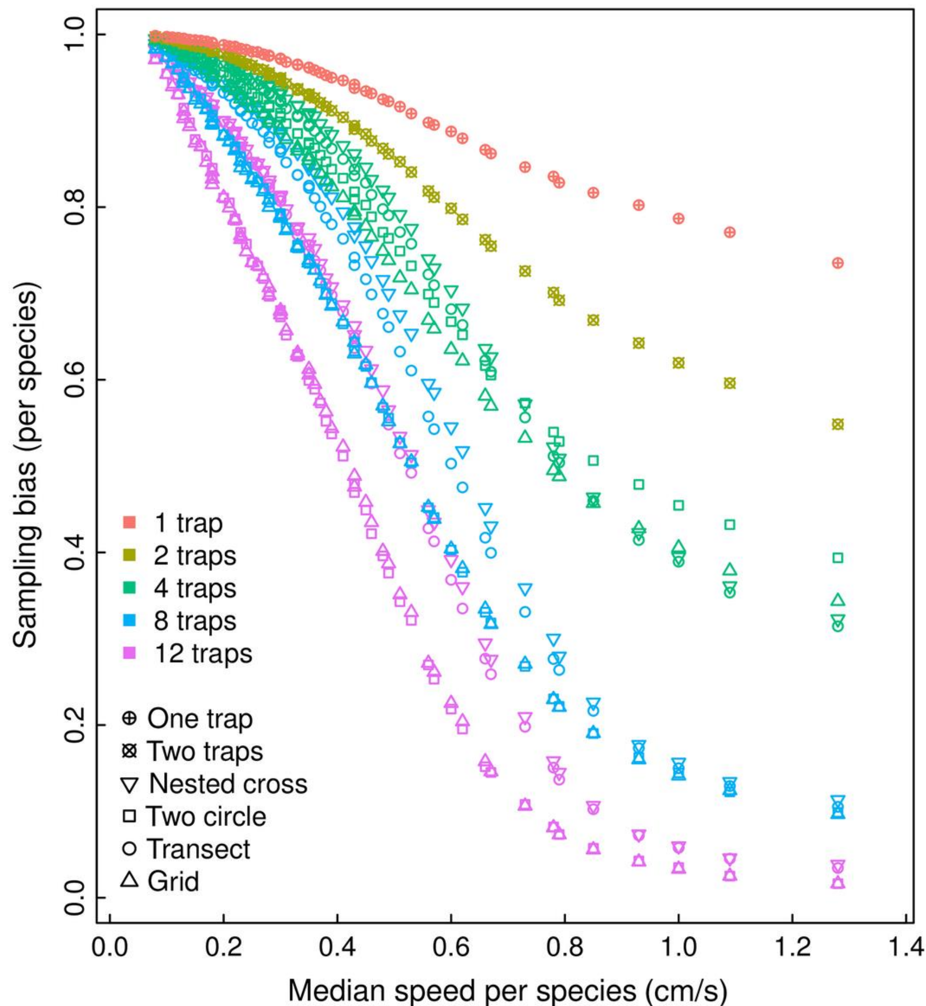


Figure 3. The relationship between the species-specific sampling bias and the median speed of the species, shown for different combinations of pitfall trap number (color-coded) and trap arrangement (symbols; applicable for 4–12 traps). Speed values are averaged across individuals per species and cover six different body temperatures (Table 1).

5.3.2.3 Temperature, population density, and body mass

The sampling bias decreased non-linearly with an increasing body temperature (Appendix S8: Fig. S1). With an increasing number of pitfall traps, the effect of body temperature on the sampling bias increased. A rise in body temperature from 15° to 30°C decreased the average sampling bias across species by about 0.1 if one trap was used and 0.5 if 12 traps were used (Appendix S8: Fig. S1).

Surprisingly, an increase in population density from 0.15 to 8 individuals per m2 had no effect on the sampling bias (Appendix S8: Fig. S2). Clearly, the absolute number of sampled individuals increased with increasing population density but the proportion of simulated individuals that were sampled remained unaffected. This can be explained as a consequence of the random distribution of the individuals at the start of each simulation experiment, which ensures a certain species-specific probability for an individual to become sampled during the simulation experiment. This probability may equal "1 minus the species-specific sampling bias" and did not depend on population density because interactions between individuals were not considered.

The body mass of a species had a non-linear negative impact on the sampling bias (Appendix S8: Fig. S3, log–log scaled). The sampling bias was non-linearly related to body mass due to the non-linear effects of body mass on maximum speed adding to the non-linear effects speed on sampling bias (Eq. 1 and Fig. 3).

5.3.2.4 Correction factor

The correction factors were linearly related to species body mass on a log–log scale (Fig. 4A; see Appendix S9: Fig. S1 for temperature effects). The fitted linear models had very high R2 values around 0.99. The slopes varied significantly between trap numbers and temperatures (Fig. 4B; Appendix S9: Fig. S2). Slopes were steeper and more negative with lower numbers of pitfall traps and temperatures indicating larger differences in the sampling bias and the correction factor between small and large species. For example, the slope was −0.44 for eight and −0.49 for four pitfall traps arranged as nested cross. Realistic estimates of unbiased relative species abundance can be obtained by applying the slope (β) to Eq. 8 and the resulting species-specific correction factor (δ) to Eq. 7.

Assuming eight traps arranged as nested cross, for example, the correction factor would be 0.105 for a species of 100 mg (100−0.49) and 0.712 for a species of 2 mg (2−0.49) yielding unbiased relative species abundance when applied to Eq. 7 The nested cross arrangement of pitfall traps may be particularly useful for estimations of unbiased relative species abundance because of the small 95% confidence interval of the slope, implying that the relation between the correction factor and the body mass is more linear than for other trap arrangements. Note that the correction factor derived from Eq. 8 is proportional to the sampling bias (see Eq. 6).

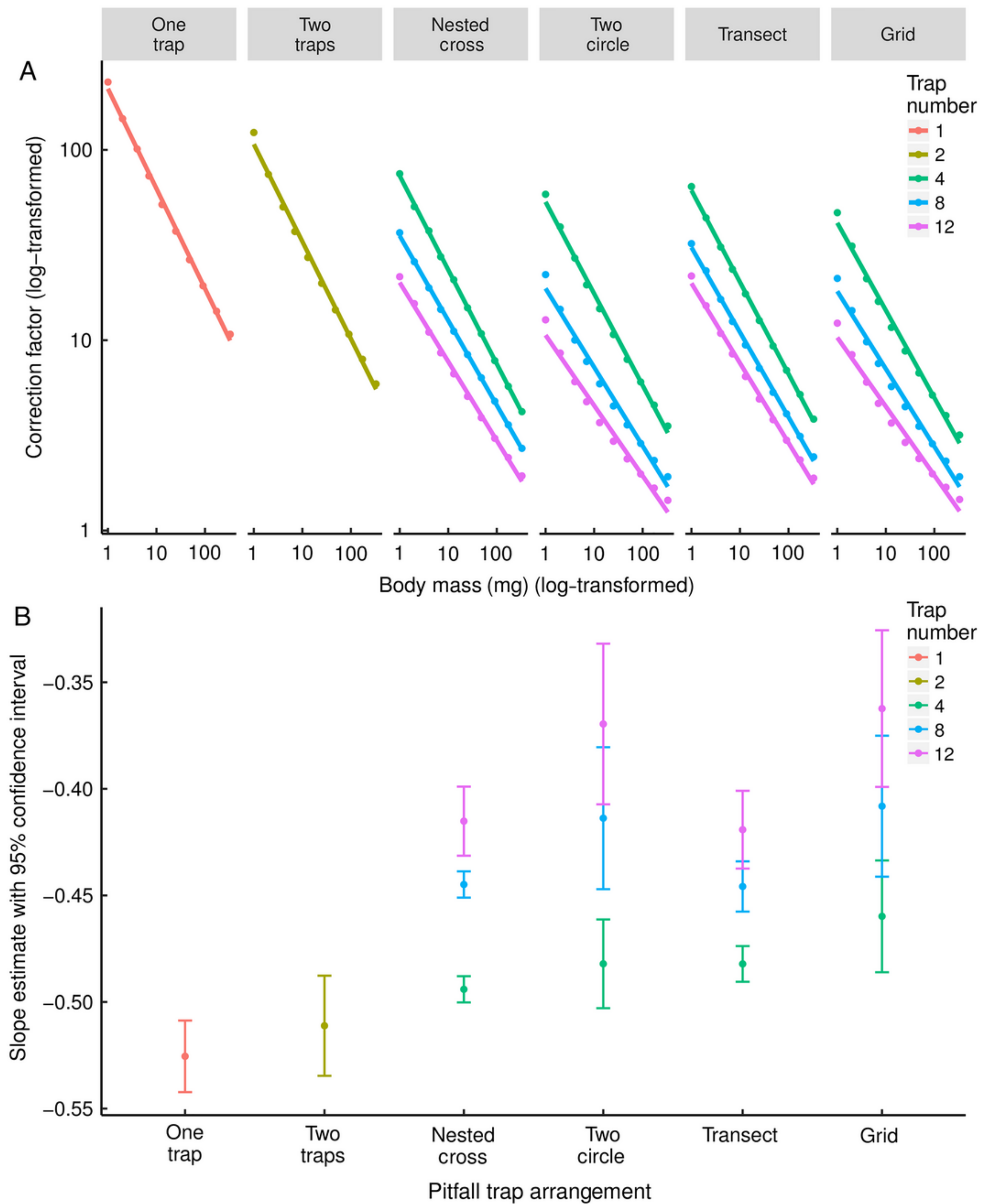


Figure 4. (A) The relationship between body mass and the correction factor for deriving unbiased relative species abundance per trap number and trap arrangement (Appendix S3: Fig. S1), averaged across six different body temperatures (15–30°C; see also Appendix S9: Fig. S1). The individual values per species (dots) and the linear regression line are shown. (B) The slope of the regression lines from panel A is shown together with the 95% confidence interval.

5.3.3 Community-level effects of the sampling bias

5.3.3.1 Rank-abundance distribution

The estimated RAD generally differed from the simulated RAD across pitfall trap numbers and trap arrangements (Fig. 5). Differences between the simulated and estimated RAD were mainly driven by the body mass–dominance relationship. The estimated RAD was flatter than the simulated RAD for arthropod communities with reasonable species abundance ranks, structured in the way that large species were rare and small species abundant (Fig. 5B, C; Appendix S5: Fig. S1). Larger, rarer species are relatively over-represented in pitfall trap catches from such arthropod communities, thus inflating diversity estimates using Shannon diversity and Fisher's alpha (Appendix S10). The opposite pattern, that is, the estimated RAD was steeper than the simulated RAD, was found when large species were abundant and small species rare (Fig. 5A). Further, the difference between the estimated RAD and the simulated RAD decreased with increasing trap numbers (Fig. 5).

5.3.3.2 Species-specific abundance rank

Pitfall trap sampling can produce a bias in the estimated abundance rank of a species (Fig. 6). We find that generally the estimated rank of small species is higher than the simulated rank, while the estimated rank of large species is lower than the simulated rank (notes: Low rank means dominance; in Fig. 5, a low rank is consistent with a lower number at the x-axis with the highest-abundant species at rank 1). The difference between the estimated and the simulated abundance rank decreased with increasing pitfall trap number and body temperature.

5.3.3.2 Classification of dominant species as subordinate

Our model simulations revealed that an average proportion of about 25% of the simulated dominant species were classified as subordinate species by pitfall trap sampling due to the bias in estimated abundance ranks (Fig. 7B). In return, this means that an equal number of simulated subordinate species was estimated as dominant species. On average, across 378 random communities (Appendix S5), this finding remains valid for changes in pitfall trap number, trap arrangement, and body temperature (see median [orange bars] and average [red dots] of communities in Fig. 7). For low body temperatures and low pitfall trap numbers in particular, the proportion of simulated dominant species that are detected as subordinate strongly varied between zero and 75%, thus depending mainly on the community structure in terms of the body mass distribution across the abundance ranks. The effect of community structure, however, generally diminished with increasing numbers of pitfall traps.

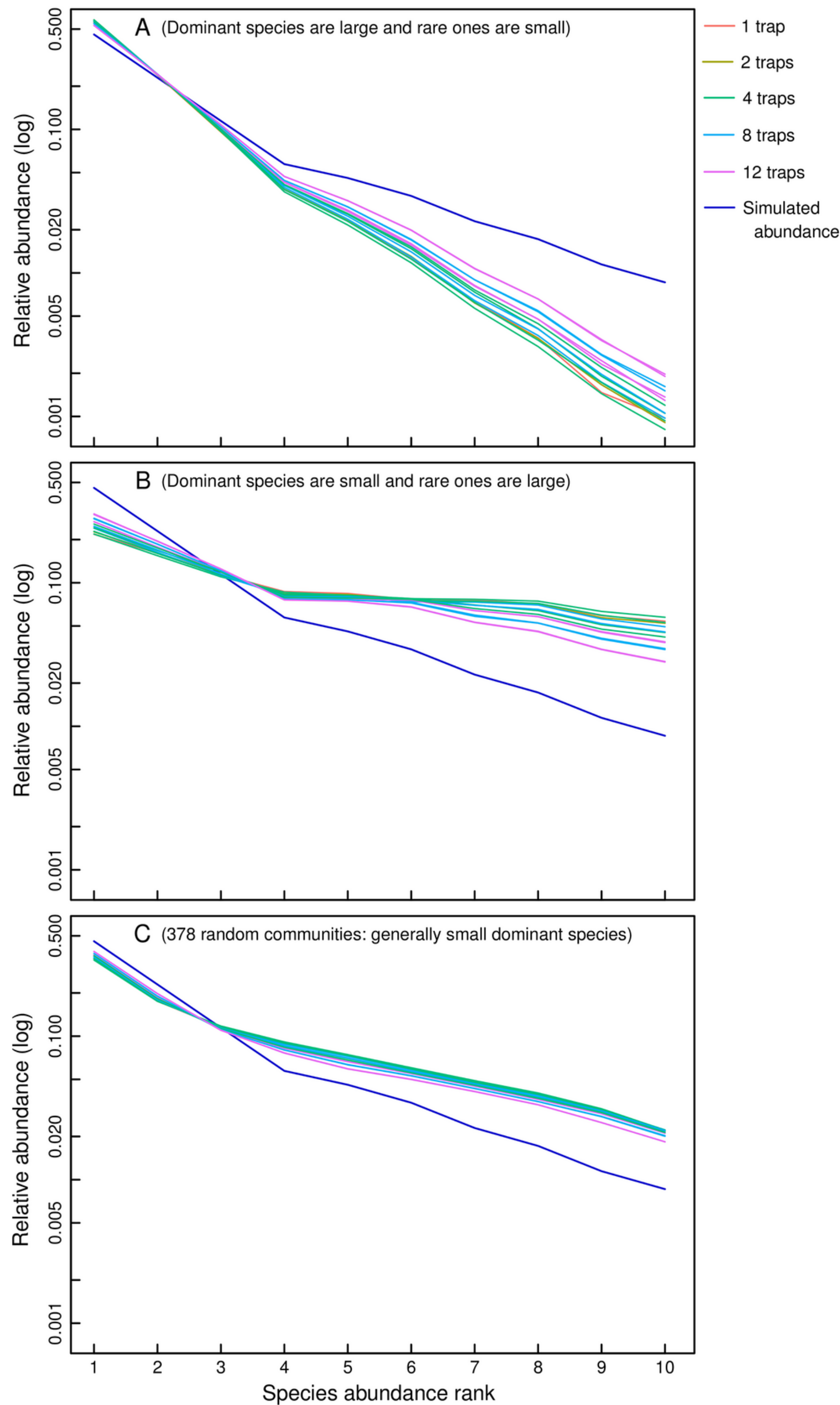


Figure 5. The rank-abundance distribution (RAD) of the simulated population abundance (dark blue; logarithmically spaced from 8 to 0.15 individuals/m2) and the observed population abundance (see legend for colors). The RAD from observed abundance is shown for each of the 14 different combinations of pitfall trap number and trap arrangement, but only differences in trap number are color-coded. The y-axis shows relative abundance per species and the x-axis the abundance rank where species with rank 1 is the species with highest relative abundance. Panels show different community structures, that is, different sequences of species body mass across abundance ranks: (A) Body mass decreases with increasing simulated abundance rank—the largest species is most abundant (rank 1); (B) body mass increases with increasing simulated abundance rank—the smallest species is most abundant; and (C) average of 378 random communities, which are characterized by a reasonable body mass–abundance relationship (smaller species having generally a higher rank than larger species, Appendix S5).

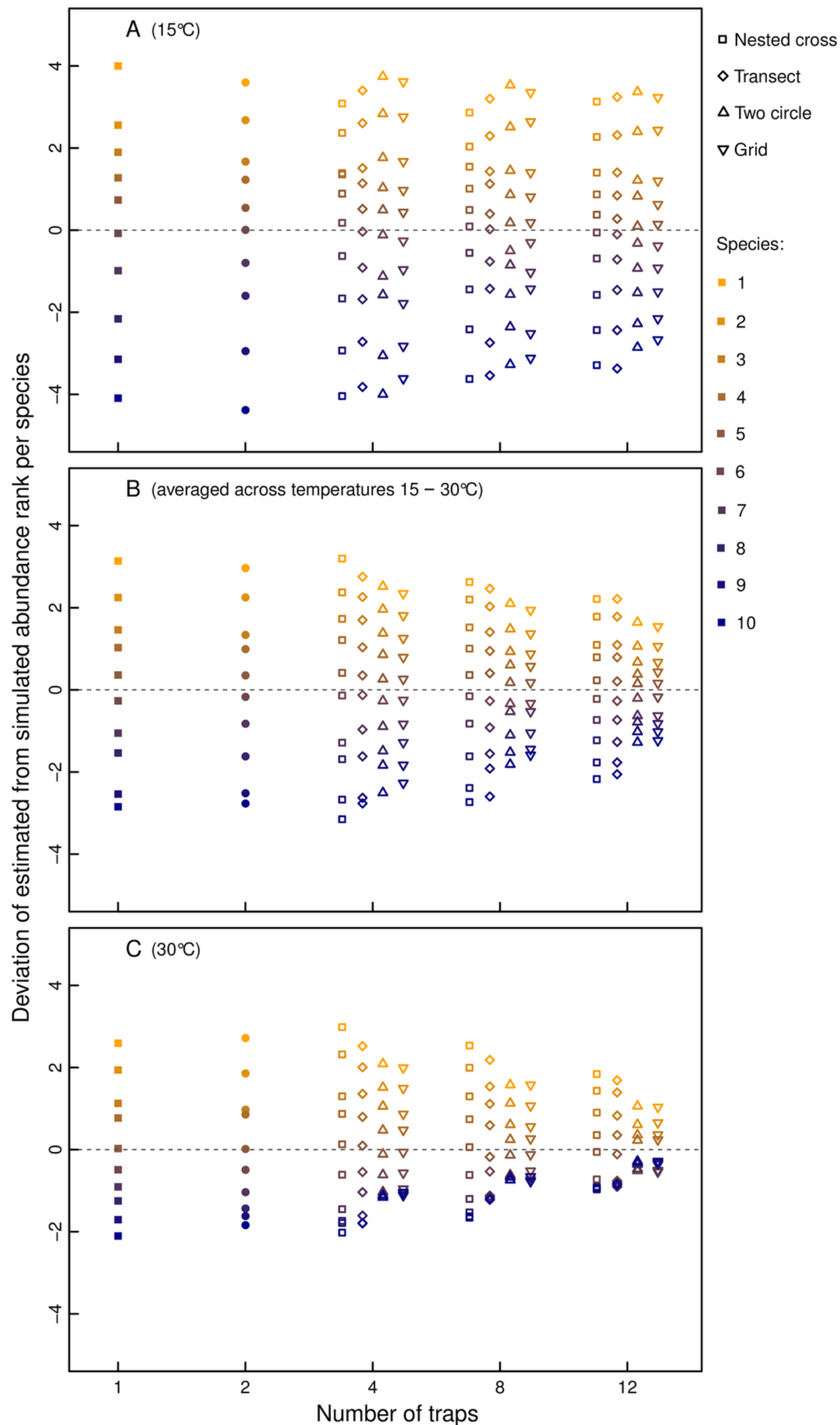


Figure 6. Difference between simulated and estimated abundance rank per species, shown for different combinations of pitfall trap number and trap arrangement (full colored symbols for "one trap" and "two traps" arrangements; see legend for open symbols). Species are color-coded (orange: species 1 with 1 mg body mass, dark blue: species 10 with 330 mg body mass). A positive value means: The estimated abundance rank from pitfall trap sampling is higher than the simulated rank; that is, the estimated relative abundance is lower than the simulated one. A negative value means the inverse, and zero means no difference between simulated and estimated ranks. Values are averaged across 378 random communities (Appendix S5). Panels show results for (A) 15°C body temperature, (B) averages across six different body temperatures spanning 15–30°C, and (C) 30°C body temperature.

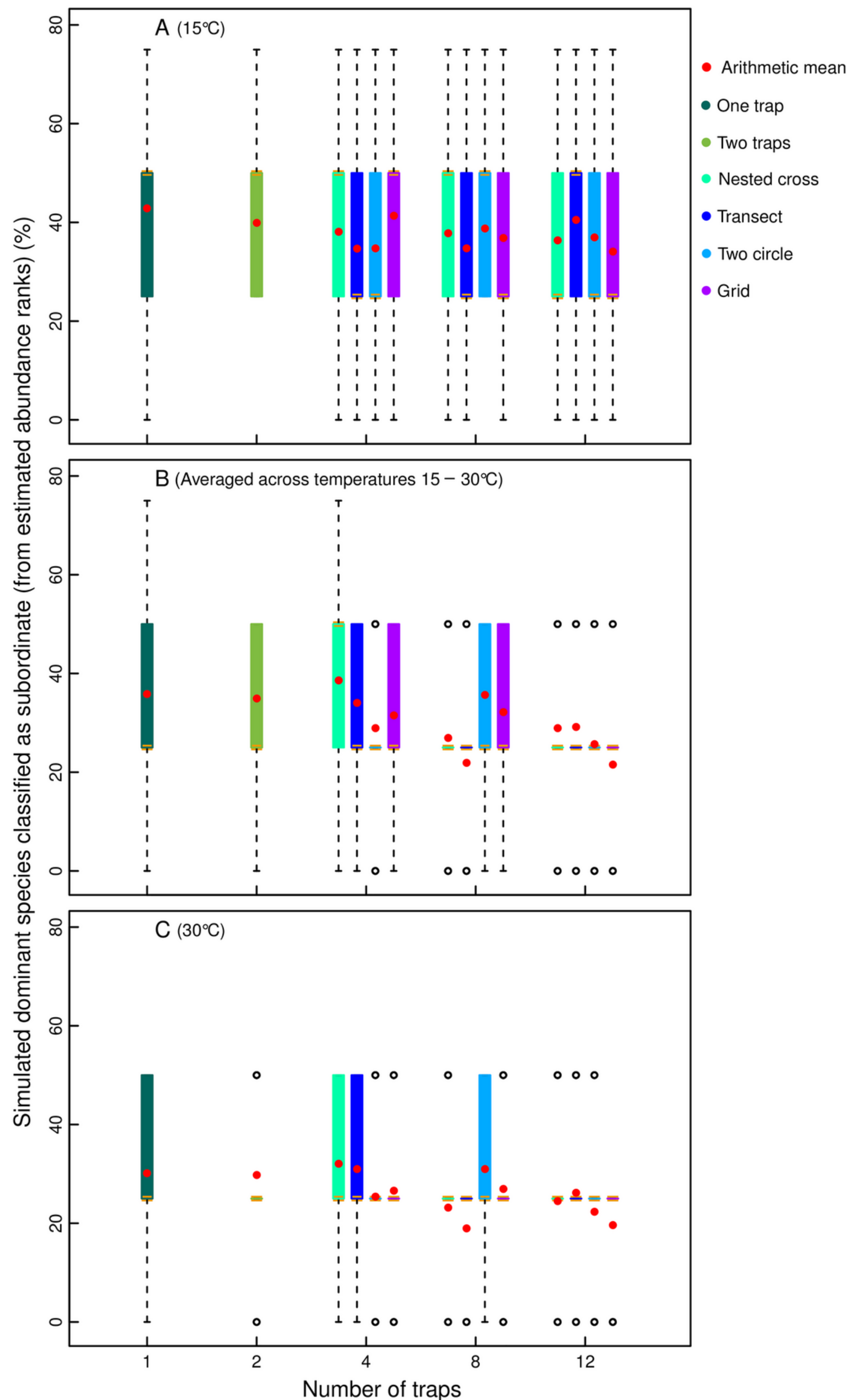


Figure 7. Boxplot of the proportion of simulated dominant species that are classified as subordinate species based on estimated abundance ranks. The values are averaged across 378 random communities (Appendix S5). Values are shown for different combinations of pitfall trap number and trap arrangement (see legend for colors). Horizontal orange bars indicate the median, and the red dots show the arithmetic mean. The colored box ranges from the upper to the lower quartile, and the dotted whiskers cover the maximum and minimum values excluding outliers (black dots, if present). For some combinations of trap number and arrangement, the box and the whiskers match with the median. Species with one of the simulated abundance ranks 1–4 were defined as dominant species (see 'Methods' section). Panels show results for (A) 15°C body temperature, (B) averages across six different body temperatures spanning 15–30°C, and (C) 30°C body temperature.

5.4 Discussion

Our study clearly shows that the pitfall trap sampling bias strongly decreases with increasing body mass, body temperature, and trap number, while the spatial arrangement of pitfall traps has only limited effects (Fig. 2). The population density does not affect the sampling bias; hence, a specific parameterization of bias correction across different studies or study years is acceptable even if the densities of sampled species vary strongly. We derived species-specific correction factors that allow unbiased estimates of relative species abundance to be derived knowing only species body mass (Fig. 4). Interestingly, our analyses reveal that the variation in the sampling bias between differently sized species scales up to a bias in community metrics that is clearly indicated by a less steep RAD, which results in an overestimation of community diversity and incorrect identification of species dominance (Figs. 5, 7). We presented an allometric individual-based model that can simulate the movement and pitfall trap sampling of multiple, actively hunting ground arthropod species across the range of body masses from 1 to 330 mg.

5.4.1 The species-level bias

Our model simulations highlight considerable changes in the movement of ground arthropods with increasing body mass. Large species move at higher speeds and change their direction of movement less often than small species do. Hence, with increasing body mass, species displace more and may be considered more active. This is consistent with the commonly known biases of activity density measures in pitfall trap sampling toward larger, faster species (Mommertz et al. 1996; Lang 2000).

Our study reveals that the spatial arrangement of pitfall traps has only limited effects on the sampling bias (Figs. 2, 3). This is an unexpected result considering the effort of previous studies in finding an optimized trap arrangement to provide reliable pitfall trap samples (Crist & Wiens 1995; Perner & Schueler 2004; Zhao et al. 2013). Yet, we find that the sampling bias is clearly lower for the two-circle arrangement than for the nested cross, which is consistent with theoretically and empirically derived findings of Zhao et al. (2013).

The number of pitfall traps strongly impacts the sampling bias. This effect varies across species revealing a trade of concerning the optimal number of pitfall traps: The mean sampling bias decreased when the number of traps increased but at the same time the differences in sampling bias between small and large species strongly increased (Fig. 2). Thus, a higher number of pitfall traps mainly reduces the sampling bias of large species and increases the importance of correcting the species-specific sampling bias. Overall, we recommend either the grid or two-circle arrangement of 4–8 traps per 400 m2 to both moderate the drawbacks of a high pitfall trap number and yield a low average sampling bias.

Environmental conditions in general and the ambient temperature in particular are important factors that constrain arthropod movement and thus affect the pitfall trap sampling bias (Melbourne 1999; Hurlbert et al. 2008; Wang et al. 2014). This is because the body temperature of ground arthropods is correlated with the ambient temperature (Casey 1976; Morgan 1985). We find accordingly that the sampling bias strongly decreases with increasing

body temperature (Appendix S8: Fig. S1). Our findings are consistent with field experiments and a statistical correlation approach showing that the number of sampled individuals increases with mean daily temperature (Brunsting 1981; Thomas et al. 1998; Saska et al. 2013; Wang et al. 2014).

The species-specific sampling bias is strongly related to the movement speed and the number of pitfall traps (Fig. 3). Knowledge of this bias could be used to estimate unbiased absolute species densities in the field. The movement speed of ground arthropod species can either be measured by observations directly in the field or estimated from body mass and body temperature. Body mass of species sampled in field studies can be measured or derived from the literature. Body temperature can be estimated if the ambient temperature is measured frequently (e.g., hourly) and knowledge on species' activity periods is available. For arthropod species with known activity periods, we propose using the species-specific median movement speed as simplifying proxy for estimating the sampling bias across trap numbers, because the movement speed in the field integrates many other factors that shape the sampling bias (Fig. 3). Further works are needed to test the practicability of this approach in field studies and the extent to which the sampling bias can be determined or reduced when compared to population densities estimated from, for example, quadrat sampling (Topping & Sunderland 1992; Spence & Niemelä 1994). For species with unknown activity periods, we suggest using body mass to calculate unbiased relative densities.

Relative species abundance is of prime importance for community ecology research being the basis for metrics such as the RAD. Our study reveals that a species-specific correction factor can be derived from species body mass alone, providing reasonable estimates of unbiased relative species abundance (similar to Hancock & Legg 2012). This correction factor offers a simple method to adjust pitfall trap data, as body mass could be easily measured for the species sampled and the sampling bias does not depend on population density (Appendix S8: Fig. S2; contrary to Perner & Schueler 2004). Bias correction would work across plots and studies along environmental and land-use gradients when taking into account potential differences in temperature and trap number and how these may affect the correction factor (Appendix S9: Fig. S2). Thus, there is potential to improve real-world data from previous and future sampling campaigns enabling much more reliable understanding of the impacts of climate and land-use change on community biodiversity.

5.4.2 The community-level bias

Our study provides one of the first attempts to quantify the impact of the species-specific sampling bias on the community-level metrics: RAD, the dominance of species, and the species diversity, that is, Fisher's alpha and Shannon diversity.

The RAD of species is one of the most commonly analyzed patterns in ecology, generally showing a few dominant species and many less abundant or rare species in a community (McGill et al. 2007; Locey & White 2013). Our model results clearly show that the estimated RAD, based on observed species abundance from pitfall trap sampling, can strongly differ from the simulated RAD or rather the true RAD of the study community (Fig. 5). We find that the simulated distribution of species body masses across the abundance ranks essentially

determines whether the estimated RAD is either more or less steep than the simulated, unbiased RAD. Generally, our results imply that a RAD estimated from pitfall trap sampling campaigns may be less steep than the real RAD (see Fig. 5B, C), because dominant species are commonly small, resulting in a high sampling bias, where the less abundant species are rather large, resulting in a comparably low bias (Siemann et al. 1999; Gossner et al. 2015). Any factor that increases the difference in the sampling bias between species may further increase the bias in the estimated RAD, such as elevating temperatures, higher trap numbers, and a larger range of body masses. The body masses we considered in our study range from 1 to 330 mg, which covers a subset of the body masses of arthropod species found by Gossner et al. (2015) in Central European grasslands. Hence, field studies that attempt to include all species occurring in a habitat may face an even larger bias in the estimated RAD as estimated from our simulations.

The RAD is clearly related to diversity indices that are important for both evaluating the condition of a community and estimating its vulnerability to environmental changes. Our results show that the estimated Shannon diversity and Fisher's alpha are generally larger than what we would expect from the simulated species abundance (Appendix S10: Figs. S1, S2). We argue that real Shannon diversity and Fisher's alpha values are generally lower than suggested from pitfall trap sampling campaigns; thus, population abundance within communities is less even and follows more the log series distribution than previously estimated. The Shannon diversity index, in particular, depends strongly on the body mass distributions across the abundance ranks, suggesting a high sensitivity to changes in the community structure. Importantly, this can jeopardize conclusions drawn from diversity analysis across communities differing in the species composition in general and the community structure in particular. Hence, the Shannon diversity of two communities can appear to be significantly different due to variations in body mass distributions. To avoid biased conclusions about differences between communities, field studies may need to apply species-specific correction factors of the sampling bias of pitfall trap data, or should test for differences in the body mass distributions.

In addition to the RAD of a community, the specific abundance rank is an important property of each single species enabling the assessment of the role it might play in ecosystem functioning. Dominant species are considered as particularly important for key ecosystem functions, while rare species may either work as insurance against future uncertainties or provide additional functions (Grime 1998; Mouillot et al. 2013). Our model simulations highlight that the estimated abundance rank of species can differ significantly from the simulated, unbiased abundance rank (Fig. 6). Generally, the relative population abundance is underestimated for small species and overestimated for large species by pitfall trap sampling. Empirical studies that analyze the arthropod community structure based on pitfall trap sampling may yield a bias in the functional importance of species underestimating small species and overestimating large species.

The classification of species into dominant and rare ones is an important tool in ecology to explore relationships between community diversity and functioning (Grime 1998). A difference between the estimated and the true abundance rank of species, however, can considerably impact the reliability of this classification. Our simulations reveal that a considerable proportion (about 25%) of the simulated dominant species are observed as

subordinate (Fig. 7). Accordingly, an equal number of subordinate species is observed as dominant. This can constitute serious implications for the conclusions of studies on ecosystem function that focused on dominant species only. Furthermore, analysis of which species traits may drive key functions may need to be tested for reliability against variation in the set of species observed as dominant, for example, by statistically testing for effects of the body mass in general or its distribution across abundance ranks in particular.

In summary, pitfall trap sampling in field studies may generally produce flattened RAD yielding overestimations of community diversity and likely providing false results for the dominance classification of some species. Our findings extend the widely accepted species-specific bias in pitfall trap sampling campaigns to community-level metrics and urge caution to previous conclusions about the diversity and structure of ground arthropod communities when solely sampled with pitfall traps without bias correction.

5.4.3 Model assumptions

We deliberately used a simple model to simulate the movement and pitfall trap sampling of ground arthropods. Yet, we expand on previous simulation studies of pitfall trap sampling by considering parameter variation of multiple factors important for both ground arthropod movement and the design of a pitfall trap sampling campaign. In our simulations, we did not cover, however, other factors that were shown to influence the pitfall trap sampling bias of individual species, such as precipitation, litter depth, vegetation density, and the design of pitfall traps (Greenslade 1964; Spence & Niemelä 1994; Melbourne 1999; Lang 2000; Work et al. 2002; Koivula et al. 2003; Thomas et al. 2006; Cheli & Corley 2010; Brown & Matthews 2016). Though, our simplifying assumptions of a homogeneous, featureless landscape and plain traps are unlikely to affect the conclusions of our study as all simulation experiments were, in this respect, equal. Future simulation studies may particularly aim at integration of a plant diversity gradient facilitating sampling bias correction of multitrophic diversity studies in grassland (Rzanny & Voigt 2012), farmland (Klaus et al. 2013) and forest (Schuldt et al. 2015). Modeling the effects of vegetation structure and density on movement of ground arthropods should include plant individuals explicitly, so that the movement paths of ground arthropods result from a combination of external factors and the internal navigational capacity (Nathan et al. 2008).

5.4.4 Implications

The results of our individual-based model simulations help field studies to increase the reliability of species-specific data and any community-level metric estimated from pitfall trap sampling. The main factors that shape the species-specific sampling bias are body mass, temperature, and the number of pitfall traps (Fig. 2). Although the arrangement of traps is of minor importance when compared to trap number, the reliability of pitfall trap sampling can be increased by distributing traps uniformly within the sampling area. The bias in community-level metrics is linked to the species-specific biases and additionally shaped by the distribution of body masses across the abundance ranks (Fig. 5). To simplify the bias correction, studies

should keep constant the trap number and trap arrangement across sampling sites and sampling periods whenever possible. Following the call of Brown and Matthews (2016) for standardized trap designs, we suggest to use either the nested cross or grid arrangement with standard trap numbers and spacing. For data analysis, species may be grouped into size classes assuming a similar bias for the species in one class and, thus, lowering the workload for bias correction across species. Species size classes may preferably cover logarithmically increasing ranges of body mass as body mass non-linearly affects the species-specific sampling bias (Appendix S8: Fig. S3).

Most research in community ecology derives diversity and community metrics based on species relative abundance, such as the RAD and species dominance within the community. One therefore may not need to correct observed species abundance on an absolute scale but rather on a proportional scale. Our analyses of the correction factor showed that species relative abundance can be re-set to unbiased values by species body mass when controlling for temperature variation, pitfall trap number and arrangement (Fig. 4B, Appendix S9: Fig. S2). For instance, a field study using two pitfall traps could correct relative abundance from species body mass with two scaling factors from the range between −0.55 and −0.37. The resulting interval in diversity and community metrics would be unbiased by pitfall trap sampling and could be used as a sensitivity test for conclusions drawn from individual studies or a meta-analysis across several studies.

We conclude that (1) the correction of the species-specific sampling bias to derive realistic absolute species abundance and (2) the use of body mass-related correction factors to derive true relative species abundance are a manageable task and necessary to reliably identify changes in species abundance and community diversity across time or habitats. Given that the species body masses are easily estimated for ground arthropods, the bias correction for true relative abundance should be a simple, practical approach for field studies to be widely adopted and tested. Also, the ambient temperatures (as proxy for body temperature) across the sampling periods are often available, facilitating estimations of the median movement speed and, subsequently, realistic indications of the sampling bias and absolute densities per species (Fig. 3). Future studies may attempt to reanalyze available “activity density” data from previous pitfall trap sampling campaigns to advance our understanding of arthropod community structure both within certain habitats and along environmental gradients.

5.5 Acknowledgements

Jan Engel and Lionel Hertzog contributed equally to this study. We thank Günter Köhler as the manuscript greatly benefited from his comments. This work was supported by the German Research Foundation (DFG) and the Technical University of Munich (TUM) in the framework of the Open Access Publishing Program. The authors declare no conflict of interest. Model simulations were done at the High Performance Computer Cluster of the Friedrich Schiller University Jena. We are grateful to the helpful comments and suggestions of two anonymous reviewers on an earlier draft of this manuscript.

5.6 Supporting Information

Appendix 1: Parameterization of ground arthropod movement

Preliminary ‘parameterization simulations’ were used to empirically parameterize the movement of ground arthropod species across body mass (1 to 330 mg) and body temperature (15 to 30°C). The set of parameter values (Table S1, Appendix 1) that provided the best fit between data from parameterization simulations and selected empirical studies was used for modeling the ground arthropod movement in the ‘simulation experiments’ of our study. Overall 3,072 parameterization simulations, each with a different set of parameter values (Table S1, Appendix 1), were simulated across the four temperatures: 15, 20, 25, and 30°C.

The parameterization simulations covered the movement of 10 species differing in body mass, each with 100 individuals across three days (3 · 8h = 24 hours) of movement activity (Table S2, Appendix 1). Across the parameterization simulations the values of six model parameters were varied that describe the ground arthropod movement in our model: a) the parameters γ and x_0 of the Cauchy distribution were used to draw the speed S_i of an individual i per time step ($0 < S_i \leq M_i$), b) the parameters γ and x_0 of the Cauchy distribution were used to draw the displacement D_i of an individual i per time step ($0 \leq D_i \leq 10 \cdot S_i$), and c) the constants a and b determined the relationship between maximum speed (M_i) and the standard deviation of the normal distribution used to draw the turning angle A_i of an individual i per time step (Eq. 2).

No consistent empirical data currently exists for parameterization of ground arthropod movement across the ranges of body mass and ground temperature considered in our study. Moreover, data on body temperature or ambient temperature, a key parameter in our model, is rarely provided by empirical studies that study arthropod movement. Thus, we based our parameterization on different studies. We used three empirical studies (Klazenga and Vries 1994; Thomas et al. 1998; Byers 2001), and additionally tested the simulated mean squared displacement, a common measure of the spatial extent over time of random motion in general, against predictions for a correlated random walk (Codling et al. 2008). If the body mass of a species was not presented by the empirical study, we calculated it from mean body length using: $\text{Mass[mg]} = e^{(-3.46 + 2.79 \cdot \ln \text{size[mm]}}$ according to Rogers et al. (1977).

Based on the information about the generality of the mean squared displacement and the three empirical studies we defined four conditions for the evaluation of the 3,072 parameter sets (details below). A parameter set was rejected *a priori* if either condition 1 was not met or the proportional difference of the simulated to empirical value was ≥35% for one of the other conditions. Subsequently, we identified the parameter set that provided the lowest proportional difference of the simulated to empirical data averaged across conditions 2 to 4. Specifically, we used the weighted average, giving the condition 2 three times the weight that conditions 3 and 4 had because the condition 2 covered three different species, while conditions 3 and 4 covered one species each.

Condition 1: The mean squared displacement (denoted $E(R_t^2)$ in Codling et al. (2008)) across each of the 10 species and across all four temperatures must increase non-linearly to differ from a random walk. Specifically, a correlated random walk is characterized by $1 < \mu < 2$, where $E(R_t^2) \sim t^{\mu}$, and the squared displacement of one individual *i* and many time steps t is calculated as $SD_{i,t} = \sum D_{i,1}^2 + D_{i,t}^2$ (Codling et al. 2008). At least 90% of the μ values across all species and temperatures must fit the range $1 < \mu < 2$, otherwise the respective parameter set was neglected independent of results for other conditions.

Condition 2: Proportional differences between the simulated and the empirical average daily displacement of the 25 % fastest individuals per species should be low. The 24 hours simulated movement data per species were scaled to 8 hours and averaged across all four temperatures. Simulated data for three species with the body mass 5, 32, and 66 mg was validated against empirical data presented in Klazenga and Vries (1994) (estimated, average displacement per day: *Pterostichus diligens* 1.6 m, *Pterostichus lepidus* 4.3 m, and *Carabus nitens* 7.8 m).

Condition 3: Proportional differences between the simulated and the empirical average daily displacement should be low, considering simulated individuals of the 60 mg species and empirical data of *Pterostichus melanarius* presented in Thomas et al. (1998). We used empirical data covering two months June and July excluding data presented for August. We removed August-data because movement is quite high when compared to June and July, likely due to behavior associated with mating that is not covered in our model. We used simulated daily displacement across individuals of the 60 mg species at 15 and 20 °C to approximate the daily ambient temperatures at the field site (Thomas et al. 1998). We scaled simulated daily displacement from 24 to 8 hours of activity and compared it to the empirical mean dispersal of 2.6 m as well as the mean coefficient of variation across individuals of 1.53. The proportional differences of the simulated to empirical data averaged across mean dispersal and mean coefficient of variation was used for validation.

Condition 4: Proportional differences between the simulated and the empirical average daily displacement should be low, considering simulated individuals of the 97 mg species and empirical data of *Eleodis extricate* calculated from Byers (2001) based on Crist et al. (1992). Empirical data were observed at soil-surface temperatures of 20 to 30 °C (Crist et al. 1992), suggesting an average daily displacement of 13.95 m (Byers 2001). Accordingly, we used simulated daily displacement across individuals of the 97 mg species at 20, 25, and 30 °C (assuming soil-surface temperature to be about equal with body-temperature) scaled from 24 to 8 hours of activity, for model validation.

Table S1. Set of parameters that determined the simulated movement of arthropods, and their values tested for differences between the simulated and the empirical movement ('parameterization simulations')

Parameter	Description	Values
Constants a and b (see Eqn. S2, , Appendix 1)	Combination of slope (a) and y-intercept (b) of the linear relationship used for calculating the standard deviation of a normal distribution, which was used for drawing species-specific turning angles per time step (Eqn. A2).	i) a: -17.24, b: 70 ii) a: -13.79, b: 66 iii) a: -10.34, b: 62
Speed x_0	The 'location' parameter of the Cauchy distribution for drawing the actual speed (S_i) of an individual *i* per time step between 0.001 cm/s and the maximum speed (M_i); x_0 is provided as proportion of M_i, e.g. 0.5 means ½ of M_i	0.625, 0.5, 0.375, 0.25
Speed γ	The 'scale' parameter of the Cauchy distribution for drawing the actual speed (S_i) of an individual *i* per time step	1, 2, 2.5, 3, 3.5, 4, 4.5, 5
Displacement x_0	The 'location' parameter of the Cauchy distribution for drawing the displacement (D_i) of an individual *i* per time step between zero and $10 \cdot S_i$; x_0 is provided as proportion of $10 \cdot S_i$, e.g. 0.5 means ½ of $10 \cdot S_i$	0.75, 0.625, 0.5, 0.375
Displacement γ	The 'scale' parameter of the Cauchy distribution for drawing the displacement (D_i) of an individual *i* per time step	1, 2, 2.5, 3, 3.5, 4, 4.5, 5

Table S2. Species specific parameters and values used for model parameterization ('parameterization simulations')

Parameter	Description	Values
Species body mass [mg]	Fixed species-specific body mass of species 1-10; some values differ from the logarithmically scaled body mass values in Table 1 to match average body mass of species from empirical studies we used for model validation	1, 5, 18, 32, 60, 66, 97, 173, 223, 330
Body temperature [K (°C)]	Fixed body temperature across the simulation	288, 293, 298, 303 (~ 15 - 30)
Number of individuals	Number of individuals per species of which we simulated the movement	100

Validated model parameter values for ground arthropod movement

According to the validated model parameters we used for our simulation experiments, most individuals move generally at about 25 % of its *maximum speed* (Fig. S1, Appendix 1; Cauchy distribution parameters: scale $\gamma = 1.0$ and location $x_0 = 0.25 \cdot M_i$). Contrary, the *displacement* per time step varied strongly between zero and maximum displacement (i.e. moving a straight line), with a slightly increased probability for an individual to displace about 37.5 % of its maximum displacement capability (Fig. S2, Appendix 1; Cauchy distribution parameters: scale $\gamma = 5.0$ and location $x_0 = 0.375 \cdot$ max displacement). The *directional persistence* across modeled time steps strongly increased with increasing body size and speed, that is, at the temporal scale of our model (10 s time steps) larger species move much straighter than smaller species (Eq. 2 parameters: a = -17.24, b = 70).

When applying these parameter values, the simulated movement of ground arthropod species differs from empirically observed ground arthropod movement by 26.03 % on average across the conditions 2, 3 and 4. This is surprisingly close to empirical data considering the simplicity of our model, such as the assumption of a featureless landscape.

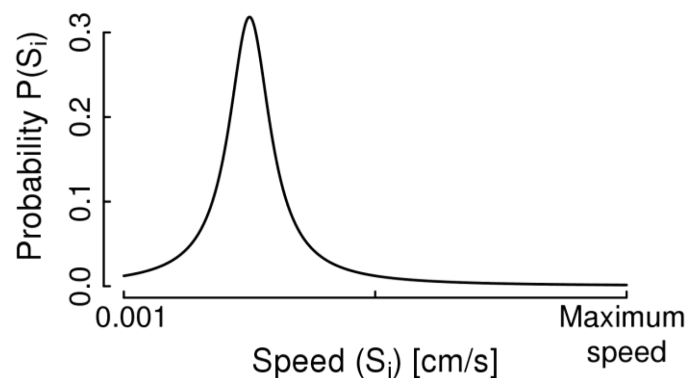


Figure S1. Cauchy distribution with parameters: scale $\gamma = 1.0$ and location $x_0 = 0.25 \cdot M_i$ (maximum speed of individual *i*), obtained from empirical parameterization and used to randomly choose a value for the movement speed (S_i) per model time step and individual (see Eq. 1).

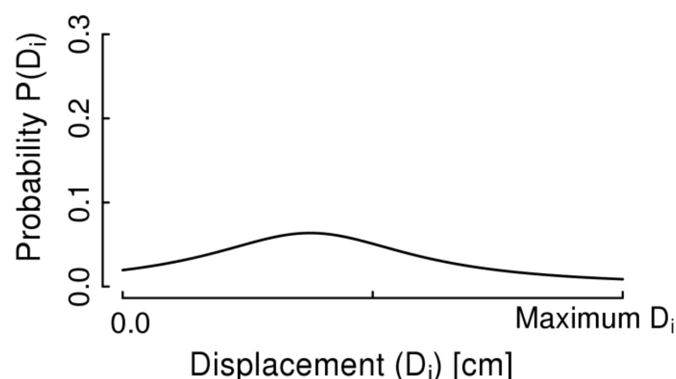


Figure S2. Cauchy distribution with parameters: scale $\gamma = 5.0$ and location $x_0 = 0.375 \cdot$ max displacement (calculated as time step length [10 s] multiplied by speed S_i), obtained from empirical parameterization and used to randomly choose a value for the air-line displacement (D_i) per model time step and individual.

Appendix 2: Sensitivity analysis of the model

A local sensitivity analysis of the model was performed varying model parameters that both are central for the simulation of ground arthropod movement and were parameterized using empirical values from published literature (Table S1, Appendix 1). We varied six parameters by +10 % and -10 % of the above reference value (Table S1, Appendix 2).

To test the impact of the variation of one of these parameters on the key model outcome of our study, the proportion of individuals sampled per species, we performed 84 different simulation experiments. This set included the following parameter values: i) pitfall trap numbers of 1, 2, 4, 8 and 12, ii) all spatial layouts (see Fig. 1A-F), iii) population densities of 0.2, 0.8, and 4 individuals/m^2, and iv) body temperatures of 291 and 300 K (~18 and 27 °C). All parameter possible combinations were simulated for 10 species with a body mass as defined in Table 1. Each individual simulation was repeated 50 times and the averaged proportion of individuals sampled per species was used for comparison.

Per parameter one figure was created showing plotting the results from simulations with the original parameter values against the results from simulations with +10 % and -10 % variation of the particular parameter (Fig. S1, Appendix 1).

Table S1. Parameters key for the simulation of ground arthropod movement and their values used in the sensitivity analysis, plus original value

Parameter	Panel in Figure S1, Appendix 2	Value -10%	Original value	Value +10%
Speed x_0	A	0.225	0.250	0.275
Speed γ	B	0.900	1.000	1.100
Displacement x_0	C	0.338	0.375	0.413
Displacement γ	D	4.500	5.000	5.500
Constant a (Eq. 2)	E	-18.964	-17.240	-15.516
Constant b (Eq. 2)	F	63.000	70.000	77.000

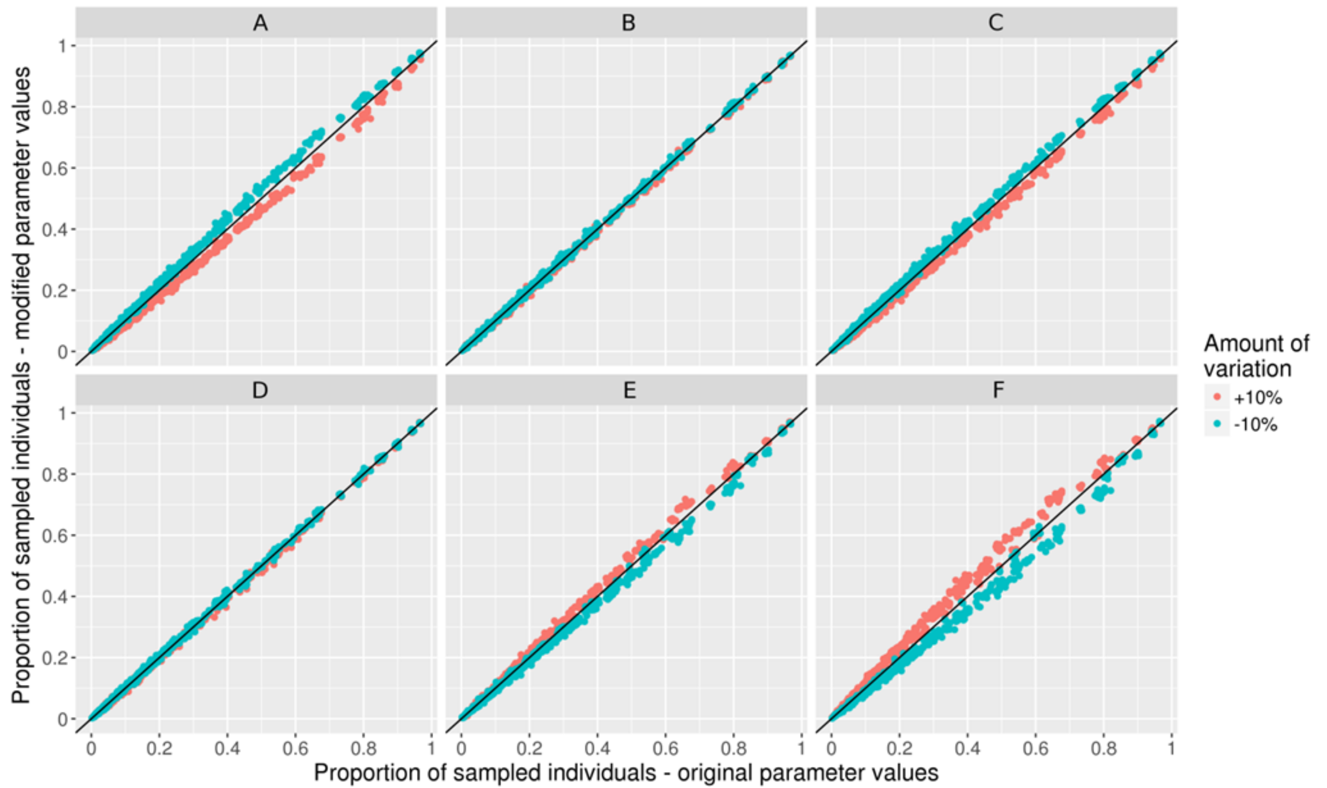


Figure S1. Proportion of sampled individuals from simulations using original parameter values against respective results from sensitivity simulations, i.e. simulations with variation in parameter values as shown in Table S1, Appendix 2. Each panel shows 1680 points, 840 for each red and blue, covering 84 different simulation experiments with 10 species (see above text for details).

Appendix 3: Spatial pitfall trap arrangement and details on pitfall traps at the simulated area

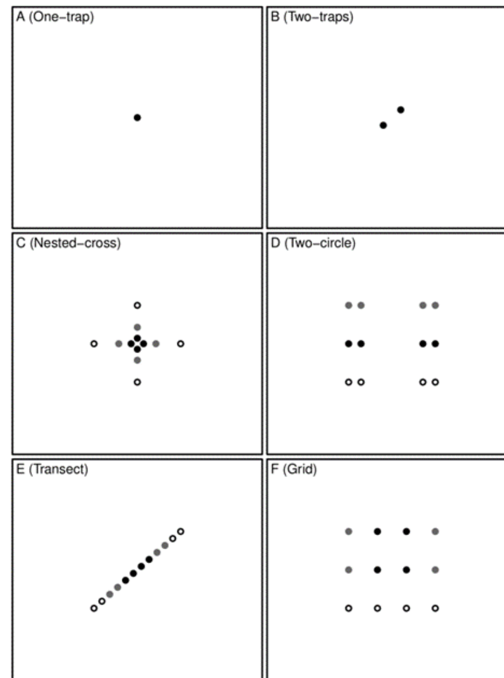


Figure S1. Six spatial arrangements of pitfall traps within a 20 x 20 m area used in this study: **A**) one-trap, **B**) two-traps: diagonally arranged with a distance of 2.0 m, **C**) nested-cross (Perner and Schueler 2004), **D**) two-circle: a grid of multiple pairs of traps (Zhao et al. 2013), **E**) transect: traps along a diagonally oriented line, and **F**) grid: individual traps uniformly distributed. Each dot represents the location of a pitfall trap. The number of pitfall traps was varied between 4 and 12 for the arrangements c-f (4 traps: black dots; 8 traps: black and dark-grey dots; 12 traps: all dots).

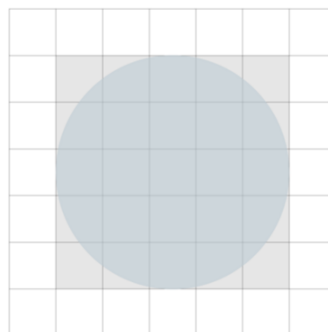


Figure S2. A round pitfall trap (slate blue circle) at a section of the simulated area. Each cell of the grid represents a real area of 1 x 1 cm. The pitfall trap has a diameter of 5 cm. Light gray cells are denoted as pitfall trap, though the probability of falling into the trap varies across these cells. The probability of an individual falling into the trap when crossing a “grey colored cell” equals about the proportion of the cell covered by the trap (i.e. proportion of slate blue circle per cell). An arthropod that moved at one of the four corner cells had a 20% probability to fall into the trap, while the probability to fall into the trap was 75 % for all cells at the edges next to the corner cells. At the remaining cells the probability to fall into the trap was 100 %.

Pitfall traps with a diameter of 5 cm were simulated, because such traps were suggested as cost-effective way to detect meaningful differences in population density across ground arthropod species (Work et al. 2002).

Appendix 4: Ambient temperatures during the vegetation period in Central Europe

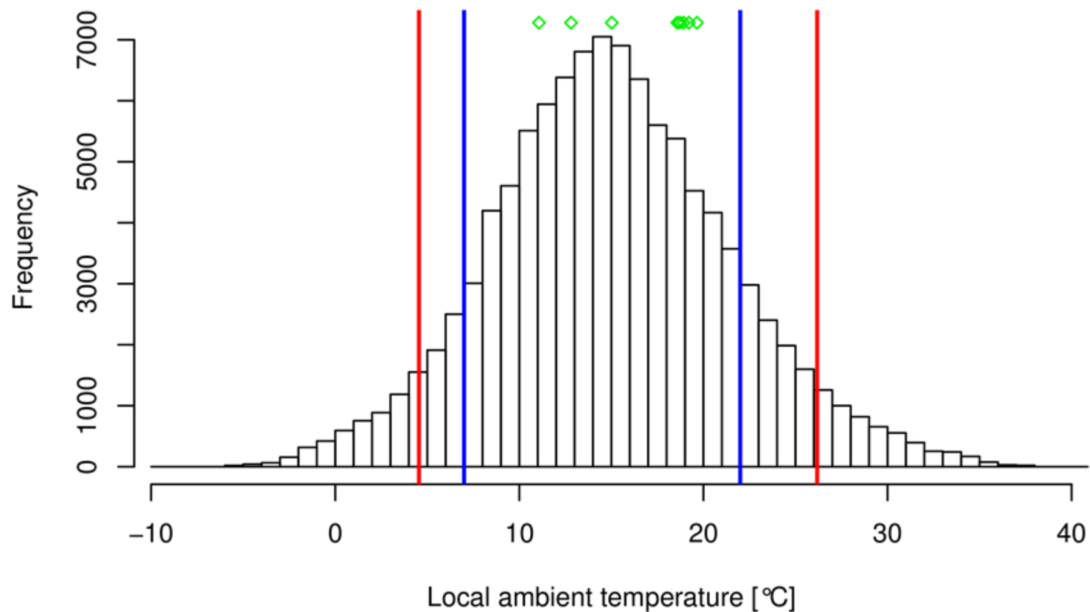


Figure S1. Histogram of ambient temperatures from April 1st to September 30th across the years 2012 to 2015 measured at a grassland biodiversity experiment in Central Europe (Roscher et al. 2004). The temperature was measured every 10 minutes yielding about 105,000 individual values for the period April 1st to September 30th across 2012-2015. Data files were downloaded at January 25th 2016 from https://www.bgc-jena.mpg.de/wetter/ (e.g. mpi_saale_2012a.zip and mpi_saale_2012a.zip for the year 2012) and values from column 'T (degC)' were used.

The two red vertical lines indicate the 5 % and the 95 % quantiles of the data. The two blue vertical lines show the minimum and maximum ambient temperature considered in our model simulations. We assumed the body temperature of actively hunting, moving ground arthropods exceeds the ambient temperature by 8 °C (Morgan 1985). Accordingly, model simulations considered body temperatures 15 to 30 °C (Table 1).

Additionally, the green squares show the mean air temperature of seven individual sampling periods across one year in a ground arthropod sampling campaign in north-east China (temperature on x-axis; y-axis has no meaning for green squares) (values from sampling periods T1-T6 and T8-T10; see Table 1 in Wang et al. 2014).

Appendix 5: Arthropod communities with reasonable species abundance ranks

Creation of random communities: For one community, each of the 10 species was assigned randomly to an abundance rank using a uniform probability distribution of integer values from 1 to 10. We repeated this random distribution of species to abundance ranks 50,000 times yielding an initial set of 50,000 random communities with a completely random relationship between body mass and abundance rank. From this initial set, we selected communities where small species have generally a higher rank than larger species. To identify such communities the sum of the species numbers (i.e. 1 to 10) across ranks 1 to 4 was subtracted from the sum of the species numbers across ranks 7 to 10 yielding a value K_c for each single random community c, where $1 \leq c \leq 50,000$. A large K_c indicates that the small species have, on average, a higher rank than large species. We selected all communities with a value of K_c higher than the 99% quantile of all 50,000 K_c values, yielding 378 communities.

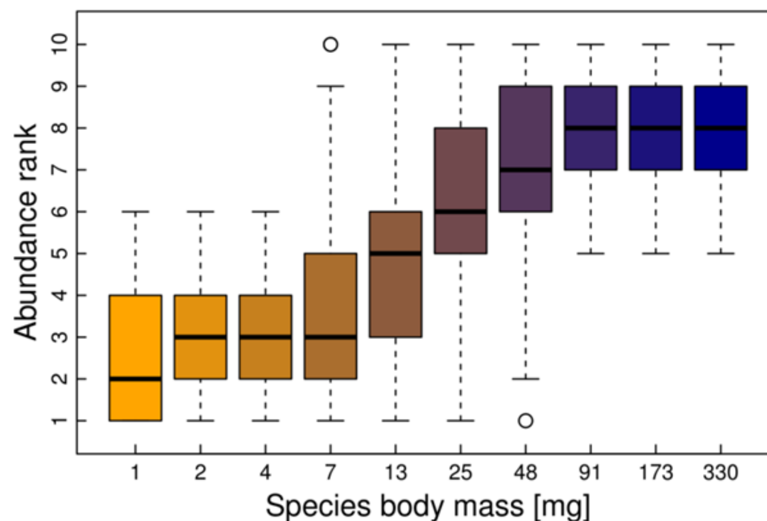


Figure S1. Boxplot showing the abundance ranks of the 10 ground arthropod species we covered in our study (species 1 to 10 with body mass 1 to 330 mg) across 378 random communities. In these random communities the abundance of each species was randomly assigned, though, small species have generally a higher rank than larger species accordingly to empirical data (i.e. smaller species are more abundant than larger species). The simulated population abundance decreases from 3200 individuals at rank 1 to 60 individuals at rank 10, logarithmically spaced (rounded to integer values, Table 1).

Appendix 6: Animated movie of ground arthropod movement and pitfall trap sampling

The *Supplement 1* provides a movie showing in top view the simulated movement of 12 ground arthropods from three different species within the central 4 x 4 m square of the simulated area (20 x 20 m). Individuals are presented as black dot, with a colored tail attached. The tail shows the movement path across the previous 11 minutes and 40 seconds (for the purpose of visualizing the movement pattern and the speed). A red tail indicates individuals of the large species (330 mg), green medium species (13 mg; the classification as 'medium' concerns the log scale of body mass and the results of the analysis of movement pattern, see Fig. G1) and blue small species (1 mg).

The movie shows the simulated arthropod movement at 20 °C body temperature across 4 hours in fast motion (runtime of 2 min). Each second of the movie represents 12 simulated time steps of 10 seconds each.

Additionally, the indicates the position of 8 pitfall traps arranged as nested cross (red open circles) and two pitfall traps of the grid arrangement (blue) for the purpose of visualization only. The traps are neither active in this simulation nor are they sized correctly (size was increased as traps with a diameter 5 cm would hardly be visible in the area of 4 x 4 m).

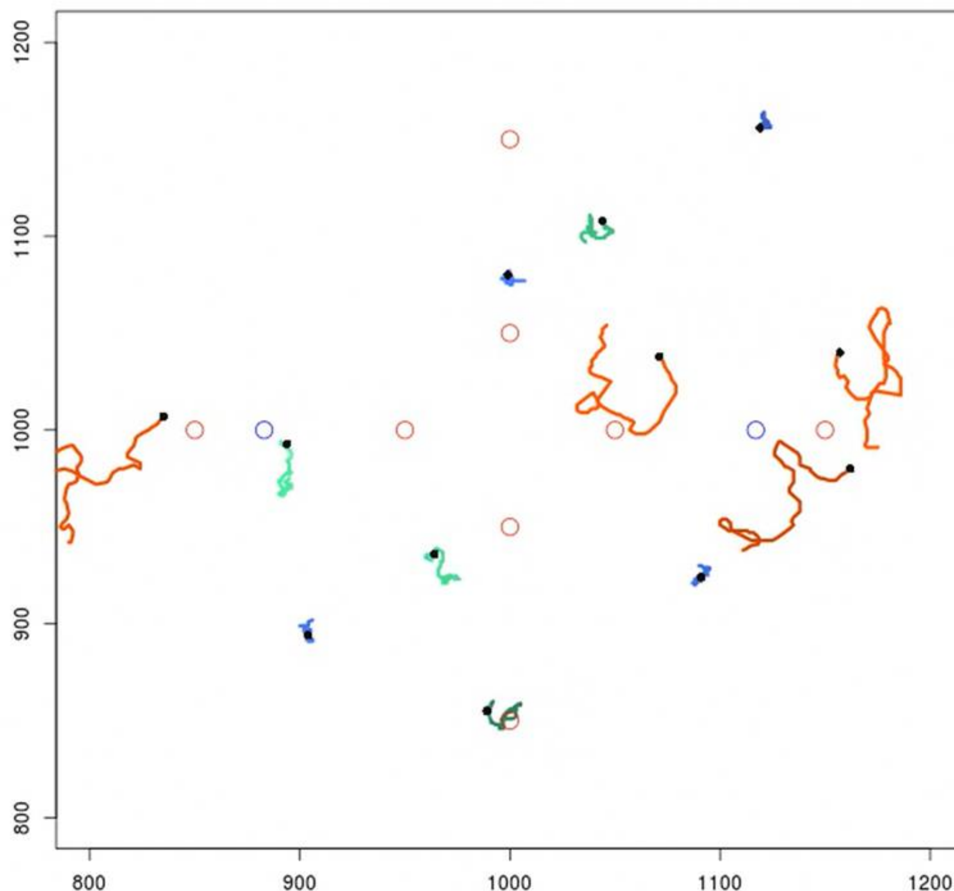


Figure S1. Screenshot from the movie provided as Supplement 1. See above text for details.

The *Supplement 2* provides a movie as in Supplement 1 but showing 10 hours of movement, plus some extra statistical analysis. The movie has increased fast motion compared to Supplement 1, with 30 model time steps per second of movie runtime (overall runtime of 2 min).

The additional statistics were calculated from two different model simulations with 10 species each (body mass 1 to 330 mg, see Table 1; 20 °C body temperature; activity period 10 hours). The one simulation applied pitfall trap sampling with 12 traps arranged as nested cross and the other 12 traps arranged as grid (Fig. S1, Appendix 3). Each species had a simulated population abundance of 800 individuals moving within the 20 x 20 m area.

The two figures at each side show the population abundance and the mean squared displacement (MSD) for three different species over time (red: 330 mg body mass; green: 13 mg; blue 1 mg; same as in the movie from Supplement 1). The MSD is the most common metric for measuring movement in general indicating the average area covered by the individuals of one species. The additional counter centered between the two figures at each side show the number of sampled individuals across all 10 species across simulation time (i.e. 8000 individuals per simulation with either nested cross or grid arrangement of pitfall traps).

The two movies were created with the R language version 3.2 (R Core Team 2015), based on the x and y coordinates of the particular individuals visualized and the information of sampled individuals per species in the particular model simulation.

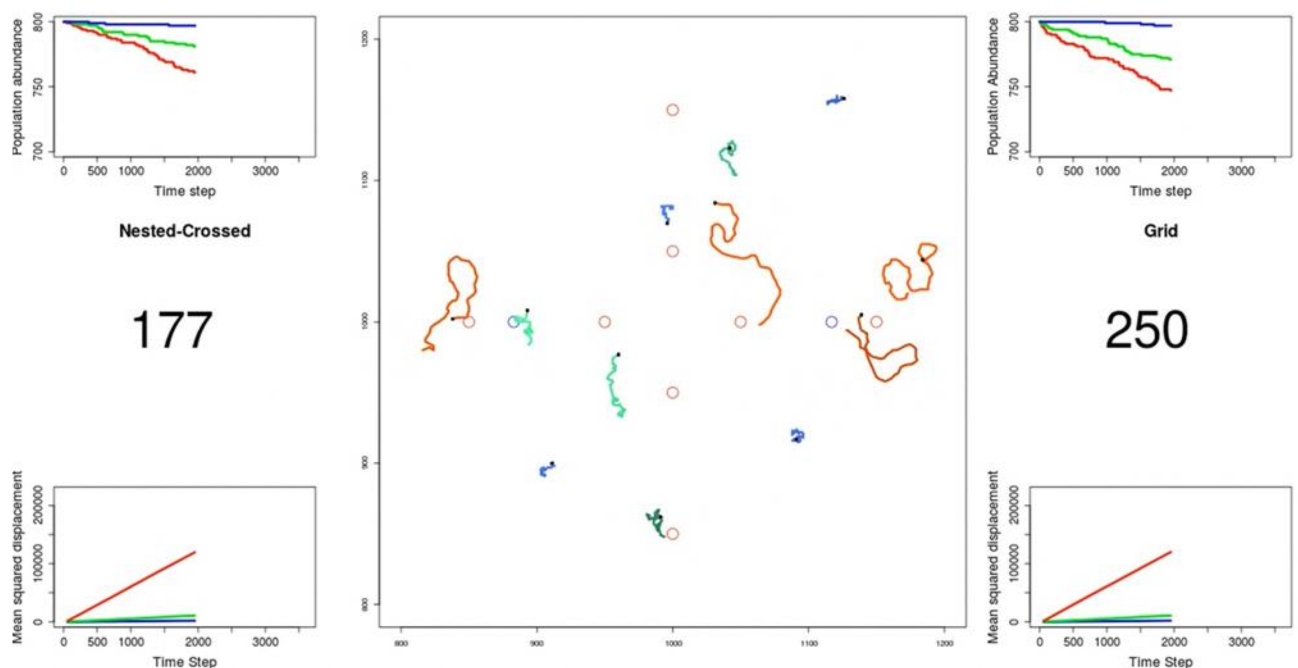


Figure S2. Screenshot from the movie provided as Supplement 2. See above text for details.

Appendix 7: Body mass related movement pattern

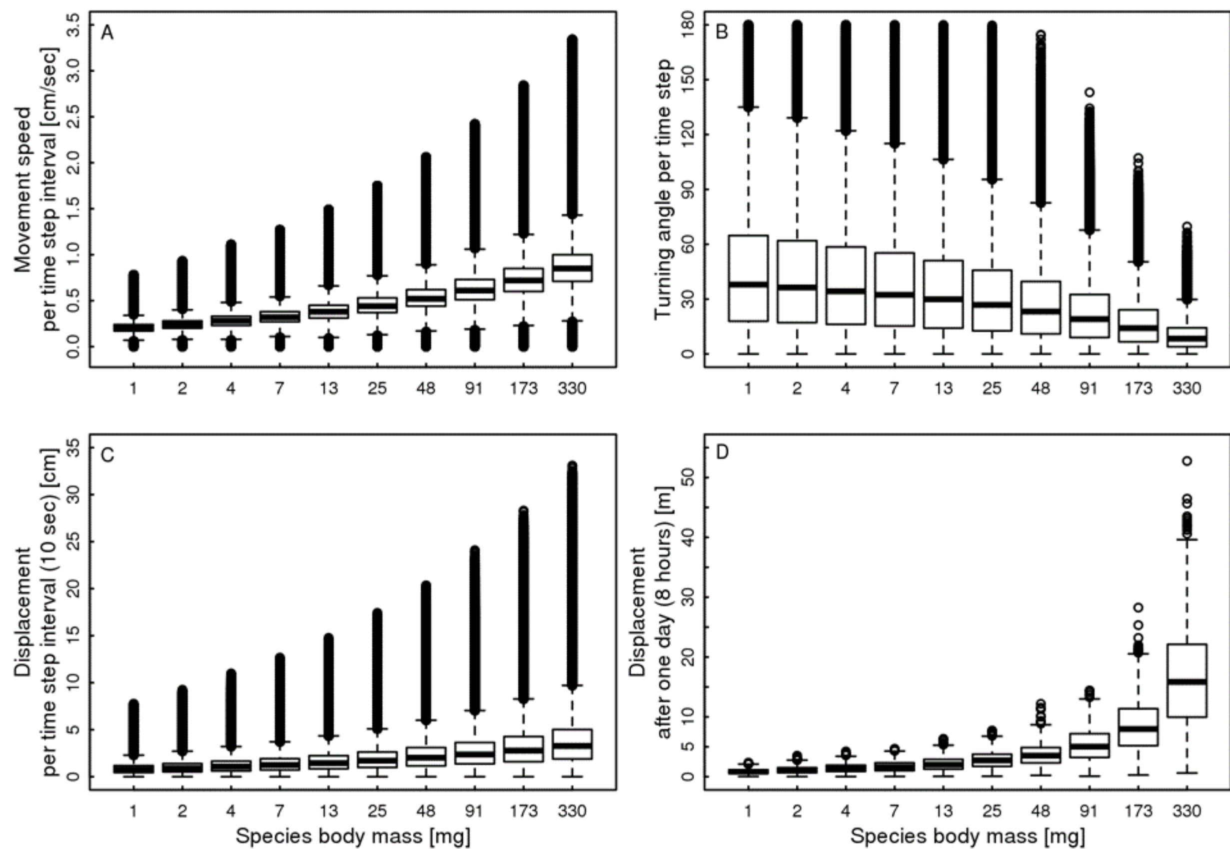


Figure S1. Boxplots of four central properties of animal movement across the 10 simulated species, with increasing body mass. Per species 1000 individuals were simulated at 24 °C body temperature across 8 hours, i.e. 2880 time steps, yielding 2,880,000 data points per species in each panel. **A)** Speed per individual in centimeter per second, for one time step (10 seconds). **B)** Turning angle per individual in degree, without differentiation between left and right turns. **C)** The air-line displacement per individual during one time step in centimeter. **D)** The air-line displacement per individual during one day, i.e. 8 hours of activity, in meter.

Appendix 8: Effects of body temperature, population density and body mass on sampling bias

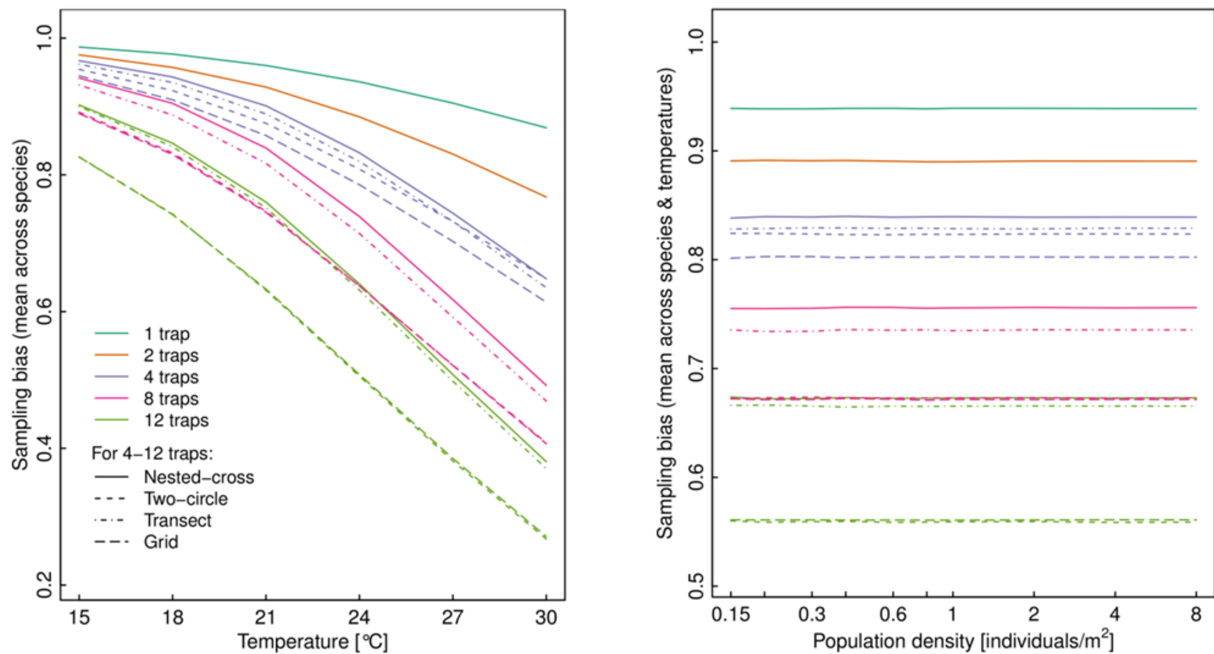


Figure S1 (left side). Sampling bias averaged across species (see Table 1) for the body temperatures considered in this study. The number of pitfall traps is color coded, and line types indicate the trap arrangement (for trap numbers four to 12.

Figure S2 (right side). Sampling bias averaged across species (see Table 1) for the population densities considered in this study (x-axis is log scaled). The number of pitfall traps is color coded, and line types indicate the trap arrangement (for trap numbers four to 12, see legend in Fig. S1, Appendix 8).

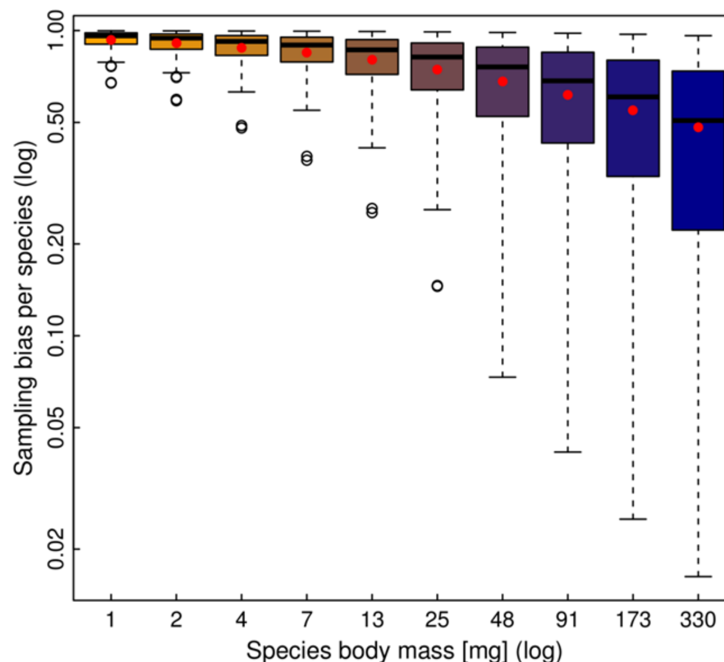


Figure S3. Boxplot showing the impact of species body mass (log scale) on the sampling bias (log scale) *across all trap numbers, trap arrangements, population densities and body temperatures* considered in this study (see section "simulation experiments" in methods). The red point within each boxplot marks the mean body mass.

Appendix 9: Correction factor for deriving unbiased relative species abundances

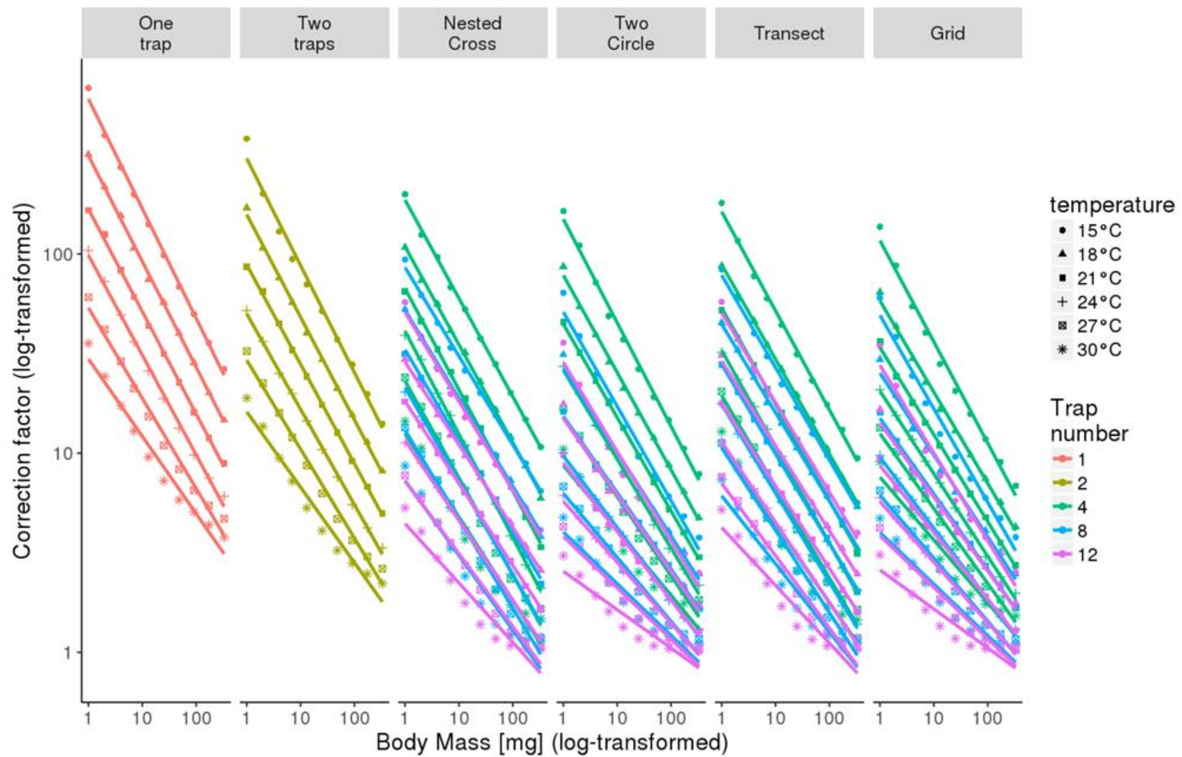


Figure S1. The relationship between body mass and the correction factor for deriving unbiased relative species abundances per trap number (Fiqs. 6-8), trap arrangement (Fig. S1, Appendix 3), and body temperatures. The individual values per species (different symbols per temperature) and the linear regression line are shown.

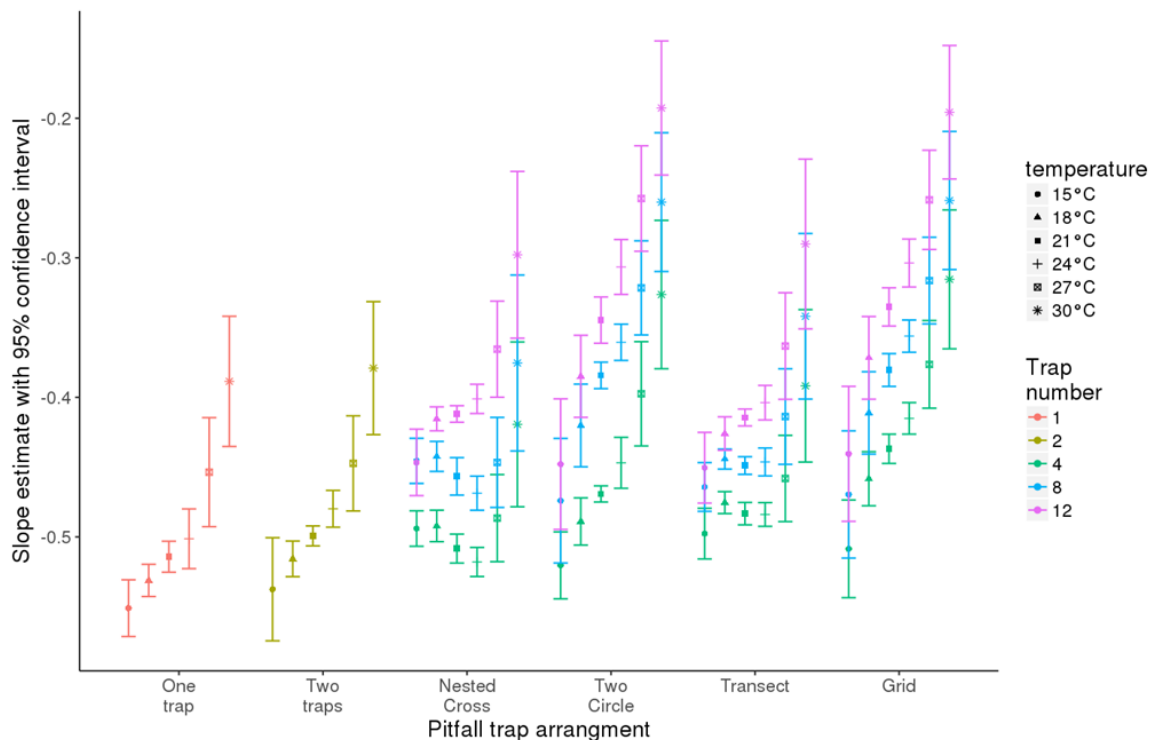


Figure S2. The slope of the regression lines from Fig. S1, Appendix 9 are shown together with the 95% confidence interval.

Appendix 10: Indices of community diversity: Shannon diversity and Fisher's alpha

Our analysis of arthropod diversity shows that pitfall trap sampling may generally lead to overestimations of certain community diversity indices. Note that in all simulation experiments all simulated species were detected, that is, the observed species richness was always 10. Accordingly, differences between the estimated and the simulated diversity resulted from changes in species evenness. The two diversity metrics used, Shannon diversity and Fisher's alpha, consistently showed a higher estimated diversity compared to the simulated diversity (Figs. S1 and S2, Appendix 10). This overestimation of biodiversity can be explained with the observed abundance distributions from pitfall trap sampling being more even than the simulated abundances that followed a log curve (see Fig. 5). The estimated Shannon diversity on average remained relatively unaffected from trap arrangement, trap number and body temperature, but varied strongly across the 378 random communities, i.e. the sequence of species body masses along the abundance ranks. Fisher's alpha was quite sensitive to pitfall trap number and body temperature but mostly resilient against changes in the community structure.

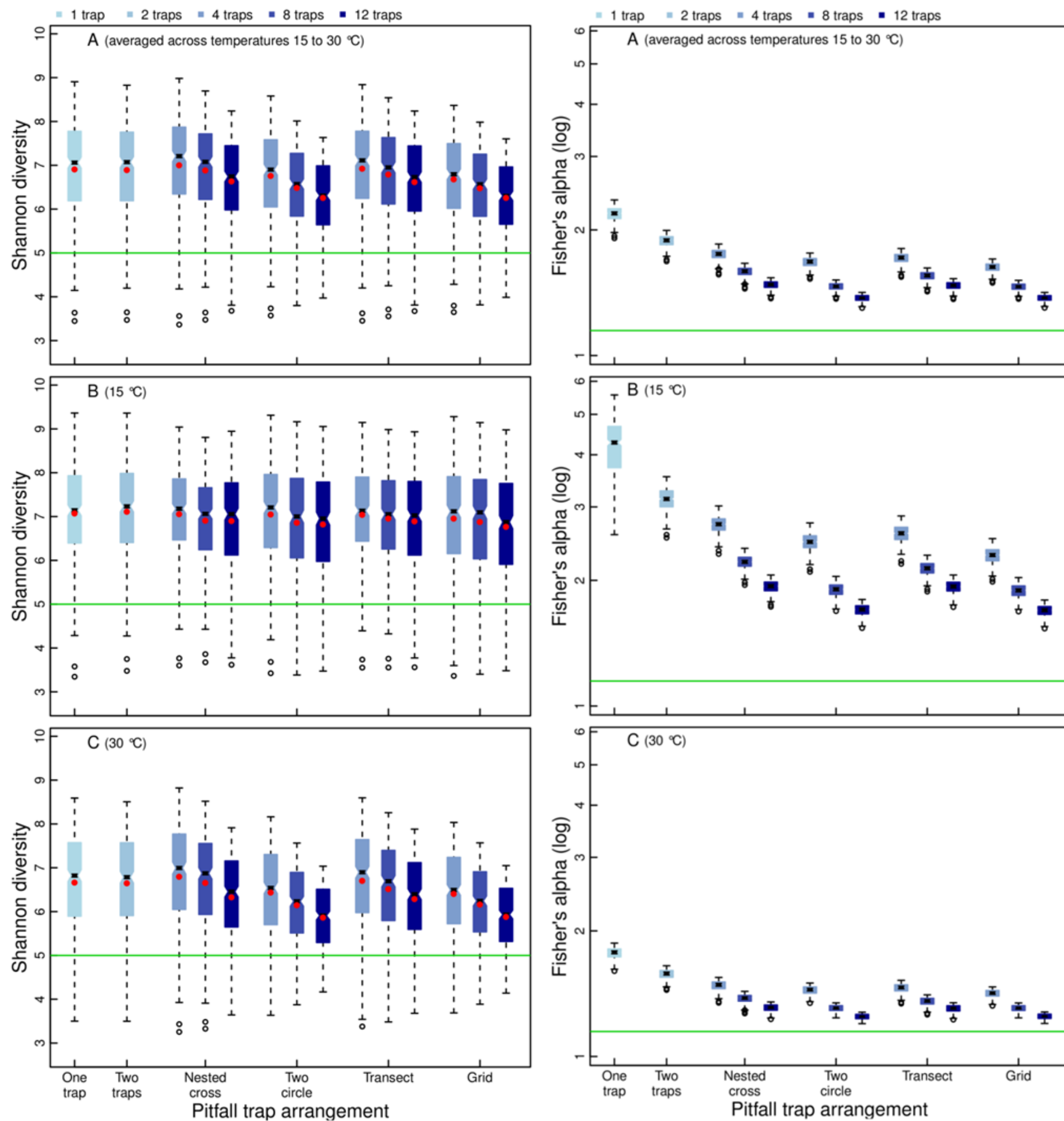


Figure S1 (left side). Boxplot of Shannon diversity calculated from the observed population abundances across 378 random communities (Appendix 6), shown for different combinations of pitfall trap number (blue colors) and trap arrangement. The mean values are shown by a red dot. The Shannon diversity of the simulated population abundances (green colored vertical line) is equal for all simulation experiments because the simulated population abundances were fixed to values logarithmically spaced from 0.15 – 8.0 individuals/m^2. Panels show results for **A)** averages across six different body temperatures (15 – 30 °C), **B)** 15 °C, and **C)** 30 °C body temperature.

Figure S2 (right side). Boxplot of Fisher's alpha calculated from the observed population abundances across 378 random communities (see Fig. S1, Appendix 6), shown for different combinations of pitfall trap number (blue color coded) and pitfall trap arrangement. The Fisher's alpha based on the simulated population abundances is colored green. The y-axis is log scaled. Panels show results for **A)** averages across six different body temperatures (15 – 30 °C), **B)** 15 °C, and **C)** 30 °C body temperature.

Supplement 1: Animation of simulated movement of three ground arthropod species (for details see Appendix 6; mp4 file type). This supporting information is available is available for this article online http://onlinelibrary.wiley.com/doi/10.1002/ecs2.1790/full.

Supplement 2: Animation of simulated movement of three ground arthropod species plus additional information about number of sampled individuals (considering simulation results for 10 species with 800 individuals each), population density and displacement (for details see Appendix 6; mp4 file type). This supporting information is available is available for this article online http://onlinelibrary.wiley.com/doi/10.1002/ecs2.1790/full.

CHAPTER 6

General discussion and implications of this thesis

6 General discussion

This thesis aimed to contribute to a better understanding of the impact of biodiversity loss on biotic interactions. Trophic and non-trophic interactions of predators and omnivores with plants, animals, fungi, protists, and bacteria were assessed in a mensurative large-scale land-use experiment and in a manipulative grassland biodiversity experiment using a range of molecular approaches. In addition, **chapter 5** of the thesis may contribute to a better assessment of biodiversity by systematically analyzing factors that affect pitfall trap efficiency employing an ecological simulation approach.

In the first study (**chapter 2**), I analyzed the performance and gut microbiota of flying predatory insects in two types of local habitats that differ in their diversity of basal resources (soybean monoculture vs. restored prairie) and that were located along a landscape complexity gradient. A key finding of this study is that even closely related predators have a species-specific set of gut bacteria that is stable over a range of environmental conditions. In a lab experiment that provided the base for the interpretation of gut bacterial diversity of field collected beetles, I found that prey diversity increased bacterial diversity in guts of predatory insect. In the field, I found that insects from soy fields had higher gut bacterial richness and lower fat content than those from prairies, suggesting the inclusion of more prey types in soy and better feeding conditions that allow a more selective foraging in prairies. Landscape context had contrasting effects on gut bacteria and body condition of exotic and native predators, which suggest inherent differences in habitat and prey use among these groups. Overall, the study illustrates the importance of both local resource and landscape-based influences on gut microbiota and their interactions with predator species-specific traits including foraging behavior and physiology.

While mensurative experiments (**chapter 2**) use environmental gradients, and have a high degree of realism, the control of independent variables is limited (McGarigal & Cushman 2002; Hadley & Betts 2016). In contrast, in biodiversity experiments like the Jena Experiment (**chapter 3 and 4**), the manipulated gradient of plant species richness is not confounded with species composition or extrinsic conditions (e.g., soil fertility, land-use management). Biodiversity experiments may lack realism to some degree but allow drawing reliable conclusions on the importance of plant species richness *per se* on multitrophic interactions (Loreau et al. 2001; Hooper et al. 2005; Weisser et al. 2017).

Further, in **chapter 2** no detailed information was available on the community of plants and animals and on other environmental variables that could influence the local pool of bacteria and mediate interactions between predators, prey, and bacteria. In contrast, the platform of the Jena Experiment (**chapter 3 and 4**) provides extensive data on the local species pool and ecological parameters. Over a decade of research in the Jena Experiment have created a great understanding of bottom-up effects from plant diversity to the consumer community and consumer mediated processes (e.g., Scherber et al. 2010; Weisser et al. 2017). Yet, the studies presented in this thesis are the first to directly and in detail measure interactions between higher order consumers and their plant and animal food resources and microbiome with next-generation sequencing-based gut content analysis.

The methods used to analyze gut bacterial communities in the first study (**chapter 2**) allowed to rapidly compare samples from multiple species and locations but does not provide information on taxon identity. In **chapter 3 and 4** I therefore tested and employed recent metabarcoding approaches to identify microbial organisms and food ingested by consumers. In the pilot study (**chapter 3**), a wide range of taxa was detected using a metabarcoding approach in regurgitates, including organisms representing trophic, phoretic, parasitic, and neutral interactions with the ground beetle *P. melanarius*. The results indicate that plant diversity and vegetation cover positively affect the richness of interactions.

A study spanning the full design of the trait-based experiment within the Jena Experiment (**chapter 4**) included three species of surface-active invertebrates with varying degree of omnivory as model organisms. The range of analyzed interaction partners was expanded from eukaryotes to prokaryotes and included not only measures of the plant community but also measures of the animal community in the experimental plots. Trophic interactions of omnivores were mainly driven by indirect effects of plant diversity through differences in the performance of the plant community or via the bottom-up food web through changes in the invertebrate community. A key finding was that the three omnivores interacted with different subsets of species in their local habitat. Their preference for different prey types was reflected by differences in the gut microbial community. This finding also confirms the assumption that dietary differences between the predators in the first study (**chapter 2**) are one of the drivers for the strong differences in their gut microbiome. These findings highlight that interactions between invertebrates and microorganisms need to be better integrated into community ecology.

Another key finding of **chapter 4** was that the density of the plot vegetation shifted the average trophic position at which the two ground beetles in the study fed, but the direction of the effect was species-dependent. In **chapter 2**, similar strong differences in the response of lady beetles to vegetation type at field and landscape scale were found. This finding illustrates that behavioral differences between species mediate their dietary response to environmental factors and that this also affects closely related species and species that are commonly assigned to the same trophic guild. Changes in the feeding behavior of higher order consumers can affect the plant and animal community via top-down effects but this has so far rarely been included in studies on biodiversity effects (Scherber et al. 2010). Metabarcoding of gut contents offers insights into the adaption of feeding behavior to changing plant diversity and is a promising novel approach to study multitrophic interactions in biodiversity experiments and ecology in general.

Studying plant diversity effects on ecological communities requires methods that reliably quantify these communities. Ground arthropods are routinely assessed with pitfall traps, although it is well known that the resulting data are biased by arthropod mobility, activity, and environmental factors (Lang 2000; Perner & Schueler 2004; Woodcock 2005; Saska et al. 2013; Brown & Matthews 2016). The virtual ecologist approach (Zurell et al. 2010), that was employed in **chapter 5**, allows the systematic analysis of factors that affect the sampling bias. A series of simulation experiments evaluated the effects of body mass and population density of differently sized arthropods, number and spatial trap arrangement of pitfall traps, and temperature. The sampling bias strongly decreased with increasing body mass, temperature,

and pitfall trap number, while population density had no effect and trap arrangement only had little effect. These results implicate that at a community level the species specific-bias can result in an overestimation of large-sized species and could lead to wrong conclusions about the trophic structure of communities. It is therefore proposed to conduct a bias correction and demonstrate how unbiased relative species abundance can be derived using correction factors that need only information on species body mass.

Overall, the results presented in this thesis show that biodiversity on a plot, field, and landscape scale can impact the interactions of invertebrates but species-specific traits including foraging behavior shape the strength and direction of these effects. Furthermore, I highlight that the approach of metabarcoding of gut contents offers novel insights into complex species interactions in multilevel communities and is most valuable when used in a well studies system with profound knowledge on the species pool. Dropping costs for sample sequencing and ever-increasing sequence databases will lead to a better integration of metabarcoding approaches in many areas of ecology. Moreover, manipulative biodiversity experiments can address fundamental questions in biodiversity research but often lack realism. To overcome this limitation, I advocate that comparisons with findings from mensurative experiments are important to test if the implications of species loss and are also applicable to real-world systems.

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CURRICULUM VITAE

The CV is available in the printet version only.

LIST OF PUBLICATIONS

Publications in peer-reviewed journals

Tiede, J., Scherber, C., Mutschler, J., McMahon, T. & Gratton, C. (2017). Gut microbiomes of mobile predators vary with landscape context and species identity. Ecology and Evolution: 2017, 00:1–13. doi:10.1002/ece3.3390.

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Conference contributions

Tiede, J., Wemheuer, B., Traugott, M., Daniel, R., Tscharntke, T., Ebeling, A. & Scherber, C. (2017). Multitrophic interactions in a plant diversity experiment analyzed by gut content metabarcoding. Ecology Across Borders: Joint Annual Meeting of the BES, GFÖ, NecoV and EEF, Gent, Belgien (Vortrag).

Tiede, J., Wemheuer, B., Traugott, M., Daniel, R., Tscharntke, T., Ebeling, A. & Scherber, C. (2017). Multitrophic interactions in a plant diversity experiment analyzed by gut content sequencing. 3rd Symposium on Molecular Analysis of Trophic Interactions (MTI-3), Uppsala, Sweden (Vortrag).

Tiede, J., Wemheuer, B., Traugott, M., Daniel, R., Tscharntke, T., Ebeling, A. & Scherber, C. (2016). Plant diversity affects multitrophic interactions in an experimental grassland. 46th Conference of the Ecological Society of Germany, Austria and Switzerland (GfÖ), Marburg (Vortrag).

Tiede J., Zwaan C., Tscharntke T. & Scherber C. (2015) Movement patterns of nocturnal ground beetles in a grassland with manipulated plant diversity. 45th Conference of the Ecological Society of Germany, Austria and Switzerland (GfÖ), Göttingen (Vortrag).

Tiede, J., Wemheuer, B., Traugott, M., Daniel, R., Tscharntke, T., Ebeling, A. & Scherber, C. (2015). How omnivores respond to plant species richness: Analyzing gut contents with next-generation sequencing. 100th Meeting of the Ecological Society of America (ESA), Baltimore, MD, USA (Vortrag).

Tiede, J., Wemheuer, B., Traugott, M., Daniel, R., Tscharntke, T., Ebeling, A., & Scherber, C. (2014). Feeding behavior of an omnivorous ground beetle in a grassland with manipulated plant diversity. 44th Conference of the Ecological Society of Germany, Austria and Switzerland (GfÖ), Hildesheim (Vortrag).

Tiede J., Mutschler, J., McMahon, K.D. & Gratton, C. (2013). Lady beetle species have different microbial communities in their alimentary tracts - is it a diet effect? 61th Meeting of the Entomological Society of America (ESA), Austin, TX, USA (Vortrag).

Tiede J., Mutschler, J., McMahon, K.D. & Gratton, C. (2013). Diet-related effects on the bacterial assemblages in the alimentary tracts of lady beetles. 43rd Annual Meeting of the Ecological Society of Germany, Austria and Switzerland (GfÖ), Gesellschaft für Ökologie, Potsdam (Vortrag).

Tiede, J., Tscharntke, T., & Scherber, C. (2013). Effects of plant species richness and identity in an experimental grassland community on omnivory of carbabid beetles. 2nd International Symposium on the Molecular Detection of Trophic Interactions (MTI-2), Lexington, KY, USA (Poster).

Tiede J. & Gratton, C. (2012). Species-specific physiological response of Coccinellidae to the impact of landscape composition. 60th Meeting of the Entomological Society of America (ESA), Knoxville, TN, USA (Poster)

Tiede J. & Gratton, C. (2012). Influence of landscape composition on the physiological condition of common Coccinellidae. 97th Meeting of the Ecological Society of America (ESA), Portland, OR, USA (Poster).

DECLARATIONS

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

The chapters 2 to 5 are a series of manuscripts that have or will be submitted to peer-reviewed journals. I am the main author of chapter 2 to 4 presented in this thesis, for which I have contributed to the development of the study design and collected and analyzed the data, made the tables, figures, and appendices, and written all manuscripts. I am a co-author of chapter 5 and was substantially involved in discussions and editing. Details about the contributions of all co-authors are stated in chapter 1 under *Original articles*.

Declaration plagiarism

I, Julia Tiede, hereby confirm that I have written this thesis independently, that I neither have used other sources than the ones mentioned in the text (no plagiarism), nor that I have used unauthorized assistance and that I have not submitted this thesis previously in any form for another degree at any university or institution.

Bochum, September 2017

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(Julia Tiede)