

Short communication

Stable isotope composition of microfauna supports the occurrence of biologically fixed nitrogen from cyanobacteria in desert soil food webs

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ABSTRACT

Biological soil crusts of the arid Southwest US are habitat for a greater diversity of microfauna than soils with absent or physically disturbed crusts. The cyanobacteria of desert soil crusts fix nitrogen biologically, but it is not known whether these cyanobacteria are consumed by microfauna and are a significant component of the soil food web. We suspect that stylet-bearing nematodes and tardigrades are the most likely microfauna to graze on cyanobacteria, so we used natural abundance stable isotopes to test whether cyanobacteria are indeed a significant component of their diet in the field. We found that stable isotope composition of microinvertebrate biomass supports the hypothesis that cyanobacteria are a potential prey item in the food chain leading up to tardigrades and dorylaim-type nematodes in desert soils, but does not support the same hypothesis for Tylenchidae in these communities. Continued development of stable isotope methods may help to refine our understanding of feeding behaviors in belowground desert soil food webs.

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1. Introduction

Biological soil crusts can represent up to 70% of the living vegetative soil cover in desert systems. Early-colonizing fungi and cyanobacteria stabilize the soil surface and facilitate colonization by later successional stages of lichens, green algae, and mosses (Belnap, 2003). Biological soil crusts are habitat for a greater abundance and diversity of microfauna than soils with absent or less well-developed crusts (Darby et al., 2007). Microinvertebrates that are best suited to feed on cyanobacteria are those that bear a piercing stylet for feeding on filaments, rather than those that ingest bacteria or particulates whole (Wood, 1973a, 1973b; Yeates et al., 1993). We suspect that several species of nematodes and tardigrades feed on cyanobacteria because specimens that we have collected from desert soils that are associated with biological soil crusts in southeastern Utah often have green to cyan-colored pigments in their intestines. We have been able to maintain cultures of tardigrades (*Haplomacrobrius utahensis*) and stylet-bearing nematodes (*Aphelenchoides* sp.) on cyanobacteria (*Microcoleus vaginatus*) in laboratory conditions. Similarly, Wood

(1973a,b) observed that Dorylaimidae and Aporcelaimidae were able to feed and reproduce on moss and “algae” (a mixture of green algae and cyanobacteria) in culture.

Visual observation of feeding activity in a captive environment helps to identify potential prey items, but it does not determine whether such a food source is necessarily a preferred or even dominant prey item in native environments. Molecular tools have limited application because nucleic acids from prey nuclei are degraded by extensive nucleases and have a short half-life in the digestive tracts of their microinvertebrate predators. Stable isotope analysis has been proposed as an alternative empirical approach to determine what food sources actually dominate a consumer's diet (Ruess et al., 2004, 2005). For example, C3 plants have a relatively depleted $\delta^{13}\text{C}$ isotope content of around -26‰ , while C4 plants have a relatively enriched isotopic composition of around -12‰ . The isotopic composition of predators is similar to that of their prey but with some enrichment in heavy isotopes (Minagawa and Wada, 1984). Carbon isotopic fractionation is around 1‰ per trophic level or less, so $\delta^{13}\text{C}$ is often considered to reflect the original source of fixation. In contrast to carbon, predators generally exhibit a greater degree of nitrogen fractionation relative to their prey, so $\delta^{15}\text{N}$ is considered a reflection of trophic level. In a review of the available literature, Vanderklift and Ponsard (2003) found that the mean trophic level fractionation of $\delta^{15}\text{N}$ enrichment in ammonotelic invertebrate herbivores was $2.5\text{--}3\text{‰}$, but also has been estimated

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as high as 5‰ (Minagawa and Wada, 1984). The natural abundance stable isotope approach has been used previously to understand the diet of several different microinvertebrate consumers (Couch, 1989; Moens et al., 2002, 2005; Neilson and Brown, 1999; Neilson et al., 1999; Riera et al., 1999).

The main objective of this project was to test the hypothesis that biologically fixed nitrogen from nitrogen-fixing soil crust cyanobacteria can be traced through the microfaunal consumer food web. We tested this hypothesis by comparing the stable isotope composition of nitrogen-fixing cyanobacteria to that of several soil microfauna, including tardigrades and stylet-bearing nematodes. The above hypothesis would be supported if the carbon isotopic composition of stylet-bearing nematodes or tardigrades was similar to that of cyanobacteria, and the nitrogen isotopic composition would be slightly enriched (3–5‰) from that of cyanobacteria. The hypothesis would fail to be supported if no microfaunal groups showed stable isotope evidence of being either primary or secondary consumers of nitrogen-fixing cyanobacteria.

2. Methods and materials

This study focuses on the soil microfauna from Arches and Canyonlands National Parks of the Colorado Plateau near Moab, Utah (38.67485 N, –109.4163 W, 1310 m). Vascular plant vegetation at this site comprises 5–20% of the total cover and is dominated by the C₃ grass *Achnatherum hymenoides* (Roem. & Schult.) (syn. *Stipa hymenoides*), but also includes the C₄ grass *Pleuraphis jamesii* (Torr.) (syn. *Hilaria jamesii*), and the C₃ grass *Bromus tectorum* (L.). Biological soil crusts at this site comprise 70–90% of total cover and are dominated by the moss *Syntrichia caninervis* Mitt., the lichen *Collema tenax* (Sw.) Ach., whose nitrogen-fixing cyanobiont is *Nostoc commune*, and the autotrophic cyanobacterium *M. vaginatus* (Vauch.) Gomont, which is incapable of nitrogen-fixation itself and therefore requires nitrogen fixed from a bacterial associate (Steppe et al., 1996). Other autotrophic cyanobacteria present, that are capable of nitrogen-fixation, include species from the genera *Scytonema*, *Tolypothrix*, and *Spirirestis* (Yeager et al., 2007).

Undisturbed soil was collected at 0–20 cm depth from outside an experimental field site (Darby et al., 2011). The nematodes used for natural abundance stable isotope analysis were extracted from these soils through an Oostenbrink extraction tray. Preliminary trials determined that 100–150 individuals were necessary for reliable C and N isotope data for large bodied nematodes (≥ 1 mm adult body length), or about 200–250 individuals for smaller nematodes (<1 mm adult body length). In the present study it was possible to collect three samples for tardigrades and samples for three groups of nematodes: five replicates of Cephalobidae (mostly from the genera *Acrobeles* and *Acromoldavicus*, thought to feed primarily on bacteria but also potentially fungal spores), nine replicates of Tylenchidae (mostly from the genus *Tylenchus*), and four replicates of Dorylaimida (mostly from the family Qudsianematidae). Individual specimens were hand-picked with an insect minuten pin into tin cups (3 mm \times 5 mm) each containing a 35- μ l droplet of sterile distilled, de-ionized water and dried overnight at 60 °C. Samples were submitted for quantification of carbon and nitrogen isotopic content (Boston University Stable Isotope Laboratory, Boston, MA). Although it was possible to clean, dry and prepare root samples from this soil for isotopic analysis, it was impossible to separate fungi or cyanobacteria from soil and organic matter. As a result, we used cultures of *Nostoc* sp., *Scytonema* sp., *M. vaginatus*, and *Microcoleus chthonoplastes* from the culture collection at the Microbial Ecology and Geomicrobiology lab at Arizona State University and submitted these samples for isotopic carbon and nitrogen analysis (Kansas State University Stable Isotope Mass Spectrometry Lab, Manhattan, KS). The cyanobacterial cultures

were monoxenic except for *M. vaginatus*, which requires a nitrogen-fixing associate to survive in nitrogen-free media. Thus, microinvertebrate samples came from specimens collected from the field, but cyanobacteria samples came from biomass cultured in the lab on media that did not contain carbon or nitrogen.

3. Results and discussion

Cephalobidae were enriched in both carbon and nitrogen relative to all other samples tested (Fig. 1), which is consistent with our understanding that they feed on primarily saprotrophs, such as bacteria and fungal spores, which will reflect the isotopic signature of soil organic matter and be enriched in both isotopic carbon and nitrogen relative to primary producers and primary consumers. We were not able to isolate enough fungi or fungivorous nematodes for stable isotope analysis, but we expect the isotopic composition of fungivores would be comparable to the isotopic composition of bacterivores like Cephalobidae.

The isotopic content of carbon ($\delta^{13}\text{C}$) for Tylenchidae was similar to that of roots (Fig. 1), as was the mean $\delta^{15}\text{N}$ for Tylenchidae in the range of 3–5‰ enriched relative to roots. We interpret this as evidence to reject the hypothesis that the cyanobacteria tested here are a significant prey item for the Tylenchidae tested here, mostly *Tylenchus* sp. This claim does not preclude the possibility that some species do consume cyanobacteria, or that they consume different species of cyanobacteria, or perhaps in different seasons. Nonetheless, we were somewhat surprised that Tylenchidae stable isotope composition bears such little resemblance to cyanobacteria (and such little support for consumption). Tylenchidae are very abundant in these soils and have previously been shown to feed on *Microcoleus* under laboratory conditions (Wood, 1973b). However, Tylenchidae also feed on roots as characterized by the “1e” or “1f” feeding type of Yeates et al. (1993), and numerous species of *Filenchus* (also in the family Tylenchidae) are known to feed and

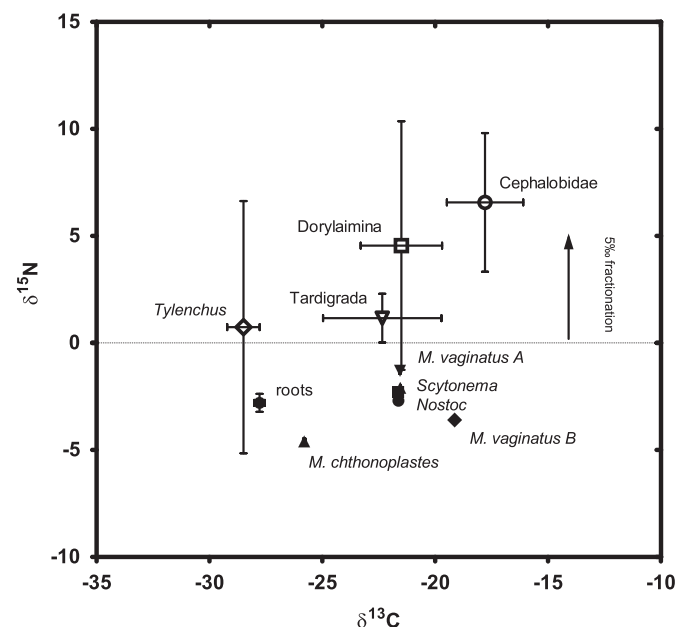


Fig. 1. Isotopic composition of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) from selected desert soil microfauna (open markers) and their potential prey items (solid markers). Markers denote the position of overall mean values; error bars denote standard error of mean. Lack of error bars indicates that standard error was within the width of the actual marker. Arrow indicates magnitude and direction of the expected 5‰ fractionation that is approximately the maximum level of $\delta^{15}\text{N}$ enrichment of consumers above their diet that is expected from literature sources.

reproduce on fungi (Okada et al., 2005). Until more data can be collected, though, we propose the working hypothesis that the bulk of these Tylenchidae (mostly *Tylenchus* sp.) in this food web are more directly part of the vascular plant primary production food chain (feeding on either vascular plant roots or root-associated fungi) than of the energy channels derived from either decomposition or non-vascular plant primary production.

Isotopic carbon content of Tardigrada and Dorylaimina was similar to that of the cultured cyanobacteria *M. vaginatus*, *Scytonema*, and *Nostoc*. Isotopic composition of nitrogen was more variable than for $\delta^{13}\text{C}$, but the mean $\delta^{15}\text{N}$ for Tardigrada and Dorylaimina was enriched in the range of 3–10‰ relative to cyanobacteria (Fig. 1). We interpret this as evidence to support the hypothesis that biologically fixed carbon and nitrogen from cyanobacteria is present in these Tardigrada and Dorylaimina, but we cannot necessarily determine whether these taxa directly feed on cyanobacteria, or feed on a grazer of cyanobacteria, or both. Personal observations of tardigrades feeding on cyanobacteria in culture and of Dorylaimida collected from the field suggest that at least some species can feed on some species of cyanobacteria.

We acknowledge that significant limitation of this study is that we were only able to include a small subset of the potential prey and predators in this food web. The sample size required to meet the minimum threshold of detection for reliable isotope signatures still limit number of taxa that can be obtained and the taxonomic resolution that can be sorted and picked by hand. Carman and Fry (2002) developed methods to decrease the sample size requirements down from 5 to 60 individual nematodes. Their procedures were similar to those used for the present study except that we found at least 100–200 individuals were necessary because the desert soil nematodes used here are smaller than the freshwater species they used. Another significant limitation is that we relied on cultured cyanobacterial biomass for stable isotope analysis even though the microinvertebrate specimens were extracted directly from the soil. The cyanobacteria were grown in media that had no organic or inorganic forms of nitrogen, so we assume that all carbon and nitrogen from the cyanobacterial biomass samples was fixed biologically and is comparable in stable isotope abundance to biomass in the field. We hope that additional methods continue to be developed so that we can overcome these limitations and continue to better characterize the feeding interactions of a greater number of belowground prey and predator groups.

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