Variations in trophic niches of generalist predators with plant community composition as indicated by stable isotopes and fatty acids

Odette González Macé1, Anne Ebeling2, Nico Eisenhauer3,4, Simone Cesarz3,4 and Stefan Scheu1,5,*

1 J.F. Blumenbach Institute of Zoology and Anthropology, University of Göttingen, Untere Karspüle 2, 37073 Göttingen, Germany
2 Department of Population Ecology, Institute of Ecology, Friedrich Schiller University of Jena, Dornburger Str. 159, 07743 Jena, Germany
3 German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Deutscher Platz 5e, 04103 Leipzig, Germany
4 Institute of Biology, Leipzig University, Deutscher Platz 5e, 04103 Leipzig, Germany
5 Centre of Biodiversity and Sustainable Land Use, University of Göttingen, Von-Siebold-Str. 8, 37075 Göttingen, Germany
* Corresponding author, e-mail: sscheu@gwdg.de

Received 5 July 2018  |  Accepted 23 July 2019
Published online at www.soil-organisms.de 1 August 2019  |  Printed version 15 August 2019
DOI 10.25674/so91204

Abstract

Arthropods are a dominant component of biodiversity in terrestrial ecosystems. They are considered pest control agents and drive important ecosystem processes like nutrient cycling. However, such ecosystem effects of arthropods may depend on the environmental context influencing nutrition and behaviour. In the framework of a grassland plant diversity experiment (Jena Experiment), we used stable isotope and fatty acid analysis to investigate intraspecific variations in the diet of two of the most abundant predatory arthropods in grasslands: the ground beetle Harpalus rufipes and the wolf spider Trochosa ruricola. The results show that the diet of H. rufipes varied significantly with plant species diversity, consuming more plant material, probably seeds, at high diversity plots, and in the presence of grasses and small herbs. By contrast, in the presence of legumes H. rufipes consumed more animal prey, presumably aphids and/or collembolans. Compared to H. rufipes, the diet of T. ruricola consisted of animal prey only and varied mainly with body size, with larger individuals occupying higher trophic position in the food web. Moreover, the diet of T. ruricola changed in response to summer flooding two months before sampling. Presumably, the availability of secondary decomposer prey as well as intraguild prey was increased in severely flooded plots. As both species are considered pest control agents, the results underline the importance of plant diversity and the composition of plant communities for biological pest control.

Keywords spider | Lycosidae | beetle | Carabidae | grassland | diet

Introduction

Knowledge of the diet of consumers is essential for understanding ecological and trophic interactions, such as niche relationships, competition, coexistence and predation (Cantor et al. 2010, Garcia et al. 2009, Vieira & Port 2007). In particular for improving biological pest control, knowledge of predator–prey interrelationships is essential (Kromp 1999, Wilby & Thomas 2002). This applies to the level of species but also of individuals as individual variation in the diet is a significant component of niche variation (Bolnick et al. 2003, Roeder & Behmer 2014, Sih et al. 2004). Thus, intraspecific variation and plasticity may provide a key mechanism promoting species coexistence (Clark et al. 2007) and plays an important role in intra-
interspecific interactions that shape population and community dynamics (Agrawal et al. 2007, McGill et al. 2006).

Generalist predators are important control agents of lower trophic level consumers such as herbivores (Romero & Harwood 2010, Wise 1993). In terrestrial ecosystems, spiders and predatory beetles are among the most important antagonists of insect herbivore species and play a major role in insect pest control (Kulkarni et al. 2015, Symondson et al. 2002). Notably, spiders and carabid beetles typically occupy different niches and complement each other in prey population control. Carabids are considered opportunistic generalist predators (Gallandt et al. 2005, Lee et al. 2001), with some of them consuming substantial amounts of plant seeds (Harrison et al. 2003, Lund & Turpin 1977). Thereby, they not only act as antagonists of insect pest species, but also may contribute to plant community composition and act as antagonists of weeds (Gallandt et al. 2005, Kulkarni et al. 2015, Menalled et al. 2006). With feeding on seeds they are likely to benefit from diverse plant communities because of the higher amount of seeds produced by diverse plant communities. Indeed, it has been shown that higher plant diversity in organic farming systems increases resource supply for seed feeding carabid beetles (Diehl et al. 2012, Graziani et al. 2012). Such increased food availability increase their potential to control weeds as well as insect pest species (Bárberi et al. 2010).

Similar to carabid beetles, spiders have been shown to effectively control insect herbivore pest species (Griffin et al. 2013, Sunderland 1999). Their wide prey spectrum allows them to occupy a variety of niches (Wise 1993). Besides prey availability, physical habitat characteristics, such as plant architecture, determine spider species diversity and composition of spider communities (Langellotto & Denno 2004, Uetz 1991). In addition to providing structure for web building, the composition of plant species also affects spider assemblages indirectly via driving the abundance and composition of herbivore prey (Dennis et al. 2001). Thereby, plant species composition may indirectly alter the control of invertebrate herbivores by spiders and in turn, top-down control of insect herbivores by spiders may alter plant community composition and e.g., enhance plant diversity (Haddad et al. 2009, Schmitz 2003, Snyder et al. 2006).

For our study, we chose two of the most abundant predatory arthropods in central European grasslands, the ground beetle Harpalus rufipes (De Geer, 1774) (Coleoptera; Carabidae) and the wolf spider Trochosa ruricola (De Geer, 1778) (Araneae; Lycosidae). Both species are geographically widespread, locally abundant and present in many natural and agricultural ecosystems (Clough et al. 2005, Freude et al. 2004, Öberg & Ekbom 2006). H. rufipes forms part of a group of large sized carabid beetles (10–17 mm) characterized by a life cycle lasting two years, autumnal breeding, nocturnal activity and good dispersal by moving on the ground but also by flying (Purtau et al. 2004, Zhang et al. 1997). Notably, H. rufipes not only feeds on animal prey but also on seeds of plants as both larvae and adults (Haritke et al. 1998, Harrison & Gallandt 2012, Shearin et al. 2008). By contrast, T. ruricola is a generalist predator feeding exclusively on living animal prey hunted during the day. As most lycosid spiders, their prey is identified by optical and tactile cues (Ford 1977) attacking virtually all mobile prey species entering their reach, however, following the attack they may reject distasteful prey (Ford 1977, Toft & Wise 1999b). Feeding on a variety of prey differing in food quality has been shown to beneficially affect lycosid spiders (Oelbermann & Scheu 2002, Toft & Wise 1999a). However, the food spectrum is assumed to be determined predominantly by prey availability (Riechert & Harp 1987, Wise 1993) and includes small soft-bodied arthropods, such as flies, springtails, bugs and spiders (Chen & Wise 1999, Kajak 1995).

Stable isotope analysis is an important and widely used tool for studying the trophic structure of animal communities and ecosystem functions (Martinez del Rio & Wolf 2005, Post 2002, Potapov et al. 2019). Although used predominantly for investigating interspecific differences in trophic niches, it allows investigating both intra- and interspecific variability in trophic relationships (Layman et al. 2012, Michener & Latjha 2007, Oelbermann & Scheu 2002). The nitrogen isotope ratio ($^{15}$N/$^{14}$N, usually expressed as $^{15}$N) increases in insects by about $2.5 \pm 1.8 \%$ (mean $\pm 1$ SD) per trophic level (Ikeda et al. 2010) thereby reflecting the trophic position of species in food webs. The carbon isotope ratio ($^{12}$C/$^{13}$C, usually expressed as $^{13}$C) changes little (about $+1 \%$; France & Peters 1997, Potapov et al. 2019), and it is commonly used to evaluate the source of carbon, often to distinguish between the flux of carbon fixed by C$_3$ plants from that fixed by C$_4$ plants (Tiunov 2007, Potapov et al. 2019). The comparison of $^{13}$C and $^{15}$N values within and among species allows assessing the degree of trophic overlap between species and among individuals of the same species (Garratt & Forbes 2006, Halaj et al. 2005, Wise et al. 2006). However, this discrimination may vary depending on a consumer’s nutritional status, diet quality, body size, age, dietary ontogeny and tissue and elemental composition (Ben-David & Schell 2001, Vanderklift & Ponsard 2003).

Another approach to investigate the diet of the animals is the analysis of fatty acids (FAs) as FAs can be traced...
from one trophic level to another, uncovering the diet of consumers and consequently food web links (Ruess et al. 2002, Ruess & Chamberlain 2010). In consumers, most of the assimilated FAs are converted into neutral lipid FAs (NLFAs) and incorporated into the fat body which may comprise a large fraction of their body mass. Since it is energetically more efficient to incorporate FAs without modification (*dietary routing*, Blem 1976, Pond 1981), NLFAs of the fat body reflect the FA composition of the food sources to a significant extent (Ruess et al. 2004) and therefore allow tracing food relationships. In addition, phospholipid fatty acid (PLFA) offers information about the microbial community composition in the soil (Ramsey et al. 2006). This information can be used to study consumer – resource interrelationships. The combination of stable isotope and FA analysis allows detailed insight into trophic niches of species but also individuals and their changes in time and between ecosystems (Ferlian & Scheu 2014, Lau et al. 2008).

We analysed variations in the diet of two of the most abundant arthropod predators in central European grasslands, i.e., the carabid beetle *H. rufipes* and the lycosid spider *T. ruricola* using natural stable isotopes and fatty acids analysis. We expected *T. ruricola* to occupy a higher trophic position than *H. rufipes* due to its strictly carnivorous diet. Further, we hypothesized the diet of *H. rufipes* to vary more with plant community composition than that of *T. ruricola* due to the omnivorous diet of the former also feeding on plant seeds.

**Material and Methods**

**Study site and experimental design**

The study site is a semi-natural temperate grassland in the Saale River floodplain near to the city of Jena (Thuringia, Germany). The site had been used as arable field for more than 40 years before the plant diversity experiment was established in 2002 (Jena Experiment, see Roscher et al. 2004). The experiment comprises 80 plots of 5 × 6 m arranged in four blocks to control for changes in soil texture with increasing distance to the river. A gradient of plant species richness (1, 2, 4, 8, 16 and 60) and plant functional group richness (1–4 functional groups: grasses, small herbs, tall herbs, and legumes) was established to represent a typical hay meadow in the region. Plots are mown twice a year and weeded three times per year (for details see Roscher et al. 2004).

In May 2013, rainfall in Germany was exceptionally high; in Jena, precipitation amounted 150 mm resulting in the experimental field being flooded for 24 days (May to 24 June). This led to anaerobic soil conditions, as shown by redox potentials ranging from -121 to 193 mV in the soil (Wright et al. 2015). Water coverage was measured for each plot daily from 31 May to 24 June and ascribed to five levels: 0, 25, 50, 75, and 100%. Flooding severity was evaluated using a continuous flooding index (sum of the percentage water coverage per day over the flooding period, Wright et al. 2015). Flooding index was used as explanatory covariable to inspect for potential effects of flooding intensity.

**Sampling**

*H. rufipes* and *T. ruricola* were collected using pitfall traps in August 2013. Two 4.5 cm diameter pitfall traps containing 3% formalin were placed in each plot. Animals were collected every two weeks, stored in 70% ethanol (for details see Ebeling et al. 2014) and identified to species (Engelhardt 1964, Schaefer 2010). Because of a high variability body size of *T. ruricola*, individuals were divided into small (from 0.1 to 0.4 cm) and large (from 0.5 to 1.0 cm) ones. *H. rufipes* individuals were highly homogenous in their body size.

**Stable isotope analysis**

Prior to analysis of natural variations in $^{13}$C/$^{12}$C and $^{15}$N/$^{14}$N ratios, *T. ruricola* and *H. rufipes* specimens were dried at 105°C for 48 h and then ground using a mortar and pestle. Ground animal tissue was transferred into tin capsules (one individual per sample), weighed and $^{13}$C/$^{12}$C and $^{15}$N/$^{14}$N ratios measured using an elemental analyser (NA 1500, Carlo Erba, Milano, Italy) coupled with a mass spectrometer (MAT 251, Finnigan, Bremen, Germany; Langel & Dyckmans 2014, Reineking et al. 1993). PD belemnite and atmospheric nitrogen served as primary standard for $^{13}$C and $^{14}$N, respectively. Acetanilide (C$_8$H$_9$NO, Merck, Darmstadt, Germany) was used for internal calibration. Isotope natural abundance was expressed by the delta notation with $\delta ^{13}$C or $\delta ^{15}$N [%] $= \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000$, where R is the ratio of the heavier isotope to the lighter isotope.

Inorganic carbon typically is isotopically heavy as compared to organic carbon and positively skews $\delta ^{13}$C values of animals if they contain carbonate-rich skeletons (Fry 1988, Haines & Montague 1979). In order to remove inorganic carbon, prior to stable isotope analysis, *H. rufipes* samples were also analysed after adding 0.1 N HCl (Hobson et al. 2002).

Stable isotope values of plants of the Jena Experiment have been studied in detail by Gubsch et al. (2011) and...
Roscher et al. (2010). δ¹⁵N values were close to 0‰ in legumes and varied between 2–4‰ in other plant functional groups; generally, they decreased slightly with plant species richness with the decrease largely being due to species specific responses (Gubsch et al. 2011). δ¹³C values of plants in the Jena Experiment vary between -30 and -32‰ reflecting typical C₄ vegetation with some assimilation of soil borne CO₂ (Roscher et al. 2010). As we focused on comparing variations in stable isotope ratios between the two predator species studied, we did not consider variations in stable isotope ratios between plant functional groups in our statistical analyses.

The data were checked for normality and homoscedasticity using Shapiro–Wilk and Fligner–Killeen tests. Linear models (ANOVA, type I sum of squares) were used to analyse the effect of Block (categorical variable, four blocks), body size of the animal (small, large; only in T. ruricola), flooding index (ranging from 1 to 23), plant diversity (ranging from 1 to 60; log-transformed, Hooper et al. 2005), and presence/absence of legumes, grasses, small herbs, and tall herbs including interactions. The Akaike Information Criterion (AIC) was used to select the best model by dropping non-significant variables from the full model in a step wise manner (Faraway 2014, Zuur et al. 2007). Variables were fitted in the following order: Block and flooding index were fitted first followed by body size and plant species richness; thereafter, presence/absence of grasses, legumes, small herbs, and tall herbs including interactions. The Akaike Information Criterion (AIC) was used to select the best model by dropping non-significant variables from the full model in a step wise manner (Faraway 2014, Zuur et al. 2007). Variables were fitted in the following order: Block and flooding index were fitted first followed by body size and plant species richness; thereafter, presence/absence of grasses, legumes, small herbs, and tall herbs, and short herbs were fitted. F-values given in text and tables refer to models in which the respective factor was fitted first (Schmid et al. 2002). Statistical analyses were performed using R 3.2.1 (R Development Core Team 2014, http://www.R-project.org).

**Fatty acid analysis**

*T. ruricola* and *H. rufipes* specimens were cleaned to remove particles attached to the body surface before extraction of total lipids from each individual, which were then extracted and fractionated into neutral lipids as described in Haubert et al. (2004). The neutral lipid fraction was dried in a rotation vacuum concentrator (50°C), and the lipids saponified, methylated and washed following the protocol for the Sherlock Microbial Identification System (MIDI Inc., Newark, USA). Fatty acid methyl esters (FAMEs) were transferred into small vials, capped and analysed by gas chromatography (CLARUS 500, Perkin Elmer, Waltham, USA). The analysis system used was equipped with a flame ionization detector (PE-5 capillary column, 30 m × 0.32 mm i.d., 0.25 mm film thickness, Perkin Elmer, Waltham, USA); helium was used as carrier gas.

FAMEs were identified by comparing retention times of samples and standard mixtures comprising unbranched and branched (bacterial) FAMEs. The following fatty acids served as biomarkers for bacteria: the methyl-branched FAs i15:0, a15:0, i16:0, and i17:0 (Gram-positive) and the cyclic FAs cy17:0 and cy19:0 (Gram-negative). The unsaturated FAs 18:1ω9 and 18:2ω6,9 served as relative plant and fungal markers, respectively (Ruess & Chamberlain, 2010). Moreover, 16:2ω6,9 and 16:3ω3,6,9 were used as biomarkers of green algae (Chlorophyceae) (Buse et al., 2013). Monoenic C20 FAs were used as indicator of a eukaryote diet (Ruess et al. 2004). All other markers, such as 10:00 and 12:00, cannot be assigned to specific taxa and were used as unspecific markers.

PLFAs were extracted from soil taken in September 2013 (93 days after the natural flood). Three soil cores of 2 cm diameter were taken from 0–10 cm soil depth and were pooled to form one composite sample. Afterwards soil was sieved with 2 mm mesh size to remove stones and animals. PLFA extraction followed the protocol by Frostegård et. al. (1991) with modifications as mentioned by Wagner et. al. (2015) using 5 g of fresh soil. The analysis system used (GC 17A, Shimadzu, Kyoto, Japan) was equipped with column DB 225MS (60 m × 0.25 mm i.d., 0.25 µm) and an autosampler (AOC 5000, Shimadzu).

Hydrogen was used as carrier gas. The software GC Lab Solution (Shimadzu) were used to assign peaks to fatty acids by comparing FAMEs to retention times of samples and standard mixtures comprising unbranched and branched (bacterial) FAMEs. The same biomarker FAs as described for NLFA were used.

Percentage values of NLFA and PLFA were logit-transformed and non-metric multidimensional scaling (NMDS) was used to compress the data. MANOVA was performed using the scores of the NMDS axes. Thereafter, if significant, differences within individual FAs were inspected by single factor analysis of variance (ANOVA). Principal components analyses (PCAs) of logit-transformed mole percentage values were performed using Canoco 5 (Ter Braak & Smilauer 2012). Flooding index, plant functional group, plant species richness and the presence of legumes, grasses, tall herbs and small herbs were included as supplementary variables. Pearson correlations were used to evaluate the relationship between logit-transformed marker FA concentrations [sum of bacterial markers, fungal marker (18:2ω6,9 and plant marker (18:1ω9)] in soil microorganisms (PLFAs) and those in *H. rufipes* and *T. ruricola* (NLFA).
Variations in trophic niches of generalist predators with plant community composition

**Results**

*Harpalus rufipes*

The δ¹⁵N signature of *H. rufipes* was on average 5.88 ‰, but the values were highly variable and ranged from 3.52 to 8.63 ‰ spanning 5.11 δ units (Fig. 1). δ¹⁵N signatures decreased significantly with increasing plant species richness (Fig. 2), whereas they were slightly increased in the presence of legumes (Table 1). The δ¹³C signature of *H. rufipes* was -23.92 ‰, but again values were highly variable, ranging from -18.28 to -29.21 ‰, i.e. spanning over 10.93 δ units (Fig. 1). δ¹³C signatures increased significantly in the presence of grasses and small herbs, but were not significantly affected by other plant community properties and flooding index (Table 1).

The FA composition of *H. rufipes* was significantly affected by the presence of legumes and marginally by the presence of grasses (Table 2). Concentrations of the plant marker 18:1ω9 increased significantly with the presence of grasses and marginally with the presence of legumes (Table 3). Similarly, the unspecific FA marker 18:0 increased significantly in the presence of legumes. The eukaryote FA markers 20:4ω6 and 20:1ω9 significantly increased in the presence of legumes. The unspecific markers 16:0 and 18:1ω7 increased significantly with the presence of grasses, whereas the unspecific FA 23:0 decreased significantly (Table 3).

Bacterial marker FAs in *H. rufipes* were negatively correlated with the bacterial marker PLFAs in soil (Table 4). By contrast, the plant FA marker in *H. rufipes* (18:1ω9) was positively correlated with the plant PLFA

**Table 1.** ANOVA table of F-values on the effect of block, body size, flooding index (FI), plant species richness (SR), presence of grasses (Gr), legumes (Leg), small herbs (SH) and tall herbs (TH) on the δ¹⁵N and δ¹³C signatures of *Harpalus rufipes* and *Trochosa ruricola*.

<table>
<thead>
<tr>
<th></th>
<th><em>H. rufipes</em> δ¹⁵N</th>
<th></th>
<th><em>H. rufipes</em> δ¹³C</th>
<th></th>
<th><em>T. ruricola</em> δ¹⁵N</th>
<th></th>
<th><em>T. ruricola</em> δ¹³C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factors</td>
<td>df</td>
<td>F</td>
<td>df</td>
<td>F</td>
<td>df</td>
<td>F</td>
<td>df</td>
</tr>
<tr>
<td>Block</td>
<td>3,49</td>
<td>2.17</td>
<td>-</td>
<td>-</td>
<td>3,45</td>
<td>2.17</td>
<td>-</td>
</tr>
<tr>
<td>Size</td>
<td>-</td>
<td>2.27</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.27</td>
<td>-</td>
</tr>
<tr>
<td>FI</td>
<td>-</td>
<td>2.51</td>
<td>-</td>
<td>-</td>
<td>1,45</td>
<td>3.28</td>
<td>-</td>
</tr>
<tr>
<td>SH</td>
<td>-</td>
<td>2.32</td>
<td>-</td>
<td>-</td>
<td>1,45</td>
<td>2.47</td>
<td>-</td>
</tr>
<tr>
<td>TH</td>
<td>-</td>
<td>2.61</td>
<td>-</td>
<td>-</td>
<td>1,45</td>
<td>2.47</td>
<td>-</td>
</tr>
</tbody>
</table>

**Table 2.** MANOVA table of F-values on the effect of block, body size, flooding index (FI), presence of grasses (Gr), legumes (Leg), small herbs (Sh) and tall herbs (Th) on the neutral fatty acid composition of *Harpalus rufipes* and *T. ruricola*, as well as the phospholipid fatty acid (PLFA) composition of soil microorganisms (based on NMDS axes; see Methods). Significant effects (P < 0.05) are given in bold and marginally significant effects (P < 0.10) in italics. ↑ Increase, respectively ↓ decrease with increasing plant species richness, body size, flooding index, or in presence of the respective plant functional group.

<table>
<thead>
<tr>
<th></th>
<th><em>H. rufipes</em> df</th>
<th>F</th>
<th><em>T. ruricola</em> df</th>
<th>F</th>
<th>PLFA df</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block</td>
<td>3,45</td>
<td>2.17</td>
<td>-</td>
<td>-</td>
<td>3,45</td>
<td>2.17</td>
</tr>
<tr>
<td>Size</td>
<td>-</td>
<td>2.51</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.51</td>
</tr>
<tr>
<td>FI</td>
<td>-</td>
<td>3.28</td>
<td>-</td>
<td>-</td>
<td>1,45</td>
<td>3.28</td>
</tr>
<tr>
<td>Sh</td>
<td>-</td>
<td>2.32</td>
<td>-</td>
<td>-</td>
<td>1,45</td>
<td>2.32</td>
</tr>
<tr>
<td>Th</td>
<td>-</td>
<td>2.61</td>
<td>-</td>
<td>-</td>
<td>1,45</td>
<td>2.61</td>
</tr>
<tr>
<td>Leg</td>
<td>1,45</td>
<td>3.28</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Gr</td>
<td>1,45</td>
<td>2.47</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
marker in soil. There was no correlation with the fungal FA marker in *H. rufipes* and the plant PLFA marker in soil.

The first four PCA axes explained 51.8% of the total variation in the FA composition of *H. rufipes*, with the first axis accounting for 20.9% and the second axis for 11.1% (Fig. 3). The first axis was separated by bacterial markers vs. fungal and plant markers. Further, the plant marker seems to correlate negatively with flooding index, plant functional group and species richness. The first axis was related to the presence of small herbs, whereas the second axis was associated with the presence of legumes. Concentrations of the bacterial markers cy17:0 and a15:0, and the unspecific markers 15:00 and 17:00 increased in the presence of small herbs. Concentrations of unspecific FA markers such as 16:1ω5 and 22:00 were higher in presence of legumes.

**Trochosa ruricola**

The $\delta^{15}$N signature of *T. ruricola* was on average 8.22‰, but the values were variable and ranged from 6.49 to 9.96‰ (Fig. 1). However, the range (3.47 δ units) was considerably lower than that in *H. rufipes*. The $\delta^{15}$N signatures of *T. ruricola* significantly increased with flooding index and body size (Fig. 4, Table 1).

Average $\delta^{13}$C signature of *T. ruricola* was -26.60‰. Contrasting *H. rufipes*, $\delta^{13}$C signatures of *T. ruricola* only varied over 1.66 δ units, i.e., from -25.95 to -27.61‰ (Fig. 2). None of the factors studied significantly affected the $\delta^{13}$C signatures of *T. ruricola* (Table 1).

The FA composition of *T. ruricola* varied significantly with body size (Table 2). The eukaryote marker FAs...
Variations in trophic niches of generalist predators with plant community composition

Table 3. ANOVA table of F-values (with degrees of freedom) on the effects of legumes (Leg), grasses (Gr) and body size (Trochosa ruricola only) on neutral fatty acids of Harpalus rufipes and T. ruricola as well as on phospholipid fatty acids (PLFAs) of soil microorganisms. Significant effects (P < 0.05) are given in bold and marginally significant effects (P < 0.10) in italics. ↑ Increase, respectively ↓ decrease with presence of legumes or grasses.

<table>
<thead>
<tr>
<th>FA</th>
<th>H. rufipes</th>
<th>T. ruricola</th>
<th>PLFA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leg F₁,₄₅</td>
<td>Gr F₁,₄₅</td>
<td></td>
</tr>
<tr>
<td>8:00</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>10:00</td>
<td>↑3.33</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>12:00</td>
<td>-</td>
<td>↑17.22</td>
<td></td>
</tr>
<tr>
<td>16:0</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>16:00</td>
<td>↑4.10</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>16:10</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>17:0</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>17:01</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>cy17:0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>17:00</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>18:00</td>
<td>↑6.04</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>20:406</td>
<td>↑4.41</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>20:503</td>
<td>-</td>
<td>↑24.70</td>
<td>-</td>
</tr>
<tr>
<td>21:00</td>
<td>↑5.39</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>23:00</td>
<td>-</td>
<td>↑4.39</td>
<td>-</td>
</tr>
</tbody>
</table>

|        | FI F₁,₄₅  |      |
| 8:00   | -          | -    |
| 10:00  | ↑12.91     | -    |
| 12:00  | -          | -    |
| i6:0   | -          | -    |
| 16:00  | ↑4.59      | -    |
| 16:10  | -          | -    |
| i7:0   | -          | -    |
| 17:01  | -          | -    |
| cy17:0 | -          | -    |
| 17:00  | -          | -    |
| 18:00  | ↑4.68      | -    |
| 20:406 | ↑11.11     | -    |
| 20:503 | -          | ↑24.70|
| 21:00  | -          | -    |
| 23:00  | -          | -    |

Figure 5. Principal components analysis of the relative abundance (mol %, logit- transformed) of individual NLFAs of Trochosa ruricola using body size (small, large), flooding index (FI), plant species richness (SR), plant functional group richness (FG), presence of grasses (Gr), legumes (Leg), small herbs (SH) and tall herbs (TH) as supplementary variables.
20:4ω6 and 20:5ω3 significantly increased with body size (Table 3). Further, the fungal marker FA 18:2ω6,9 increased marginally with body size. Unspecific marker FAs 10:00 and 12:00 decreased significantly, while the bacterial marker FA 18:1ω7 increased significantly with body size (Table 3). The FAs 10:00 and 16:00 decreased in the presence of small herbs. Further, the unspecific FAs 16:00 (significantly) and the bacterial marker FA 16:1ω7 (marginally) decreased in presence of tall herbs. FAs of *T. ruricola* were not correlated significantly with any microbial PLFA markers in soil (Table 4).

The first four PCA axes explained 67.4% of the total variation in the dataset, with axis 1 accounting for 30.5% and axis 2 for 15.4% (Fig. 5). The first axis of the PCA reflected variations in FAs of *T. ruricola* with body size (small, large), with the concentration of eukaryote markers 20:4ω6 and 20:5ω3 increasing in larger individuals. By contrast, small individuals contained higher concentrations of unspecific FAs such as 10:00 and 12:00. The second axis of the PCA was related to the number of plant functional groups and the presence of small herbs, but the effect was small. Concentrations of the FAs 18:2ω6,9 and 18:3ω6 increased at sites with higher number of plant functional groups and in the presence of small herbs.

### Microbial PLFAs in soil

MANOVA results based on NMDS scores indicated Block (significantly), flooding index (marginally) and tall herbs (marginally) to affect the composition of microbial PLFAs in soil (Table 2). The bacterial marker i16:0 and the plant marker 18:1ω9 increased significantly in heavily flooded plots (Table 3). By contrast, concentrations of the fungal marker 18:2ω6,9 significantly decreased with

| Table 4. Pearson correlation coefficients of regressions between logit-transformed marker phospholipid fatty acid (PLFA) concentrations [sum of bacterial markers (see Methods), fungal marker (18:2ω6,9), and plant marker (18:1ω9)] of soil microorganisms and the respective neutral fatty acids in *Harpalus rufipes* and *Trochosa ruricola*. Significant effects (P < 0.05) are given in bold. |
|---|---|---|
| **PLFA markers** | **Bacterial** | **Plant** | **Fungal** |
| **H. rufipes markers** | | | |
| Bacterial | -0.45 | 0.48 | 0.01 |
| Plant | 0.07 | -0.03 | -0.15 |
| Fungal | -0.06 | 0.02 | 0.16 |
| **T. ruricola markers** | | | |
| Bacterial | -0.03 | 0.04 | 0.03 |
| Plant | 0.10 | -0.11 | 0.03 |
| Fungal | 0.07 | -0.05 | -0.10 |

**Figure 6.** Principal components analysis of the relative abundance (mol%, logit-transformed) of individual PLFAs of soil microorganisms using flooding index (FI), plant species richness (SR), plant functional group richness (FG), presence of grasses (Gr), legumes (Leg), small herbs (SH) and tall herbs (TH) as supplementary variables.
increasing flooding index. The unspecific marker FAs 17:00 increased significantly at higher flooding index, whereas FAs 18:00 and 21:00 significantly decreased. The bacterial marker cy19:0 and the unspecific FA marker 16:00 increased significantly in the presence of tall herbs (Table 3).

The first four PCA axes explained 83.0% of the total variation in PLFAs. The first axis accounted for 39.2% and the second axis for 20.2% (Fig. 6). The distribution of the FAs varied with plant functional groups. The first axis represented the presence of grasses and legumes, whereas the second axis represented the presence of tall herbs and small herbs. In presence of tall herbs concentrations of the eukaryote marker FA 21:00 and the bacterial marker cy17:0 increased, whereas in the presence of small herbs the bacterial marker 19:00 increased.

Discussion

As we expected, stable isotope analysis suggests that *T. furcata* occupies a higher trophic position than *H. rufipes*. As indicated by fatty acid analysis, the diet of *H. rufipes* includes plant seeds. The more variable diet of *H. rufipes* also is reflected by the wider range of stable isotope values as compared to *T. furcata*, which is only feeding on animal prey, pointing to an omnivorous diet with substantial contribution of plant seeds. The variation in the relative contribution of plant and animal dietary components in *H. rufipes* is reflected in particular in the wide range of δ13C values spanning > 5 δ units which exceeds considerably the commonly assumed 3.4‰ per trophic level suggesting that some individuals live on a rather pure plant (seed) diet, whereas others exclusively feed on animal prey including intraguild predators.

As we hypothesized, the diet of *H. rufipes* varied significantly with plant community composition. Its diet markedly depended on the available plant resources, as indicated by the close correlation between the FA plant marker in the soil and in body tissues. *H. rufipes* consumed more plant resources in more complex and species-rich plant communities as also indicated by the decrease in δ15N values with plant species diversity. Seed predation by insects has been shown to increase with increasing plant species richness (Preukuschas et al. 2014, Vockenhuber et al. 2013). The phenology of plants differs among species, and therefore more diverse communities provide seeds over a longer period of time, presumably benefiting *H. rufipes*. In fact, beetles of the genera *Harpalus* were shown to become more abundant in the presence of higher numbers of weed plants in agricultural fields (de Snoo et al. 1995, Kota 1988). Also, *H. rufipes* has been shown to benefit from a diet comprising a diversity of seeds rather than only seeds of one plant species (Bilde & Toft 1994, Brygadyrenko & Reshetniak 2014), and seeds generally have been shown to be of high food quality for granivorous carabid beetles (Fawki & Toft 2005).

In the present study, the presence of grasses and small herbs increased the δ13C signatures of *H. rufipes*. In the Jena Experiment, grasses produce their seeds earlier during the vegetation period than most tall herbs and legumes, and generate larger amounts of seeds than herbaceous plants (C. Roscher, pers. comm.). This may explain the increased contribution of plants (seeds) to the diet of *H. rufipes*. Besides, fatty acid analyses indicated that in the presence of legumes the diet of *H. rufipes* shifted towards more animal prey, presumably including aphids and collembolans (Kielt et al. 1999). Legumes are considered key plant species (Miele et al. 2008), which suffer more from herbivore attack than other plant functional groups (Loranger et al. 2014). Further, legumes favour detritivore soil invertebrates by providing nitrogen-rich litter material (Spahn et al. 2002). Indeed, in the Jena Experiment the density and diversity of Collembola increased in the presence of legumes (Sabais et al. 2011) and Collembola are important prey of carabid beetles, in particular of juveniles (Eitzinger & Traugott 2011, Mundy et al. 2000).

The δ13C values of *H. rufipes* spanned a markedly wide range including values considerably higher than those of C3 plants suggesting that its body carbon originated from food sources other than the C3 plants dominating at the study site. Primary producers characterized by high δ13C values include algae (Akamatsu et al. 2004, Ruess et al. 2004), and potentially *H. rufipes* may have fed on algivore prey species such as soil surface dwelling Collembola (Potapov et al. 2018), but no algae FA markers were detected in *H. rufipes*. Further, high δ13C values may have been due to *H. rufipes* feeding on seeds of C4 plants or on herbivores feeding on C4 plants. Although not included in our sampling regime, there are two plots of 10 × 20 m with a C4 plant species (*Amaranthus retroflexus* L.) in the Jena Experiment. For identifying if seeds or herbivores of these plants may have contributed to the diet of *H. rufipes* we investigated if the enrichment in δ13C values in *H. rufipes* was more pronounced close to the two *A. retroflexus* plots. In fact, however, distance to the *A. retroflexus* plots did not significantly correlate with δ13C values in *H. rufipes* arguing against this explanation. Nevertheless, this does not rule out this explanation as carabid beetles such as *H. rufipes* are able to fly long distances (Zhang et al. 1997) and also move fast on the soil surface in a random way (Galis & Jong 1988). Although *A. retroflexus* produces small seeds, feeding efficiency (i.e., seeds eaten per distance travelled) in *H. rufipes* is particularly high for seeds of this species (Harrison & Gallandt 2012).
Moreover, seed production of *A. retroflexus* is excessive with 5000 to 300,000 seeds per plant (Costea et al. 2003), suggesting that the observed high δ¹³C values in *H. rufipes* may have been due to feeding on plants of this species. Irrespective of the exact source responsible for the high δ¹³C values in *H. rufipes*, the marked variations in δ¹³C values reflect that this species acquired a substantial fraction of its body carbon from other locations than the plots they were captured. In fact, carabid beetles such as *H. rufipes* are characterized by larger home ranges than the size of our plots of 5 × 6 m; however, variations in stable isotope ratios and fatty acid composition in *H. rufipes* with plot specific plant characteristics also reflect that they used local resources from the plots they were caught.

In contrast to *H. rufipes*, the diet of *T. ruricola* did not vary with plant species richness, being more independent of the diversity and structure of plant communities than *H. rufipes*, suggesting that it may be independent of variations in environmental factors and plant associated prey communities (Ebeling et al. 2017). However, the lack in variations in the diet of *T. ruricola* with plant community composition may also have been due to the small size of the plots of 5 × 6 m and reflect that *T. ruricola* acquired most of its prey from larger areas of the Jena Experiment field site. Rather than with plot specific plant characteristics, it changed markedly with body size, indicating dietary changes during developmental stages, from first towards secondary predators (i.e., decomposers and predators with higher δ¹⁵N values). The fact that larger juveniles and adults of *T. ruricola* occupy higher trophic positions in the food web than smaller individuals suggests that with increasing body size intraguild predators and/or cannibalism becomes more important (König et al. 2011, Schneider et al. 2012). Indeed, spiders are known to be voracious intraguild predators (Denno et al. 2004, Lensing & Wise 2004) and the larger the top predator the more it is feeding on larger other predator species (Riede et al. 2011, Schneider et al. 2012). As a consequence, larger predators may switch from exploitative competition with similar sized predators over intraguild predation to occupying higher trophic level with the largest predator at the top. High δ¹⁵N values in *T. ruricola* adults therefore may reflect its position as second order predator. Notably, in severely flooded plots, the δ¹⁵N values of *T. ruricola* were higher than in plots little affected by the flood. Earlier studies showed that flooding decreased bacterial biomass, whereas it increased fungal biomass, reflecting the elevated availability of dead plant biomass in severely flooded plots (Wagner et al. 2015, Wright et al. 2015), which probably increased the abundance of detritivore prey to *T. ruricola*. Overall, therefore, the diet of *T. ruricola* may have consisted mainly of secondary decomposers such as Collembola in severely flooded plots.

Conclusions

Carabid beetles and spiders are among the most abundant and important predators in grasslands. Stable isotope and fatty acid analysis of *H. rufipes* and *T. ruricola* yielded novel insights into factors driving trophic interrelationships of these species and their role in controlling herbivores. Notably, the study allowed investigating the role of variations in plant species richness, plant functional group richness and presence/absence of functional groups of plants on intraspecific dietary variation of these predator species. The results indicate that the diet of *H. rufipes* varied with the availability of plant resources and increased with increasing plant diversity as well as in the presence of grasses and small herbs. By contrast, presence of legumes increased the consumption of animal prey, presumably aphids and collemobolans. In contrast to *H. rufipes*, the diet of *T. ruricola* comprised only animal prey and was independent of plant species richness. Interestingly, however, it varied markedly with body size with larger individuals occupying higher trophic positions as second order predators highlighting the importance of IGP. Moreover, the diet of *T. ruricola* changed due to flooding, presumably by increased availability of secondary decomposers as well as intraguild prey in severely flooded plots. Our results reinforce the view that there are feedback loops between plant and invertebrate predator communities with more effective control of pest species in more diverse plant communities. Notably, generalist predators of different taxonomic and functional groups, such as carabid beetles with a wide diet including also seeds and wolf spiders with animal prey only, are likely to complement each other in controlling insect herbivore species and thereby herbivore–plant interactions.

Acknowledgements

We thank the Deutsche Forschungsgemeinschaft (German Research Foundation) for financial support (FOR 1451) as well as the Friedrich-Schiller-University Jena and the Max-Planck-Institute for Biogeochemistry Jena for financial and logistical help with the set-up and maintenance of the Jena Experiment. We also thank the gardeners, in particular Gerlinde Kratzsch, Steffen Eismann, Heike Scheffler, Katja Kunze, Silke Hengelhaupt, and Ute Köber. Laura Sánchez Galindo, Ting-Wen Chen, Juan Armando Torres Munguia and Tamara Hartke for their support and helpful comments. SC and NE are supported by the German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena.
Leipzig, funded by the German Research Foundation (FZT 118). Also I thank Mutua Madrileña for the financial support.

References


Brygadyrenko, V. V. & D. Y. Reshetniak (2014): Trophic variations in trophic niches of generalist predators with plant community composition. – SOIL ORGANISMS 91 (2) 2019


Odette González Macé et al.


R Development Core Team (2012): R: A language and environment for statistical computing.


