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Biomarker function and nutritional stoichiometry of neutral lipid fatty acids and amino acids in oribatid mites

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ABSTRACT

Biomarkers (e.g. fatty acids, amino acids, stable isotopes, and molecular barcodes) have become increasingly important for investigating food web structure and nutrient flow in soil ecosystems. While the biomarker function of fatty acids has been investigated for some soil animal taxa (e.g. collembolans and nematodes), their role in soil-dwelling oribatid mites remained unknown. Here, we investigate the biomarker function and nutritional stoichiometry of neutral lipid fatty acids (NLFA) and amino acids in oribatid mites. We reared the opportunistic model oribatid mite species *Archegozetes longisetosus* on ten different resources of animal, bacterial, fungal and herbal origin. We analyzed the neutral lipid fatty acid and amino acid compositions of resources and consumers with gas chromatography/mass spectrometry (GC/MS) and ion-exchange chromatography (IEC), respectively. We found diet-dependent amounts and compositions of NLFA in the oribatid mites, but amino acids were stable and independent of diet. Consumer NLFA composition could be used as a reliable predictor of diet using data mining approaches (i.e., Random Forest), while amino acid profiles reflected diet-independent intrinsic physiological properties and confirm the homeostatic protein stoichiometry hypothesis for oribatid mites.

1. Introduction

Understanding food web structure, food sources and resource specialization is fundamental to reveal multichannel feeding, reticulated organization and flow of soil organic matter as well as to understand the enigmatically high diversity of soil animals in terrestrial ecosystems (Anderson, 1975; Scheu and Setälä, 2002; Wolkovich, 2016). The role of microarthropods as important participants structuring the soil, e.g. the formation of soil aggregates via the high production of fecal- and necrobiomass or the degradation of soil structure caused by high local abundances, as well as contributing to energy and material flow (e.g. carbon and nitrogen) has received increasing attention (e.g. Scheu et al., 2005; Maaβ et al., 2015; Soong and Nielsen, 2016). To clarify soil microarthropods' functional role as decomposers, scavengers and potentially predators, biomarker techniques like molecular gut barcoding (e.g. Juen and Traugott, 2006), stable isotope ratios (e.g. Scheu and Falca, 2000; Pollierer et al., 2009; Maraun et al., 2011) and fatty acid profiles (Ruess and Chamberlain, 2010 and references therein) have been used.

Among soil microarthropods, especially the ubiquitous, diverse and particle-feeding acarine order Oribatida is of particular interest to draw a more conclusive picture of resource-consumer interactions in soils (Norton, 2007; Wehner et al., 2016). While only some pioneer studies on gut barcoding are available (Remén et al., 2010; Heidemann et al., 2011), oribatid mites represent a group which has been continuously examined regarding stables isotopes, providing information about food niche differentiation, trophic food web structure and isotopic fragmentation (e.g. Schneider et al., 2004; Maraun et al., 2011; Heethoff and Scheu, 2016). Stable isotopes have helped to understand general food web structure regarding oribatid mites, but failed to identify distinct food sources (Maraun et al., 2011). Therefore, the use of biomarker molecules, such as amino acids for nitrogen and fatty acids (e.g., neutral lipid fatty acids, NLFA) for carbon may provide more in-depth information about their feeding ecology.

While trophic transfer of potential biomarker fatty acids has been studied intensively in other soil animal taxa, such as collembolans, nematodes or centipedes (e.g. Ruess et al., 2002; Chamberlain et al., 2004, 2005; Ruess et al., 2005; Haubert et al., 2006; Pollierer et al., 2010), there are no studies testing

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incorporation of fatty acids into body lipids of oribatid mites. Only one study considered oribatids in a broad carbon flux field experiment using stable isotopes of fatty acids (Pollierer et al., 2012), providing information about energy and carbon flow in a below ground food web. However, this setup was inadequate to show if and how resource-fats were transferred into oribatid mites, because analyses of field-collected animals do not provide information on dietary routing and biomarker reliability (Chamberlain et al., 2005). Beside fatty acids, there is only one short note about amino acids in oribatid mites (Butler and Tonn, 1963) - and no information about trophic transfer and nutritional stoichiometry of amino acids are available at all. Therefore, one of the main organic-nitrogen storage forms in soils (e.g. Jones and Kielland, 2002; Jones et al., 2002) with a potential biomarker function has been ignored. For the direct stoichiometric transfer of amino acid signals from resources to consumers, Anderson et al. (2004) assumed that homeostasis of body protein (and therefore amino acid composition) may be a highly conserved pattern, because cellular proteins are similar in each individual of one species, since they are based on the same genetic information, and should not differ because of different diets. On the other hand, however, there is some evidence, from microbial and marine systems showing that amino acids may also possess biomarker function (Boschker and Middelburg, 2002; Arthur et al., 2014). Hence, more controlled studies about protein/amino acid stoichiometry and composition in different animal taxa are needed to test the validity of these hypotheses.

Based on the need of more laboratory experiments regarding biomarker reliability and nutrient stoichiometry (e.g. Anderson et al., 2004; Chamberlain et al., 2005; Maraun et al., 2011), we set up a no-choice feeding experiment using the parthenogenetic model oribatid mite species *Archegozetes longesitosus* Aoki (Heethoff et al., 2013). *A. longisetosus* is an opportunistic particle feeder and culturable on very different resources (see Heethoff and Scheu, 2016). We used a broad spectrum of ten resources of animal, bacterial, fungal and herbal (algae, seeds, pollen and vegetative tissues) origin. We used gas-chromatography/mass-spectrometry (GC/MS) and ion exchange chromatography to analyze patters of fatty acids and amino acids, respectively, in both resources and oribatid mites. We aimed to answer the following questions: i) are neutral lipid fatty acids (NLFA) reliable nutritional biomarkers for oribatid mites? and ii) are amino acids also suitable as trophic markers or are they in homeostasis?

2. Materials and methods

2.1. Experimental design

Archegozetes longesitosus ran (Heethoff et al., 2007; hereafter "mites") were reared at approx. 28 °C and 80–85% relative humidity in constant darkness. The mites were fed three times a week with: animal resources such as blood meal (blood; Common Baits, Rosenfeld, Germany) and bone meal (bone; Canina Pharma GmbH, Hamm, Germany); one bacterial resource, *Spirulina* sp. powder (spirulina; Interaquaristik, Biedenkopf-Breidenstein, Germany); fungal resources, such as shiitake fungus, *Lentinula edodes* powder (fungi; Arche Naturprodukte GmbH, Hilden, Germany) and grinded dry yeast, *Saccharomyces cerevisiae* (yeast; Rapunzel Naturkost GmbH, Legau, Germany); and a variety of herbal resources, such as *Chlorella* sp. powder (chlorella; Naturya, Bath, UK), hemp, *Cannabis sativa* protein powder (hemp; Naturya, Bath, UK), sweet lupine, *Lupinus albus* flour (lupine; Govinda Natur GmbH, Neuhofen, Germany), grinded mixed pollen grain (pollen; Ascopharm GmbH, Wernigerode, Germany) and wheat grass, *Triticum* sp. powder (wheat; Naturya, Bath, UK). The food was provided *ad libitum* as recommended by Raubenheimer and Simpson (1997) for nutritional transfer experiments. For each resource, specimens were cultured in three separated plastic boxes (100 × 100 × 50 mm) grounded with 2 cm mixture of plaster of Paris/activated charcoal (9:1) for 11 month and numerous generations (where the mites exclusively fed on one of the resources) prior to the analysis. For the measurements, 60 (±15, depending on size) mite individuals were randomly removed from each of the 30 culturing containers and transferred into ten new boxes (one for each resource). The mites were kept in these boxes without food for 3 days. Then, 60 ± 15 individuals with an empty alimentary tract (visual inspection) were randomly taken from these boxes and frozen at −28 °C until chemical analysis. This procedure was replicated with new mites three times at intervals of two weeks.

2.2. Fatty acid analysis

Total lipids were extracted from the resource powders and mites (4–7 mg) using 1 mL of chloroform:methanol, 2:1 (V/V) according to Folch et al. (1957) over a period of 24 h. Animals were directly refrozen after the extraction and subsequently dried, weighted with a microbalance (Mettler Toledo, XS3DU, Columbus, USA; with 0.1 μg readability and 1 μg repeatability) and stored until amino acids analyses. Extracts were purified and separated according to the method described by Frostegård et al. (1991). Briefly: SiOH-columns (Chromabond® SiOH, Macherey-NagelGmbH & Co. KG, Düren, Germany) were washed and conditioned with 6 mL hexane. Subsequently, samples were applied on the column and elution of neutral lipids was accomplished with 4 mL chloroform. Afterwards the chloroform fractions were evaporated to dryness under gentle nitrogen gas flow and residuals were redissolved in 200 μL dichloromethane:methanol, 2:1 (V/V). 100 μL for animals and 20 μL for resources were transferred to new glass vials with a conical inlet (150 μL) and 20 μL of internal standard (C19:0 in methanol; 220 ng/μL) were added and evaporated to dryness again. Subsequently samples were derivatized to fatty acid methyl esters (FAMEs) with 20 μL TMSH (trimethylsulfonium hydroxide; 0.25 M in MeOH from Fluka, Sigma-Aldrich, St. Louis, USA) reagent. TMSH is a methylation agent which directly transesterifies free fatty acids or lipids such as triglycerids to the corresponding FAMEs. TMSH in methanol can be directly injected and residual non-FAME compounds pyrolyze within the injector. All solvents were analytical grade chemicals for gas chromatography (>99.8% purity) purchased from Merck KGaA, Darmstadt, Germany.

FAME samples of neutral lipid fatty acids (NLFAs) were analyzed with a QP2010 Ultra GC/MS (Shimadzu, Duisburg, Germany). The gas chromatograph (GC) was equipped with a ZB-5MS fused silica capillary column (30 m × 0.25 mm ID, df = 0.25 μm) from Phenomenex (Aschaffenburg, Germany). One microliter sample aliquots were injected by using an AOC-20i autosampler-system from Shimadzu, Duisburg, Germany into a programmable temperature vaporizing-split/splitless-injector (Optic 4, ATAS GL, Eindhoven, Netherlands), which operated in splitless-mode. The injection-temperature was 70 °C (5 s hold) which was raised to 300 °C with a heating rate of 30 °C sec^{-1} and then an isothermal hold for 59 min. Hydrogen was used as carrier-gas with a constant flow rate of 2.89 mL/min. The temperature of the GC oven was raised from initial 60 °C for 1 min, to 150 °C with a heating-rate of 15 °C min^{-1}, to 260 °C with a heating-rate of 3 °C min^{-1}, to 320 °C with a heating-rate of 10 °C min^{-1} and then an isothermal hold at 320 °C for 10 min. Electron ionization mass spectra were recorded at 70 eV from m/z 40 to 650. The ion source of the mass spectrometer and the transfer line were kept at 250 °C.

Methyl esters of the NLFAs (hereafter "fatty acids") were identified by comparing gas chromatographic retention times and mass spectrometric fragmentation patters with those of the Supelco® 37