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SHORT COMMUNICATION

Where are the decomposers? Uncovering the soil food web of a tropical montane rain forest in southern Ecuador using stable isotopes (^{15}N)

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Trophic relationships among animals, plants and microflora are the basis for the construction of terrestrial and aquatic food webs, but both the structure and dynamics of food webs remain contentious. Examples of issues include how the overall nutrient status of a system affects the number of trophic levels, whether trophic-level omnivory and intraguild predation are rare or important, if different animal species can be aggregated into functional groups according to their taxonomic affiliation, how large numbers of decomposer animal species can coexist and why there are so many parthenogenetic taxa in soil.

The relationship between ecosystem characteristics and the number of trophic levels has been the subject of controversy. It has been proposed that the number of trophic levels increases with productivity and resource availability by increasing population density at higher trophic levels (Persson *et al.* 1992). On the other hand, theoretical considerations suggest that since nutrient-poor systems (such as tropical forests) are species rich, the large number of interactions between species results in more trophic levels (Vander Zanden *et al.* 1999). Indeed, Reagan *et al.* (1996) found evidence for about five trophic levels in a tropical rain forest in Puerto Rico whereas Ponsard & Arditi (2000) and Scheu & Falca (2000) identified only two and three to four trophic levels, respectively, in soil food webs of temperate forests.

The aim of this study was to contribute to our understanding of the soil food web of a tropical montane rain forest and to estimate the number of trophic levels in that system by analysing natural variations in stable isotope ratios (^{15}N/^{14}N). Stable isotope signatures of animals have been shown to be a powerful tool in evaluating the trophic structure of animal communities (Minagawa & Wada 1984, Ponsard & Arditi 2000, Post 2002, Scheu & Falca 2000, Schneider *et al.* 2004, Vanderklift & Ponsard 2003, Vander Zanden *et al.* 1999, Wada *et al.* 1991). Empirical evidence indicates that animal tissues are more enriched in ^{15}N than their food source (DeNiro & Epstein 1981) by a constant 3.4 δ units per trophic level (Post 2002).

In soil a large number of saprophagous animal taxa co-exist despite the homogeneity of the habitat, and despite the lack of direct co-evolutionary interactions between decomposers and their resources (Anderson 1975, Maraun *et al.* 2003). It has been suggested recently that several species of putative litter-feeding oribatid mites are not primary decomposers but mainly feed on fungi or are predatory or necrophagous (Schneider *et al.* 2004), and similar results have been obtained for collembolans (Chahartaghi *et al.* 2005). This suggests that litter-feeding decomposer animals are less diverse than previously assumed. To prove this hypothesis we investigated the affiliation of tropical soil animal taxa with the principal trophic groups, i.e. phycophages, saprophages, mycophages and predators.

The study site is part of the Reserva Biológia San Francisco (3°58′S, 79°5′W), located in Zamora-Chinchipe province, near the city of Loja in southern Ecuador. The reserve is situated in the easternmost montane chain (Cordillera del Consuelo) of the Southern Ecuadorian Andes at the northern border of the Podocarpus National Park at 1850 m asl. The region is covered with mostly undisturbed montane rain forest. Melastomataceae are the most abundant plants in this region (Homeier *et al.* 2002). The climate is semi-humid with 8–10 humid months, the average annual

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precipitation is 2031 mm and the average annual temperature is 15.7 °C (P. Emck, unpubl. data). The soil types are mainly Aquic and Oxaquic Dystropepts (Schrumpf *et al.* 2001), the organic soil layer is thick and the pH ranges between 4 and 4.5 (Wilcke *et al.* 2002).

In March 2003 ten replicates of L/F litter material (*Graffenrieda emarginata* (Ruiz & Pav.), Melastomataceae) from about 1 m^2 each were sampled. In addition, three replicates of a mixture of L/F litter material of different species of Melastomataceae were sampled. Both litter materials were transferred to the laboratory, where animals were extracted from the *Graffenrieda emarginata* litter material using a modified high-gradient extractor (Kempson *et al.* 1963), then transferred to 70% ethanol and identified to different taxonomic levels. Voucher specimen are deposited at the University of Darmstadt, Department of Zoology. Storage in ethanol does not significantly affect the $^{15}N/^{14}N$ signature of arthropods (Fabian 1998). For analysis of $^{15}N/^{14}N$ ratios animals were placed into tin capsules and dried at 70 °C. After 48 h the samples were weighed and stored in a desiccator until analysed. Replicates of species or higher taxa were analysed if possible. Each sample consisted of pooled individuals (1–150 individuals) to obtain sufficient material for ^{15}N analysis. In addition, litter material from the Melastomataceae mixture and from the *Graffenrieda emarginata* litter (three replicates) was analysed. Samples were dried (70 °C), milled, weighed in tin capsules and stored in a desiccator until analysed.

The $^{15}N/^{14}N$ ratios of animals and litter material were determined by a coupled system of an elemental analyser (NA 1500, Carlo Erba, Milan) and a mass spectrometer (MAT 251, Finnigan). The system is computer controlled allowing on-line measurement of ^{15}N. Stable isotope abundance is expressed using the δ notation with $\delta^{15}N$ (‰) $= (R_{sample} - R_{standard})/R_{standard} \times 1000$. R_{sample} and $R_{standard}$ represent the $^{15}N/^{14}N$ ratios of the sample and standard, respectively. For ^{15}N, atmospheric N_2 served as the primary standard and acetanilide (C_8H_9NO, Merck, Darmstadt) for internal calibration. The mean standard deviation of samples of 10–200 μg N, the range of the samples measured, is 0.2‰ (Reineking *et al.* 1993).

To allow a grouping of the animals in different trophic levels we set the baseline similar to the study of Schneider *et al.* (2004). From that and other studies (Vanderklift & Ponsard 2003) it appears that primary decomposer animals, feeding on litter material, are not enriched in ^{15}N by 3.4 delta units per trophic level as are other consumers. Therefore, the signatures of the primary decomposers were assumed to vary around those of their resource (± 1.7‰).

The $\delta^{15}N$ signature of the litter material (L/F layer) of the mixture of different Melastomataceae trees was − 1.23 (SD = 0.16). The $\delta^{15}N$ signature of pure *Graffenrieda emarginata* litter material was very similar (− 1.15; SD = 0.13). We used the *Graffenrieda emarginata* litter as the baseline for the food web because all animals used for ^{15}N-analysis were extracted from that litter material.

$\delta^{15}N$ signatures of the animals ranged between − 1.33 and 8.16 (Figure 1) forming a gradient of about 9.5 δ units. The taxa were ascribed to four trophic groups spanning over 3.4 δ units each. Only two species had low $\delta^{15}N$ signatures similar to those of *G. emarginata*, *Rostrozetes ovulum* Sellnick (Oribatida) and Diplopoda sp. 1; they were ascribed to the primary decomposer group, i.e. species that feed mainly on litter material that is little colonized by micro-organisms. The $\delta^{15}N$ signatures of the secondary decomposers (animals that feed mainly on fungi but may also ingest litter material), which include a number of oribatid mites and one isopod (*Ischioscia andina* Vandel) ranged between 1.53 (Diplopoda sp. 2) and 4 (*Beckiella arcta* Perez-Inigo & Baggio, Oribatida). $\delta^{15}N$ signatures of adults and juveniles of the oribatid mite *Hermannobates monstruosus* Hammer were similar (1.53 and 1.80, respectively). The $\delta^{15}N$ signatures of the first group of predators ranged between 4.00 and 6.66 and comprised staphylinid and pselaphid beetles, some oribatid mites and Mesostigmata (Gamasina and Uropodina). A second group of predators included four taxa with $\delta^{15}N$ signatures > 7.5, mainly Mesostigmata (Gamasina and Uropodina) but also Trombidiidae (Prostigmata).

This is the first investigation of the soil food web of a tropical montane rain forest using stable isotopes (^{15}N). The results suggest that the number of trophic levels exceeds neither that in temperate soil systems (Ponsard & Arditi 2000, Scheu & Falca 2000) nor that in aquatic or terrestrial tropical lowland systems.

The number of trophic levels in terrestrial and aquatic food webs is subject of intense discussion. It has been speculated that the number of trophic levels in food webs is correlated with either productivity or habitat complexity (Persson *et al.* 1992), with low-productivity systems having fewer trophic levels (Havens 1991). However, the number of trophic levels ascribed to terrestrial food webs may not reflect the actual situation due to the low resolution of trophic species (Martinez 1991). Using stable isotopes (^{15}N) the number of trophic levels in terrestrial food webs in tropical and temperate systems has been shown to be rather similar, usually 3–4 levels (this study, Scheu & Falca 2000). This suggests that productivity and energy flow do not significantly affect the number of trophic levels. Presumably, the number of trophic levels is limited due to the low energy-use efficiency of consumers in detrital systems rather than by productivity.

A surprising result of this study was that the number of primary decomposers (= animals that feed mainly

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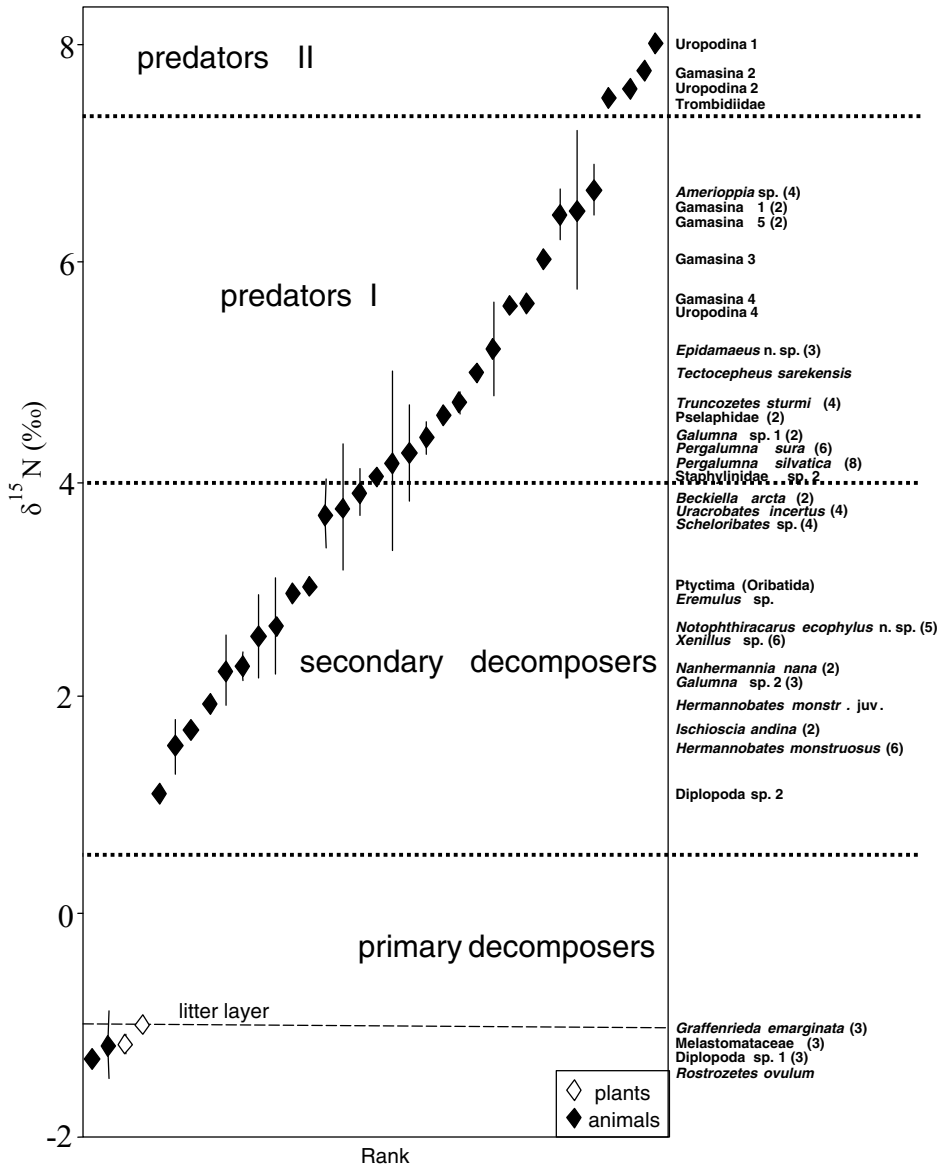

Figure 1. Variation of δ^{15}N signatures of soil animals from *Graffenrieda emarginata* leaf litter from a tropical montane rain forest in southern Ecuador. Single measurements (without SD) and means of 2–8 (numbers shown in parentheses) replicates with SD. Species were ordered according to increasing δ^{15}N values. All species identified to genus or species level are oribatid mites except *Ischioscia andina* (Isopoda).

on litter) appears to be low. Only one oribatid mite (*Rostrozetes ovulum*) and one diplopod had ^{15}N signatures close to that of litter suggesting that they function as primary decomposers. Empirical evidence from other studies also suggests that primary decomposers are not or only slightly enriched in ^{15}N compared with the resource they feed on (Scheu & Falca 2000, Vanderklift & Ponsard 2003). The reason for this low enrichment is unknown but may be due to the fact that primary decomposers are limited by the availability of nitrogen (cf. Yoneyama *et al.* 1997). Considering that the organic layer of the study site is thick (mean of 16 cm, Wilcke *et al.* 2002) low numbers of primary decomposer species are puzzling. Possibly, the quality of the litter is low (Cornejo *et al.* 1994). Low litter quality is also indicated by low microbial biomass and strong nutrient limitation of the soil microflora at the study site (J. Illig, S. Scheu, M. Maraun, unpubl. data). Also, low pH at the study site may be responsible for the low number of primary decomposers. However, this does not apply to decomposer mesofauna (especially oribatid mites) since they are not sensitive to low pH (Maraun & Scheu 2000).

A number of oribatid mite species, one diplopod and one isopod species (*Ischioscia andina*) were ascribed to

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secondary decomposers, i.e. animals that mainly feed on fungi and bacteria but also consume litter to access the micro-organisms. Especially the grouping of Ptyctima, *Hermannobates monstruosus* and *Xenillus* sp. is consistent with earlier observations that their juveniles feed inside decomposing litter material with fungi being probably their main food (Hansen 1999).

A surprising high number of oribatid mite species (seven) was ascribed to predators/necrophages. Oribatid mites generally have been assumed to be primary or secondary decomposers, however, the study of Schneider *et al.* (2004) documented that oribatid mites cannot be aggregated in one or two trophic groups but rather occupy four. This is supported by the present study with similar taxa being grouped to similar trophic groups as in the study of Schneider *et al.* (2004), e.g. Oppiidae and Galumnidae were grouped to predators/necrophages in both studies. The food of these mites is unknown, but most likely they feed at least in part on nematodes. The density and size of nematodes at the study site is high (L. Ruess, unpubl. data) and certain oribatid mite species have been shown to feed on nematodes (Muraoka & Ishibashi 1976, Rockett 1980). This first group of predators also includes some of the typical predators of forest ecosystems, such as staphylinid and pselaphid beetles and mesostigmate mites (Uropodina, Gamasina). Pselaphid beetles feed on oribatid mites but also use other arthropods as prey (Park 1947), whereas the prey of most Mesostigmata is mainly nematodes and collembolans (Walter & Proctor 1999).

Four taxa (two uropodid mites, one gamasid mite and one prostigmate mite species) were ascribed to second-level predators. Their high ^{15}N signatures indicate that they feed on other predators, i.e. for gamasid mites it is known that they feed on other predatory mites and some Prostigmata are known to feed on eggs of soil invertebrates (Walter 1988). Interestingly, no evidence for phycophagous species was found in this study. Phycophagous species are characterized by very low ^{15}N signatures (cf. Schneider *et al.* 2004). This is surprising since epiphyllous algae and lichens are probably an abundant food on the surface of the litter.

The grouping of the animals into functional groups has to be considered with care. There is no objective way to adjust the lines that separate trophic groups. We used the knowledge on the ecology of the species, data on ^{15}N signatures of oribatid mites from temperate forests and evidence from other studies to group the animals into different feeding guilds. There is evidence that litter and fungal feeders do not have $\delta^{15}N$ signatures that are uniformly 3.4 δ units higher than their food (Kohzu *et al.* 1999, Scheu & Folger 2004). However, it seems likely that the trophic position and the number of trophic levels are properly reflected by the ^{15}N signatures as has been shown in a number of studies (Post 2002, Schmidt *et al.* 2003, Vanderklift & Ponsard 2003).

Results of this study suggest that oribatid mite species occupy distinct trophic niches as has been shown for temperate forests (Schneider *et al.* 2004). $\delta^{15}N$ signatures of oribatid mites ranged over 9 δ units indicating that they feed on very different resources. The different trophic niches of oribatid mite species help in explaining the high diversity of decomposer invertebrates in forest ecosystems. However, the enigma of soil animal species diversity (Anderson 1975, Maraun *et al.* 2003) is misleading in part since it applies to the high diversity of primary decomposers which are confronted with rather uniform dead resources. Results of the present study and other recent studies (Chahartaghi *et al.* 2005, Schneider *et al.* 2004) suggest that species-rich decomposer taxa, such as oribatid mites and collembolans, do not consist mainly of species which primarily feed on dead organic matter, rather most species appear to feed on fungi and other prey, including other invertebrates, i.e. in fact they are predators.

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