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Reliability of isotopic fractionation ($\Delta^{15}N$, $\Delta^{13}C$) for the delimitation of trophic levels of oribatid mites: Diet strongly affects $\Delta^{13}C$ but not $\Delta^{15}N$

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ABSTRACT

Stable isotope analysis has become an analytical tool of central importance in trophic ecology. The actual degree of isotopic fractionation, however, remains a black-box in most studies. Hence, mean values for trophic enrichment from other taxa are commonly used to delimitate trophic levels. On the other hand, resource composition as well as consumer physiology both influence fractionation patterns. Especially in soil food webs, high variability of isotopic fractionation has been found for both ^{15}N and ^{13}C. Here, we investigate effects of diet on trophic enrichment in a no-choice feeding experiment with the oribatid mite model species *Archegozetes longisetosus* and a set of fungal, animal, algal and plant resources (seeds and vegetative tissues). We found consistent trophic enrichment for nitrogen irrespective of diet ($\Delta^{15}N = 3.9‰$), but no reliable fractionation for carbon being negative for fungi ($\Delta^{13}C = -1.8‰$), neutral for algae, and positive for the remaining plant and animal resources ($\Delta^{13}C = 2.3‰$). The results suggest that $\delta^{15}N$ is a reliable marker for delimiting the trophic level of oribatid mites and presumably other soil detritivores, while understanding $\delta^{13}C$ signals needs *a priori* knowledge about isotopic fractionation with respect to consumed resources.

1. Introduction

Stable isotope analysis has become an important tool in trophic ecology (Wada et al., 1991; Gannes et al., 1997; Post, 2002; Tiunov, 2007; Martinez del Rio et al., 2009; Boecklen et al., 2011). The principle is based on the presence or absence of a reproducible physiological discrimination of stable isotopes between resources and consumers. While mainly $\delta^{15}N$ and $\delta^{13}C$ have been used for diet reconstruction (Boecklen et al., 2011), sulfur ($^{32}S/^{34}S$) and hydrogen ($^{2}H/^{1}H$) might also be suitable markers (Tiunov, 2007). The difference between resource and consumer stable isotope ratios (isotopic fractionation factor or trophic enrichment factor TEF, $\Delta^{15}N$ and $\Delta^{13}C$) seems, with exceptions, to be quite stable or at least predictable for $\Delta^{15}N$ (Post, 2002; Vanderklift and Ponsard, 2003; Tiunov, 2007), but varies significantly for $\Delta^{13}C$ (Post, 2002; Caut et al., 2009; Pollierer et al., 2009). The variation in carbon stable isotope signals has been explained by differences of $\delta^{13}C$ values between C3 and C4 plants (Martin et al., 1990) and by variation among tissue compounds (e.g., lipids, sugars, cellulose), and can be a valuable tool to identify the most relevant carbon sources for consumers (Pollierer et al., 2009). Furthermore, $\Delta^{13}C$ (Klarner et al., 2014) and $\Delta^{15}N$ (Vanderklift and Ponsard, 2003) can be influenced by resource C-to-N ratios (Gannes et al., 1997). Both isotope ratios can significantly differ among tissues of vertebrates and invertebrates, but due to their small size mostly whole animals were analyzed from the latter (Vanderklift and Ponsard, 2003; Tiunov, 2007; Tsurikov et al., 2015). Overall mean estimates of TEFs, were positive for both, $\Delta^{13}C$ (0.4‰) and $\Delta^{15}N$ (3.4‰) (Post, 2002), or $\Delta^{13}C$ (0.75‰) and $\Delta^{15}N$ (2.75‰) (Caut et al., 2009), and not influenced by the environment (freshwater, marine or terrestrial), at least for invertebrates (Caut et al., 2009). Some physiological, geographical and ecological factors (e.g., excretion type, soil type, temperature, pH), however, can significantly correlate with stable isotope signals and fractionation (Vanderklift and Ponsard, 2003; Corral-Hernández et al., 2015). Furthermore, some statistical artifacts may have led to some questionable interpretations of isotopic fractionation patterns (Auerswald et al., 2010), although this has been a controversial topic (Caut et al., 2010). Hence, a reliable determination of nitrogen and carbon TEFs is essential for the reconstruction of trophic interactions and food-webs using stable isotope ratios (Vanderklift and Ponsard, 2003; Martinez del Rio

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et al., 2009).

Stable isotopes have also been used intensely to unravel the enigmatic trophic ecology of soil organisms such as nematodes, collembolans and oribatid mites (Scheu and Falca, 2000; Ruess et al., 2004; Schmidt et al., 2004; Schneider et al., 2004a; Tiunov, 2007; Pollierer et al., 2009; Maraun et al., 2011; Digel et al., 2014; Klarner et al., 2014). Due to characteristic properties of soil food-webs, such as a high degree of omnivory (Scheu and Setälä, 2002; Digel et al., 2014), stable isotope signatures need to be interpreted with caution, and studies have shown that $\Delta^{15}N$ in soil organisms can span almost 13 units, from −3.2 to 9.5 (Tiunov, 2007). Hence, the appeal for more laboratory experiments to understand isotope fractionation holds true especially for the soil system (Gannes et al., 1997; Martinez del Rio et al., 2009; Feldhaar et al., 2010).

Oribatid mites represent an intensely studied group of soil organisms regarding stable isotope data (Schneider et al., 2004a; Erdmann et al., 2007; Fischer et al., 2010; Maraun et al., 2011; Perdomo et al., 2012; Corral-Hernández et al., 2015). Some studies provided evidence for oribatid mite feeding niche differentiation, spanning over three to four trophic levels with about 13 δ-units for ^{15}N and 7 δ-units for ^{13}C (Schneider et al., 2004a; Erdmann et al., 2007; Pollierer et al., 2009; Corral-Hernández et al., 2015), while others (Schneider and Maraun, 2005) described oribatid mites as "choosy generalists" (although based on feeding experiments rather than stable isotope analyses). Furthermore, stable isotope signatures show some intraspecific variability in the trophic position of oribatid mites. *Hermaniella dolosa* and *Paradamaeus clavipes*, for example, spanned about 5–8 units in $\delta^{15}N$, depending on forest location, host tree species and calibration method (Erdmann et al., 2007; Corral-Hernández et al., 2015). It remains unknown whether these patterns result from plasticity in feeding or in stable isotope discrimination since TEFs have not yet been determined experimentally for this taxon. Instead, "standard values" of e.g. 3–4‰ and 0.5–1‰ for $\Delta^{15}N$ and $\Delta^{13}C$, respectively, have been used (Post, 2002; Schneider et al., 2004a; Pollierer et al., 2009), although their applicability to soil organisms has been questioned (Erdmann et al., 2007; Tiunov, 2007; Maraun et al., 2011). Since oribatid mites play a key role in soil food webs (Maraun et al., 2003, 2007; Schneider et al., 2004b; Heethoff et al., 2009; Maraun et al., 2011; Wehner et al., 2016), and stable isotopes have been used intensely to unravel their trophic interactions, yet provide highly controversial results, it seems mandatory to empirically estimate values of $\Delta^{15}N$ and $\Delta^{13}C$ in this group. To do this, we used a parthenogenetic oribatid mite species to exclude factors resulting from genetic or ecological variability. The *Archegozetes longisetosus* ran lineage (Heethoff et al., 2007) was established from a single gravid female and has become a model for oribatid mites (Heethoff et al., 2013). *A. longisetosus* is an opportunistic feeder and can be reared on very different kinds of food. Here, we used a broad spectrum of fungal, animal, algal and plant resources (seeds and vegetative tissues) to empirically determine nitrogen and carbon TEFs.

2. Material and methods

2.1. Animals and resources

Archegozetes longisetosus ran (Heethoff et al., 2007) was reared in darkness at about 28 °C and 80–85% relative air humidity. Animals were fed with yeast (Y; Rapunzel Naturkost GmbH, Legau, Germany), blood meal (BM; Common Baits, Rosenfeld, Germany), *Chlorella* (Ch; Naturya, Bath, UK), hemp protein (HP; Naturya, Bath, UK), sweet lupin flour (Lu: Govinda Natur GmbH, Neuhofen, Germany) or wheat grass (WG; Naturya, Bath, UK). Compositional information on these resources is given in Table 1. For each resource, three small round plastic containers (5 cm diameter, 8 cm height), with about 2 cm plaster of Paris/activated charcoal (9:1) were initially set up with ~30 adult individual mites each (18 containers for 6 resources in total). Food (in form of powders) was supplied *ad libitum*.

After about two months and a complete life cycle, adults from the second generation (i.e. all developmental stages were fed exclusively with one of the different resources) were sampled. For sampling, about 20 individuals were randomly taken from each of the three containers of each resource, pooled, and then randomly divided into three groups with each ten pooled individuals (0.15 ± 0.03 mg dw) for stable isotope analyses ($\delta^{13}C$, $\delta^{15}N$). Stable isotope signatures were also measured in triplicate from the diets.

2.2. Stable isotope analyses

Samples were dried, weighed and transferred into tin capsules. Stable isotope and C-to-N ratios were determined using a coupled system of an elemental analyzer (NA 1500, Carlo Erba, Milan, Italy) and a mass spectrometer (MAT 251, Finnigan, Bremen, Germany) (Reineking et al., 1993; Langel and Dyckmans, 2014). Isotope signatures were calculated by $\delta X\ (‰) = (R_{sample} - R_{standard})/R_{standard} \times 1000$, with X the target isotope and R the ratio between the heavy and light isotopes ($^{13}C/^{12}C$ and $^{15}N/^{14}N$). Nitrogen in atmospheric air served as standard for $\delta^{15}N$ and Vienna PD Belemnite as standard for $\delta^{13}C$ measurements; acetanilide was used for internal calibration.

2.3. Data analyses

Variation among replicates mainly reflects measurement errors (i.e., weighing and stable isotope measurement errors), since animals were pooled and resource replicates originated from the same product charges. However, these pseudoreplicates can be used to test wether effects were higher than measurement errors (ANOVA and *t*-tests), and hence are of biological relevance. Correlation analyses of TEF with resource parameters were performed using Pearson's r analyses based on means. Due to the multiple comparisons, p-values were Bonferroni corrected leading to a significance threshold of $p < 0.005$ (for ten comparisons).

TEFs were determined by subtracting the isotopic mean values of the resource measurements from the mean values of the consumer samples:

$$TEF\ (\Delta^{13}C) = \delta^{13}C_{consumer} - \delta^{13}C_{resource}$$

$$TEF\ (\Delta^{15}N) = \delta^{15}N_{consumer} - \delta^{15}N_{resource}$$

Statistical analyses were performed in PAST 3 (Hammer et al., 2001).

Table 1
Resource characterization. Compositional data were taken from the product information of the supplier. Y: yeast, BM: blood meal, Ch: *Chlorella*, HP: hemp protein, Lu: lupine, WG: wheat grass. *Compositional data of blood meal was not available from the manufacturer and was estimated based on (Donkoh et al., 1999) and references therein.

	Y	BM*	Ch	HP	Lu	WG
Energy [kJ/g]	13	13	14	19	15	8
Fat [%]	6	1	8	12	12	2
Carbohydrate [%]	11	0	7	26	10	22
Protein [%]	41	80	61	50	40	21
Fibre [%]	29	1	16	21	28	42