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SHORT COMMUNICATION

Trophic relationships among terrestrial molluscs in a Hawaiian rain forest: analysis of carbon and nitrogen isotopes

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Soil and adjacent leaf-litter environments support a diverse decomposer fauna. This has led to what is known as 'the enigma of the soil fauna', or the question of how it is possible for such large numbers of species to coexist without obvious biotic mechanisms, such as competitive exclusion, limiting coexistence (Anderson 1975). Dietary specialization or effective partitioning of food resources could be a mechanism to avoid niche overlap among sympatric soil/litter species (Chahartaghi *et al.* 2005, Jennings & Barkham 1975). However, unravelling the complexities of trophic relationships can be difficult, especially in soil/leaf-litter habitats where both consumers and prey are small, diverse and often unidentifiable (Scheu & Falca 2000). As such, the trophic relationships among species in these habitats typically remain unresolved.

Stable isotope ratios, particularly $^{15}N/^{14}N$ and $^{13}C/^{12}C$ ratios (expressed as $\delta^{15}N$ and $\delta^{13}C$), are increasingly used to identify trophic relationships among organisms in soil and leaf-litter habitats (Chahartaghi *et al.* 2005, Ponsard & Arditi 2000, Scheu & Falca 2000, Schmidt *et al.* 2004). Nitrogen ratios ($\delta^{15}N$) are used to estimate trophic positions of organisms because stepwise enrichment of ^{15}N (3.4‰ ± 1.1‰) is usually observed with each trophic transfer (Post 2002). Carbon ratios ($\delta^{13}C$) are used to determine the dietary source because carbon ratios typically vary among primary producers but change little with trophic transfers (0.4‰ ± 1.4‰) (Ostrom *et al.* 1997). Together $\delta^{15}N$ and $\delta^{13}C$ can identify subtle differences in trophic position among litter invertebrates (Schmidt *et al.* 2004).

Our goal was to use $\delta^{15}N$ and $\delta^{13}C$ to investigate the trophic relationships among terrestrial mollusc species (native and non-native) in the leaf-litter of a Hawaiian rain forest. We focus on terrestrial molluscs because they are major components of Hawaiian terrestrial biodiversity (Cowie *et al.* 1995). We evaluated two hypotheses: (1) mollusc species partition food resources and occupy distinct trophic niches and (2) $\delta^{15}N$ increases during the decomposition process. While differences in $\delta^{13}C$ are used to determine dietary source, differences in $\delta^{15}N$ between the leaf-litter and fresh plant material may allow identification of contributions of the above-ground and below-ground food webs to the various molluscs.

Molluscs, other soil invertebrates and plants were collected from a 20 × 20-m area in the Upper Waiakea Forest Reserve (19.33479°N, 155.15023°W) on the eastern (windward) side of the island of Hawaii. The site is a young (the last lava flow to cover the area was ~ 2100 y ago) wet forest with dense canopy cover that typically receives an annual rainfall of 2500 to >5000 mm (Meyer & Cowie 2010a). The plant community is characterized by the dominant native tree, *Metrosideros polymorpha* Gaudich., mid-canopy tree ferns, *Cibotium* spp., and understorey plants such as *Broussaisia arguta* Gaudich. The only common native mollusc in the litter is *Succinea cepulla* Gould. Other *Succinea* species occur in the area but are typically found on plants and rarely seen in the litter (Meyer 2009). Common invasive molluscs in the study area are the slugs *Arion intermedius* Normand, *Limax maximus* Linnaeus and *Deroceras laeve* Müller, and the predatory snail *Oxychilus alliarius* Miller, all of which are widely distributed within the Hawaiian Islands (Meyer & Cowie 2010a).

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To identify the isotopic baseline, leaf-litter (leaves collected after abscission) were allowed to decompose for 0, 6 and 12 mo at the site, and leaves (pre-abscised) were collected from the three most common plant species as determined by numbers of individuals. Leaf-litter consisted of leaves collected just after abscission between June 2007 and December 2007. Leaves from all plant species at the study site were collected and mixed thoroughly to ensure a random mixture of species. To evaluate if isotopic ratios change as litter decomposes, leaf-litter was placed in bags that were deployed in the field. Each bag consisted of a $10 \times 10 \times 8$-cm plastic-mesh food carton (to provide a three-dimensional framework) covered with plastic screen (2.0-mm mesh). Each bag contained 4.0 g (mean air-dry mass) of leaf-litter and had two holes (2.5 cm diameter) cut in the mesh on opposite sides of the bags to allow macro-invertebrates access. The leaf-litter decomposition experiment ran from 20 January 2008 to 9 January 2009. In total, 20 bags were released at the site and six and eight litter bags were recovered after 6 and 12 mo, respectively. Ten 4.0-g leaf-litter samples were not placed in the field and were used to assess the isotopic signatures of fresh litter. Fresh leaves of the three most common plant taxa (*Metrosideros polymorpha*, *Cibotium* spp., *Broussaisia arguta*) were collected from six individual plants within the study area in August 2008 to evaluate differences among plant species and between pre-abscised and abscised leaves.

Molluscs and non-molluscan invertebrates were collected in July 2008. Molluscs were collected by hand. Non-molluscan invertebrates were extracted from the leaf-litter that had been allowed to decompose for 6 mo. The litter bags were collected in the field and their contents immediately transferred into Berlese funnels for 48 h to extract invertebrates (temperatures of litter in the funnels did not exceed 30°C). All invertebrates collected were preserved in 95% ethanol. Non-molluscan invertebrates included one oligochaete species (earthworm) and the dominant arthropod groups: Acari (mites), Aranaeae (spiders), Collembola (springtails), Diplopoda (millipedes) and Isopoda (isopods). We used one-way ANOVAs and Tukey's multiple comparison tests to determine differences in N and C isotopic signatures among terrestrial molluscs and potential food sources (three plant species and three leaf-litter treatments). Nitrogen isotopic ratios of non-mollusc invertebrates were used to help confirm placement of terrestrial molluscs into feeding guilds. Isopod, millipede, springtail and earthworm samples were used to identify primary decomposers, those probably feeding on litter/detritus with adhering lichens, algal mats, fungi and bacteria. The mite sample, which included both predatory and detritivorous mites, was used to identify secondary consumers, a group feeding either on a mixture of litter and other invertebrates, or microbes that can be enriched in ^{15}N (Högberg *et al.* 1999). Spiders were used to identify predators/scavengers or those species feeding on either live or dead invertebrates.

Most efforts to elucidate diets of terrestrial molluscs typically involve direct observations of feeding behaviour, gut analysis or food choice experiments, and conclude that most molluscs are general grazers, feeding indiscriminately on leaf-litter and associated microbes (Barrada *et al.* 2004, Cook & Radford 1988, Jennings & Barkham 1975). In contrast, our study provides evidence of food partitioning and indicates that mollusc species occupy distinct trophic niches and multiple trophic levels (Table 1), suggesting that dietary specialization or effective partitioning of food resources may help explain their coexistence.

Assuming an enrichment of δ^{15}N of $\sim$ 3.4‰ per trophic level (Post 2002), a δ^{15}N range of 4.94‰ (−9.5‰–1.5‰) indicates the presence of at least two trophic levels. Comparing mollusc isotopic ratios to the ratios of non-mollusc invertebrates for which we had a priori assumptions of their trophic guild, we assigned terrestrial molluscs to three feeding guilds: (1) primary consumers/decomposers, (2) omnivores/secondary consumers or (3) predators/scavengers. The native snail *S. cepulla* and the introduced slug *A. intermedius* were assigned as primary consumers because individuals were enriched in δ^{15}N $\sim$ 3.4‰ above the leaf-litter, suggesting these species feed on litter/detritus with adhering fungi and bacteria (Table 1). The δ^{15}N values for *Cibotium* spp. and *M. polymorpha*, the two most abundant plant species at our study site, are $\sim$ 5‰ lower than any mollusc species indicating that *S. cepulla* and *A. intermedius* probably feed primarily on leaf-litter, not fresh plant material. However, using isotopes to distinguish between above-ground and below-ground food webs is complicated in our system because live tissue from *B. arguta* had a similar δ^{15}N to that of the leaf-litter (Table 1). However, it is unlikely that these species consume live plants because they were always found in the litter (pers. obs.). Increased degree of decomposition of food sources can result in δ^{15}N enrichment of soil animals (Schmidt *et al.* 2004). However, we only observed a slight, non-significant, trend for δ^{15}N to increase (0.7‰) during the decomposition process (Table 1). As such, larger differences in δ^{15}N values between litter and live plant tissue are most likely the result of differences in proportions of ^{14}N and ^{15}N reabsorbed before abscission, because fresh litter (composed primarily of *Cibotium* spp. and *M. polymorpha* leaves) had significantly higher δ^{15}N values than tissues collected from living *Cibotium* spp. and *M. polymorpha* (pre-abscission).

The invasive molluscs, *D. laeve*, *L. maximus* and *O. alliarius*, feed higher in the food web (Table 1). *Deroceras laeve* was classified as an omnivore or secondary consumer because δ^{15}N values were intermediate between those of primary decomposers and predators/scavengers.

Table 1. Sample description and isotopic values (δ^{15}N and δ^{13}C) for all treatments. Values denoted with the same superscript letter do not differ significantly.

Substrate	Tissue used	No. of samples	No. of individuals per sample	Mean sample weight (mg)	δ^{15}N	δ^{13}C	C:N
Plants							
Metrosideros polymorpha	Leaf	6	1	2.11	-9.5 ± 0.8^{A}	-30.7 ± 1.5^{A}	62.3 ± 10.6
Cibotium spp.	Leaf	6	1	2.17	-9.1 ± 0.9^{A}	-26.5 ± 1.3^{C}	32.1 ± 8.5
Broussaisia arguta	Leaf	6	1	2.45	-6.9 ± 1.1^{B}	-29.3 ± 0.9^{AB}	24.5 ± 4.9
Leaf litter							
Fresh	Leaf	10	1	4.58	-7.2 ± 0.6^{B}	-29.3 ± 0.3^{AB}	50.3 ± 7.9
Decomposed 6 mo	Leaf	6	1	2.70	-7.0 ± 0.7^{B}	-29.4 ± 0.4^{AB}	42.3 ± 5.5
Decomposed 12 mo	Leaf	8	1	2.80	-6.5 ± 0.3^{B}	-29.2 ± 0.5^{B}	33.5 ± 6.9
Terrestrial molluscs							
Succinea cepulla	Foot muscle	8	1	0.42	-4.1 ± 0.7^{C}	-26.3 ± 0.6^{C}	4.39 ± 0.7
Arion intermedius	Foot muscle	9	1	0.35	-4.4 ± 1.6^{C}	-24.1 ± 0.6^{D}	4.13 ± 0.8
Deroceras laeve	Foot muscle	9	1	0.37	-1.5 ± 1.8^{D}	-24.6 ± 0.8^{D}	4.08 ± 0.7
Limax maximus	Foot muscle	10	1	0.43	0.54 ± 1.3^{E}	-23.9 ± 1.2^{D}	3.72 ± 0.1
Oxychilus alliarius	Foot muscle	10	1	0.40	0.44 ± 1.3^{E}	-23.4 ± 0.5^{D}	3.47 ± 0.2
Non-mollusc invertebrates							
Primary consumers							
Oligochaetes (earthworms)	Whole body	1	1	0.29	−3.0	−25.7	4.68
Collembola (springtails)	Whole body	1	~ 100	0.45	−4.0	−24.7	3.65
Diplopoda (millipedes)	Whole body	1	1	0.63	−3.1	−22.8	5.70
Isopoda (isopods)	Whole body	1	1	0.58	−2.6	−24.1	4.10
Secondary consumers							
Acari (mites)	Whole body	1	~ 50	0.37	−2.0	−24.7	3.71
Predators/scavengers							
Aranaeae (spiders)	Whole body	1	~ 10	0.12	1.5	−23.7	3.43

Deciphering the diet of *D. laeve* at a finer scale is difficult using stable isotopes alone. For instance, intermediate δ^{15}N values may indicate omnivory (feeding on both animal and plant resources) which is prevalent in soil food webs (Eggers & Jones 2000), or may indicate that *D. laeve* is selectively feeding on microbes in the litter, which can in some ecosystems be enriched in δ^{15}N compared with the surrounding environment (Högberg *et al.* 1999). Analysis of the diet of a congeneric species, *Deroceras reticulatum*, indicated that a small proportion of its diet consisted of animal material (Barrada *et al.* 2004). *Limax maximus* and *O. alliarius* were classified as predators/scavengers, because individuals enriched in δ^{15}N~3.4‰ above primary decomposers. *Oxychilus alliarius* preys on snails (Meyer & Cowie 2010b) and δ^{15}N was therefore expected to be more enriched than the other molluscs. However, we were surprised by the high δ^{15}N values associated with *L. maximus*, which indicates that plant material does not form a significant proportion of its diet. This contradicts Cook & Radford (1988) who, examining faecal pellets, found the diet of *L. maximus* in Northern Ireland consists primarily of vascular plant material. Explanations for this discrepancy include: (1) novel feeding behaviours by *L. maximus* in its non-native range or (2) that *L. maximus* does ingest a large portion of plant material, but may only assimilate material from the invertebrates and microbes on that plant material. *Limax maximus* has been observed consuming a variety of food items other than plants including dung, dog food, dead/crushed molluscs and rat bait in Hawaii (pers. obs.).

Values of δ^{13}C are typically expected to change little with each trophic transfer (0.4‰ ± 1.4‰) (Ostrom *et al.* 1997). However, in our study, all invertebrates were enriched in δ^{13}C compared to plant and litter food resources (Table 1). Enrichment between invertebrates and plant material was less for *Cibotium* spp. (0.2‰–3.7‰) than for *B. arguta* (3.0‰-6.5‰), leaf-litter (3.0‰–6.5‰), and the dominant tree species *M. polymorpha* (4.4‰–7.9‰). Using the convention that δ^{13}C is expected to change little with trophic transfers (0.4‰ ± 1.4‰), we would conclude that *Cibotium* spp. contribute to the leaf-litter food web but that the other two plants contribute little. However, it seems inconceivable that the dominant tree species, *M. polymorpha*, would not contribute to the leaf-litter food web in Hawaii. Primary consumers in our system may be elevated in δ^{13}C relative to primary producers for a variety of reasons. Litter invertebrates may be feeding primarily on fungi and bacteria for which isotopic values are often not determined here but can have higher δ^{13}C values than the detritus (Gregorich *et al.* 2000) or litter invertebrates may only be able to assimilate certain fractions of the litter (Martin *et al.* 1992). Elevated δ^{13}C values may have also resulted from the preservation technique used (invertebrates preserved with 95% ethanol) which can increase δ^{13}C values (Kaehler & Pakhomov 2001). Regardless of reasoning, δ^{13}C values currently cannot reliably provide an estimate for the contributions of different food sources in our leaf-litter food web because δ^{13}C enrichment differs considerably from what is expected for the first trophic transfer.

Although, dual analysis of δ^{13}C and δ^{15}N can identify subtle feeding differences among species (Schmidt *et al.* 2004), we suggest that differences in δ^{13}C among molluscs should be interpreted as hypotheses that still require further testing. Significant differences in δ^{13}C between the primary decomposers *A. intermedius* and *S. cepulla* suggest these species use different food resources (Table 1). *Succinea cepulla*, the only native snail examined, differs from all the invasive molluscs in δ^{13}C suggesting little dietary overlap. However, evidence for competition has been observed between the primary consumers *A. intermedius*, *S. cepulla* and millipedes at our study site, but not between millipedes and other molluscs that feed higher in the food web (W.M. Meyer unpubl. data), suggesting that species at similar trophic levels, as defined here by δ^{15}N, may compete for food resources despite differences in δ^{13}C values.

In our study, we demonstrate that stable isotopes, particularly nitrogen, can be used to define trophic relationships among molluscs and examine the roles of resource partitioning and competition in structuring soil/litter mollusc communities. Isotopic ratios provide evidence of food partitioning among mollusc species and indicate that these species occupy distinct trophic niches, suggesting that partitioning of food resources may help explain coexistence. In addition, our study underscores that classification of terrestrial molluscs as general grazers is not appropriate as they occupy a wide trophic breadth that ranges from primary to secondary consumers to predators/scavengers. Since many of the same non-native species examined here have also become established on other Hawaiian and Pacific islands (Cowie 2001, Cowie *et al.* 2008), determining the trophic relationships of these species can have wide-ranging implications for understanding resource utilization and competition among mollusc species and other soil invertebrates throughout the Pacific.

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