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Application of stable isotopes and lipid analysis to understand trophic interactions in springtails

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Abstract. The combination of nitrogen and carbon isotope ratios with fatty acids (FA) analysis is a useful tool for food web studies in cryptic belowground systems. The study investigates trophic niche diferentiation of Collembola communities from the litter of two Romanian beech forests and determines the origin of posible food sources consumed by springtails using fatty acid technique. $\delta^{15}N$ signatures of Collembola taxa in the investigated food web spanned over 10δ units, which implies a wide range in food sources used. Assuming a shift in ^{15}N of 3‰ per trophic level, the results indicate a range of three to four trophic guilds: phytophages, primary decomposers, secondary decomposers and carnivores. The lipid composition of their potential food resources was assessed in order to gain insight into food web linkages from the forests. Among the 19 fatty acids detected in the PLFA fraction, nine were ascribed as bacterial, one derived from fungi, 2 as plant and 6 occurred in various organism groups, and had ''other'' origin. Most species were either plant litter or fungal feeders, as indicated by high proportions of oleic (18:1w9) or linoleic (18:2w6) acid, but we can say that Collembola feed unselectively on a wide variety of food materials.

Keywords: Collembola, nitrogen and carbon stable isotopes, feeding guilds, fatty acids, phospholipids.

Introduction

Analysis of the natural variation in stable isotope ratios in Collembola tissue has been shown to be a powerful tool in evaluating feeding guilds (Scheu & Falcă, 2000, Chahartaghi et al. 2005, Hishi et al. 2007, Ladygina et al. 2008, Pollierer et al. 2009, Okuzaki et al. 2009, Oelbermann & Scheu 2010, Semenina & Tiunov 2011, Huang et al. 2012). $\delta^{15}N$ can be used as an indicator of trophic levels of organisms in food chains, and $\delta^{13}C$ can be used to indicate relative contributions to the diet of different potential primary carbon sources in a trophic network (Pereira et al. 2010). Animal tissues are more enriched in ^{15}N than their food resources by a constant value of 3 δ units per trophic level (Minagawa & Wada, 1984). Trophic levels usually are assumed to consist of animal guilds feeding on similar resources (detritivores, carnivores) with the distance of adjacent levels being equivalent to that between a consumer and its resource. Consumers feeding on living animal tissue are aggregated to carnivores and those feeding on living plant material to phytophages. Detritivores feed on detrital materials originating from all trophic levels of the food web, i.e. plant and animal tissues, bacteria, algae and fungi (Oelbermann & Scheu 2010).

The combination of nitrogen and carbon isotope ratios with fatty acids (FA) analysis is a useful tool for food web studies in cryptic belowground systems (Chamberlain et al. 2005, Haubert et al. 2009). The use of lipid signature biomarker analysis for tracing trophic relationships in soil food webs is still developing. Lipids constitute a significant part of the total carbon flux through soil food webs, and they can be applied to investigate belowground trophic interactions (Ruess et al. 2007). Specific FAs can serve as *biomarkers* to assess feeding strategies of the soil fauna (Chamberlain et al. 2005, Haubert et al. 2009). Despite these recent advances, consumer–resource relationships in soil are still poorly understood (Ruess & Chamberlain 2010). Generally, soil detritivores are regarded as food generalists with a low degree in nutritional specialisation (Scheu & Setälä 2002). Studies on feeding strategies in Collembola concluded that the majority of euedaphic and hemiedaphic species feed unselectively on a wide variety of food materials. Most of them are mainly regarded as saprophageous or microphytophageous, but more likely, are food generalists (Hopkin 1997). Depending on the resources available, they ingest bacteria, fungi, algae, mosses, plant litter, or other soil animals, such as protozoa, nematodes, rotifers, and enchytraeids (Rusek 1998). Edaphic species are considered to be primarily fungivorous (Hopkin 1997, Rusek 1998). Additionally, some collembolan species such as *Heteromurus nitidus* preferentially feed on nema-

todes rather than soil algae when offered a choice (Fiera 2014).

Collembolans are known to occupy different trophic niches as indicated by stable isotope analysis ($^{15}N/^{14}N$), depending on habitat and availability of resources (Chahartaghi et al. 2005), but the exact nature of trophic niches is still unknown.

The present study investigates trophic niche diferentiation of Collembola species from the litter of two Romanian beech forests and determines the origin of posible trophic resources consumed by Collembola. Therefore, we measured the natural variation of stable isotope ratios ($^{15}N/^{14}N$, $^{13}C/^{12}C$) of 14 species of springtails to evaluate whether Collembola species occupy distinct trophic guilds with respect to food resources. We expected trophic differentiation in springtails species to be low since they are thought to be generalist opportunistic feeders and trophic guild omnivores predominate (Scheu & Setälä 2002).

The objectives of the study were: (i) to determine the origin of potential food resources which could be consumed by Collembola; (ii) to indicate the feeding strategies of Collembola as revealed by their stable isotope ratio ($^{15}N/^{14}N$ and $^{13}C/^{12}C$); (iii) to establish the feeding guilds of springtails communities from forests according to stable isotope ratios.

Materials and Methods

Site description

Collembola were sampled from two beech forests located in the Bucegi National Park, belonging to the Romanian Southern Carpathians (45°21′N, 25°32′E). The tree layer consisted mainly of beech (*Fagus sylvatica* L.) and *Abies alba* Mill, at 1.050 m a.s.l., with slopes of 25-30°. The average annual temperature is 3.7°C and the average annual precipitation amount is 900 mm.

Site 1. Buşteni (Prahova County) at 860 m *alt.*, 45°24'48"N; 25°32'37"E;

Site 2. 10 km from Sinaia (Prahova County) at 1400 m *alt.*, 45°21'2"N, 25°33'29"E.

Sampling and processing of Collembola and litter

Within an area of about 1000 m² 12 randomly distributed samples of litter and mineral soil (0–5 cm soil depth) were taken in August 2009, using a soil corer of a diameter of 10 cm. Animals were extracted by heat at 45°C for two days. Animals were collected in water and separated under a dissecting microscope. Collembola were stored in ethanol 75% until identification and further processing. Litter samples were frozen -20°C until analysis. For analysis of food resources of Collembola, three litter replicates from each forest studied were taken from samples after extraction of the animals, dried at 60°C for 24 h and ground to powder (Pollierer et al. 2009).

Stable isotope analysis

For C and N stable isotope ratio analysis, the specimens were transferred into tin capsules and dried at 60°C for 48 h. Samples were weighed and stored in a desiccator. Generally, three replicates were analysed; depending on body size samples consisted of 1 and 150 individuals each to obtain sufficient material for ^{15}N and ^{13}C analysis. In addition to animals, appropriate amounts of dried and ground leaf litter, fine roots and soil were weighed into tin capsules. Samples were analysed with a coupled system consisting of an elemental analyser (NA 1500, Carlo Erba, Milan, Italy) and a mass spectrometer (MAT 251, Finnigan, Bremen, Germany). The computer controlled system allowed on-line measurement of stable isotopes (^{13}C and ^{15}N). Their abundance (δX) is expressed using the δ notation with:

$$X(‰) = (R_{sample} - R_{standard})/R_{standard} \times 1000$$

R_{sample} and $R_{standard}$ represent the $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios of samples and standard, respectively. For ^{13}C PD belemnite (PDB) and for ^{15}N atmospheric nitrogen served as the primary standard. Acetanilide (C_8H_9NO, Merck, Darmstadt, Germany) was used for internal calibration.

Analysis of FAs from litter

Lipid extraction and separation. FAs from 2 g of plant tissue (dry weight, three replicates each) were extracted using the procedure described by Bligh & Dyer (1959) and modified by Frostegård et al. (1993) by adding 9,5 ml Bligh/Dyer solvent (chloroform, methanol, citrate buffer (pH 4); 1:2:0.8) for 2 h with repeated shaking. Samples were shaken for 5-10 sec. and centrifuged at 2500 rpm for 10 min. Top phase of the solvent was then removed to new tubes and samples were washed with 2.5 ml Bligh/Dyer solvent, vortex and centrifuged again at 2500 rpm for 10 min. Extraction solvents of both steps were combined and 3.1 ml chloroform and 3.1 ml citrate buffer were added, samples shaken and centrifuged at 2500 rpm for 10 min. The second phase of the solvent was collected in new tubes (10 ml). A double quantity of solvent was used for soil phospholipid (PLFA) extraction from 4 g of soil, following the same procedure as for litter lipid extraction. The chloroform fraction of each sample was dried in the vacuum evaporator (RVC 2-25, CHRIST®, Buddeberg, Mannheim) for 1 hour- at 40°C. Samples were kept at -20°C until analysis.

Lipid fraction. Litter and soil lipid extract were transferred to a silica acid column (0.5 g silicic acid, 3 ml; HF BOND ELUT – SI, Varian, Inc.) and lipids were eluted with 5 ml chloroform (NLFAs), and 5ml methanol (PLFAs). For beech litter NLFA and PLFA fractions were dried in the vacuum evaporator. Each sample was dissolved in 1 ml methanol–toluene solvent (1:1) and 30 µl internal Standard C19:0 (5.77 mg methyl-nondecanoate in 25 ml isooctane) was added. Basic methanolysis of lipids was conducted in 1ml 0.2M methanolic KOH (2.8 g KOH in 250 ml methanol) with incubation for 15 min at 37 de-

grees C. The FA methyl esters (FAMEs) were extracted with 2 ml hexane–chloroform solvent (4:1), 0.3ml 1M acetic acid and 2 ml demonized water. Samples were shaken and centrifuged at 2500 rpm for 10 min. The organic (top) phase was transferred to new tubes and FAMEs were re-extracted with 2 ml hexane–chloroform solvent. Extraction solvents of both steps were combined and dried in the vacuum evaporator for 1 hour at 40 degrees C. Samples were dissolved in 100 µl isooctane and kept at -20°C until analysis (Ruess et al. 2007).

Quantification and identification of fatty acids. FAMEs of litter were analyzed by gas chromatograph (PerkinElmer Corporation, Norwalk, USA), with a flame ionization detector (FID). The FAMEs of all samples were identified on the basis of their retention times and quantified using the Perkin Elmer Software TotalChrom Navigator (PE Nelson-Version 6.3.2, 2008). The separated fatty acid methyl esters of litter were identified using standard qualitative bacterial acid methyl ester mix (Supelco) that ranged from C11 to C24. For each sample the abundance of individual fatty acid methyl esters was expressed in nmol per unit dry litter sample. The fatty acid nomenclature as described by Frostegård et al. (1993) was used. The sum of nine fatty acids (i15:0, a15:0, 15:0, i16:0, 16:1v7, 17:0, i17:0, cy17:0 and cy19:0) was used to represent bacterial PLFAs (bactPLFAs) (Frostegård & Bååth 1996) and 18:2v6 (Frostegard et al., 1993) was used as an indicator of fungal biomass. The ratio of fungPLFA to bactPLFAs was taken to represent the ratio of fungal to bacterial biomass (Frostegård & Bååth 1996).

Statistical analysis. Data on body weight, N and C content, $\delta^{15}N$ and $\delta^{13}C$ were subjected to correlation analysis using Pearson's correlation coefficient. If significant differences were found between the abundance of isotopes at Collembola taxa, pairs of treatments were compared by Student's t test. Statistical analyses were performed using SPSS 10.0 (SPSS Inc., 1999).

Results

The origin of possible trophic resources which could be consumed by Collembola

In total, 28 different FAs were extracted from litter with a chain length from 14 to 24 in phospholipid fractions. Some FAs were detected bellow 1% only in some replicates and are not presented in the paper. Among the 19 fatty acids left, nine were ascribed as bacterial (Frostegård & Bååth 1996), one derived from fungi (Frostegård & Bååth, 1996), 2 as plant (Volkman et al., 1980), and 6 occurred in various organism groups, i.e. had ''other'' origin (Chamberlain et al., 2005, Ruess et al., 2007) (Table 1).

The total amount of PLFAs was similar in both studied forests (1000 nmol/DW in the litter of Sinaia şi 925 nmol/g DW in the litter of Buşteni) (Table 1). The PLFAs of litter from both sites were dominated by linoleic acid (18:2w6) - marker for fungi, the unsaturated oleic (18:1w9c) and palmitic acid (16:0). Litter showed greater mole fraction of identified linoleic acid (18:2w6) at Sinaia (31%) and lower mole fraction of the same fatty acid at Buşteni (24%), so the ratio of fungPLFA to bactPLFAs was higher at Sinaia beech forest. Gram-negative bacteria had a higher proportion of phospholipid fatty acids at Sinaia (8.5 %) than Gram-positive bacteria (5.6 %). At Buşteni both Gram-negative bacteria and Gram-positive bacteria had participated equally in decomposition process (Fig. 1).

Stable isotope signatures of potential food sources and Collembola

Mean $\delta^{13}C$ signatures of beech litter collected in August 2009 were similar in both studied forests with -27.11 ± 0.4‰ in Buşteni and -27.17 ± 0.8‰ in Sinaia. In contrast, mean $\delta^{15}N$ signatures differed between Buşteni (-3.9 ± 0.6‰) and Sinaia (-2.9 ± 0.1‰). A total of 14 different Collembola species were used for isotope analysis (Table 2). The mean biomass of individuals varied markedly from 1.1 to 169 µg ind^{-1}. The smallest species were *Isotomiella minor* and *Parisotoma notabilis* and the biggest *Thaumanura caroli.*

Of the total Collembola species analysed five occurred at both sites (*Folsomia ksenemani, Folsomia quadrioculata, Protaphorura fimata, Isotomiella minor, Parisotoma notabilis*) (Fig. 2). Total N and $\delta^{15}N$ in Collembola had a weak negative relationship (r= -0.267, P= 0.356). Body weight and $\delta^{15}N$ had a positive weak correlation (r= 0.341, P = 0.233).

Comparably body weight and nitrogen content were significantly correlated at 0.01 level (r= -0.746, P= 0.002). Total C and $\delta^{13}C$ showed a good negative correlation (r= -0.513, P= 0.061); body weight and C content had a significant negative correlation (r= -0.768, P=0.001 at 0.01 level); body weight and $\delta^{13}C$ had a significant positive correlation (r= 0.780, P= 0.001).

To allow comparison of ^{15}N and ^{13}C values for species from both forests, the ^{15}N and ^{13}C values of the animals were calibrated to that of the ^{15}N and ^{13}C signature of the litter layer in Buşteni (-27.11‰ ^{13}C, -3.9‰ ^{15}N) and Sinaia (-27.17 ‰^{13}C, -2.9‰ ^{15}N). Mean $\delta^{15}N$ signatures of Collembola species spanned over 10.6 δ units, ranging from -8.4‰ for *Ceratophysella sylvatica* to 2.2‰ for *Kalaphorura paradoxa.* Mean $\delta^{13}C$ signatures of Collembola species spanned over 6.2 δ units from -25.4‰ for *Protaphorura fimata* to -19.2‰ for *Thaumanura caroli.*

Table 1. Proportion (% ± s.d.) and total amount (nmol/gDW ± S.D.) of phospholipid fatty acids (PLFAs) in litter of two Romanian beech forests; with bold - fatty acids which were dominant.

	Fatty acid	Sinaia	Buşteni
		Bacteria	
Frostegård & Bååth 1996	i15:0	2.6 ± 0.9	4.5 ± 0.7
	a15:0	1.6 ± 0.6	2.6 ± 0.5
	15:0	1 ± 0.1	1.3 ± 0.3
	i16:0	1 ± 0,3	2.1 ± 0.6
	16:1w7	4.4 ± 0.8	5.1 ± 1.4
	i 17:0	0.4 ± 0.2	0.6 ± 0.3
	cy 17:0	1.4 ± 0.4	2.5 ± 0.4
	17:0	1 ± 0.1	1 ± 0.5
	cy 19:0	0.7 ± 0.5	0.1 ± 0.2
		Fungi	
Frostegård & Bååth, 1996	18:2w6	31.2 ± 1.9	**24.6 ± 3.8**
		Plants	
Volkman et al. 1980	22:0	0.7 ± 0.2	1 ± 0.1
	24:0	0.4 ± 0.2	0.7 ± 0.2
		Other origin	
(see the rewiew in Ruess et al. 2007)	14:0	0.7 ± 0.2	1.1 ± 0.03
	16:0	**18.3 ± 1.7**	**18.7 ± 2.7**
	18:0	2.3 ± 0.4	0.5 ± 0.8
	17:1	1.3 ± 0.1	0.8 ± 0.4
	18:1w9c; 18:2w6t	**24.7 ± 9.1**	**20 ± 4.8**
	18:1w9t	**6.2 ± 1.7**	**12.2 ± 2.7**
	20:2	0.1 ± 0.02	0.6 ± 1.03
	Total amount (nmol/gDW)	1002.7 ± 329.4	924.4 ± 207.8
	Fungal: bacterial PLFAs	2.25 ± 0.3	1.25 ± 0.18

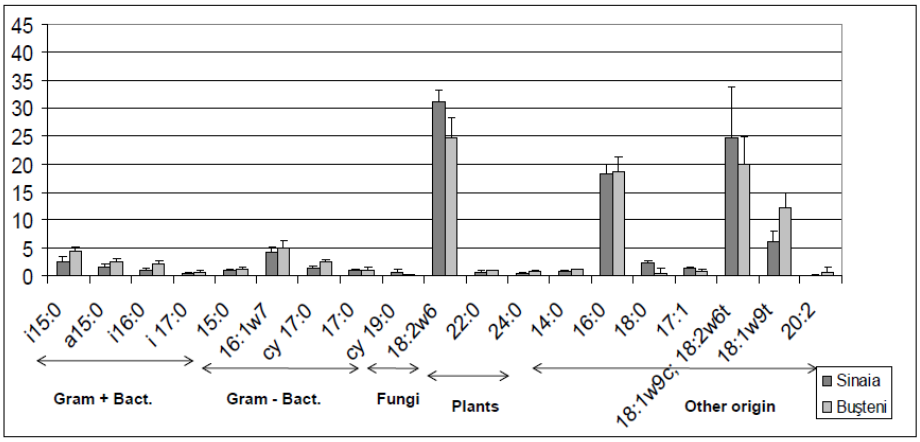
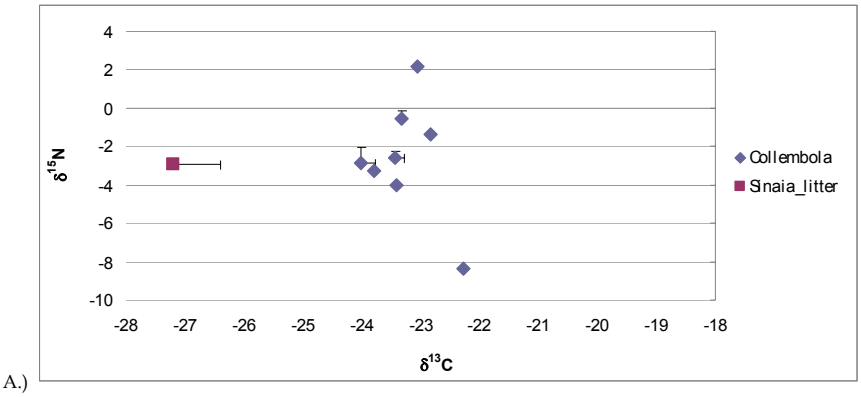


Figure 1. Proportion (% ± s.d.) of phospholipid fatty acids (PLFAs) in litter of the two studied forests (Sinaia and Buşteni) with evidence for the origin of possible trophic resources from natural environment available for springtails.

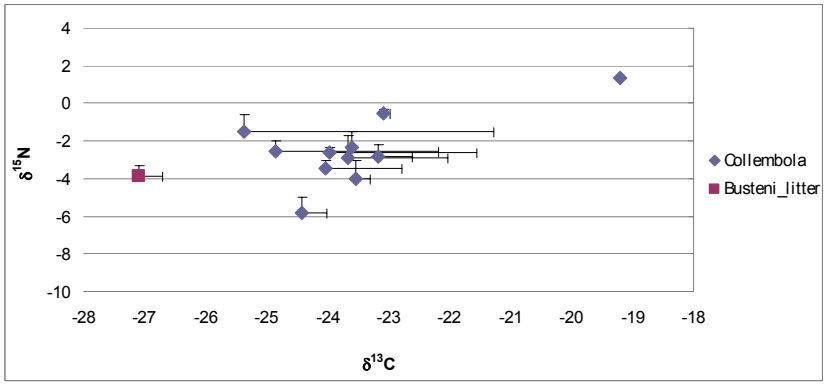
The pattern in $\delta^{15}N$ and $\delta^{13}C$ values of Collembola species differed between the Buşteni and Sinaia (Fig. 2). At Buşteni $\delta^{15}N$ and $\delta^{13}C$ values of the 11 species formed a gradient from *Pogonognathellus flavescens,* the most depleted species ($\delta^{15}N$ = -5.8‰ and $\delta^{13}C$ = -24.4‰) to *Thaumanura caroli,* the most enriched species ($\delta^{15}N$ = 1.4‰ and $\delta^{13}C$ = -19.2‰). In contrast, at Sinaia $\delta^{15}N$ and $\delta^{13}C$ values of the 8 species present at this site varied between *Ceratophysella silvatica* being most depleted ($\delta^{15}N$ =

Table 2. Dry weight per indivdiual (µg ± sd), total amount of nitrogen and carbon (% ± SD), $\delta^{15}N$ and $\delta^{13}C$ (‰ ± sd) in 14 Collembola species. (In the brankets are number of replicates).

Species	Dry weight/ ind (µg)	N content (%)	$\delta^{15}N$ (‰)	C content (%)	$\delta^{13}C$ (‰)
Ceratophysella sylvatica (1)	2.5	14.3	-8.4	38.8	-22.3
Morulina verrucosa (1)	88	5.29	-4	15.7	-23.4
Thaumanura caroli (1)	169	6.3	1.4	22.09	-19.2
Kalaphorura paradoxa (1)	16.1	12.4	2.2	46.3	-23.1
Tetrodontophora bielanensis (2)	16.2 ± 2.6	12.4 ± 5.2	-2.9 ± 1.2	42.7 ± 18.4	-23.7 ± 1.6
Hymenaphorura sp. (3)	8 ± 2.8	11.5 ± 0.9	-2.6 ± 0.3	46.9 ± 3.8	-24.0 ± 2.4
Protaphorura fimata (6)	4.7 ± 0.8	11.8 ± 0.7	-1.0 ± 0.8	41.7 ± 1.2	-24.3 ± 2.8
Isotoma anglicana (2)	3.9 ± 1.1	12.2 ± 0.5	-2.8 ± 0.6	41.5 ± 2.5	-23.2 ± 0.6
Isotomiella minor (4)	1.1 ± 0.1	10.2 ± 0.6	-2.5 ± 0.5	39.3 ± 1.3	-23.5 ± 0.1
Parisotoma notabilis (4)	1.5 ± 0.3	8.4 ± 2.2	-3.8 ± 0.9	30.6 ± 4.2	-23.6 ± 0.2
Folsomia ksenemani (3)	2.8 ± 0.1	12.1 ± 0.4	-0.8 ± 0.5	42.9 ± 0.8	-23.0 ± 0.2
Folsomia quadrioculata (5)	1.8 ± 0.6	11.4 ± 0.8	-2.7 ± 0.6	41.5 ± 1.6	-24.5 ± 2.0
Pogonognathellus flavescens (2)	23.7 ± 7.8	12.4 ± 0.3	-5.8 ± 0.9	40.5 ± 1.4	-24.4 ± 0.4
Seira domestica (3)	2.5 ± 0.2	13.1 ± 3.0	-3.5 ± 0.4	42.7 ± 3.8	-24.0 ± 1.3



A.)



B.)

Figure 2. Variation in $\delta^{15}N$ and $\delta^{13}C$ values (± SD, n= 1-3) of Collembola taxa from two Romanian beech forests. Data are calibrated to the litter layer of each site: A. Sinaia; B. Bușteni.

-8.4‰ and, $\delta^{13}C$ = -22.3‰) and *Kalaphorura paradoxa* being the most enriched ($\delta^{15}N$ = 2.2‰ and $\delta^{13}C$ = -23.1‰). There was no significant difference in $\delta^{15}N$ values between species from both sites as indicated by two-tailed unpaired Student's t test; 95% significant level, α= 0.05 (t= 0.116, df= 17, p=

0.909).

Species which occurred at both forests differed little in $\delta^{15}N$ and $\delta^{13}C$ values (*Parisotoma notabilis, Isotomiella minor* and *Folsomia quadrioculata*) (two-tailed paired Student's t test; t = -0.210, df = 2, p= 0.853 for $\delta^{15}N$; t = -0.770, df = 2, p=0.522 for $\delta^{13}C$), whereas others differed considerably, being more depleted at Buşteni (*Protaphorura fimata*) and at Sinaia (*Folsomia ksenemani*) (t = -0.053, df=1, p= 0.967 for $\delta^{15}N$; t= -1,276, df=1, p= 0,423 for $\delta^{13}C$). There was no significant difference in $\delta^{15}N$ and $\delta^{13}C$ values between species which were common for both sites (two-tailed paired Student's t test; 95% significant level, α= 0.05) (t= - 0,173, df= 4, p= 0,871 for $\delta^{15}N$, t = -1.505, df = 4, p=0.204 for $\delta^{13}C$). Our study determined the origin of possible trophic resources which could be consumed by Collembola which are available in the natural environment. Of the 28 different PLFAs obtained from the litter layer of the forest sites 19 could be ascribed to a predominant resource (presented in the paper). These were derived from bacterial, fungal, plant or had "other" origin according to previous works (see the Table 1). Some of these marker FAs may be found, although in smaller amounts, in the lipids of other diets, too. 53% of the total amount of PLFAs could not be assigned to a specific source. This high proportion is due to the fact that PLFA profiles derived from whole soil communities contain many FAs common to different organisms and, moreover; FA indicators have often not been determined, in particular for the soil fauna (Zelles et al. 1992, Chamberlain et al. 2005). Taking into account the large number of organisms in an environmental sample and the potential multiple sources of FAs, the usage of soil PLFA patterns to characterize potential food sources in Collembola is hampered by this great abundance of ''common'' FAs across taxonomic and functional groups (Ruess et al. 2007).

Discussion

Trophic structure of Collembola communities as revealed by stable isotopic analyses

Food source partitioning along decomposition gradients is one of the main drivers of species diversity and composition in Collembola communities (Hishi et al. 2007). However, in contrast to above-ground systems the role of trophic niche differentiation to account for the diversity of decomposer communities remains obscure. Molecular tehnicques based on PCR, fatty acid analysis, food choice experiments and the examination of gut contents are often used for elucidate the diets of Collembola (Castaño-Meneses et al. 2004, Jørgensen et al. 2005, Ruess et al. 2007, Endlweber et al. 2009). The results of these studies indicate that there is little specialisation in food resources. It may be easy to demonstrate that a particular species of Collembola will show a preference for a specific type of food in the laboratory, but it is more difficult to prove that it will be chosen under field conditions. It is difficult to avoid the conclusion that Collembola feed unselectively and that their gut contents represent a random selection of the components of their environment. Indeed, the opportunistic nature of the feeding behaviour of many species of Collembola may be one reason for their success (Hopkin 1997).

This study is the first to apply stable isotopic analyses to the study of the food webs of Romanian terrestrial ecosystems, although analogous studies have been successfully applied on marine communities on the Black Sea of Romanian coasts (Banaru et al. 2006). Our results show that Collembola species occupy different trophic guilds. $\delta^{15}N$ signatures of Collembola taxa in the investigated food web spanned more than 10 δ units, which is similar to other temperate forests (Scheu & Falcă, 2000, Pollierer et al. 2009). Assuming an enrichment of 3 ‰ per trophic level (Minagawa & Wada 1984), this is equivalent to three to four trophic guilds (Fig. 3, Table 3). However, ascribing decomposer invertebrates to trophic levels by using $^{15}N/^{14}N$ ratios is not straightforward, for example, $\delta^{15}N$ values of decomposer animals may reflect feeding guilds rather than trophic groups (Oelbermann & Scheu 2010).

Carnivores/omnivorous springtails

This group includes two taxa *Thaumanura caroli* and *Kalaphorura paradoxa* which were distinguished by the others feeding guilds by distinctly higher $\delta^{15}N$ values, 1.4‰ and 2.2‰, respectively. Some authors Chahartaghi et al. (2005), Pollierer et al. (2009) found $\delta^{15}N$ to be higher in carnivores than in primary consumers. *Thaumanura caroli* belong to the Neanuridae where several species are reported to be carnivorous and prey on the eggs of other Collembola or ingest tardigrades and rotifers (Hopkin 1997).

In addition to carnivorous capacity, the genera from Neanuridae have piercing-sucking mouthparts, that means they also feed largely on fungal

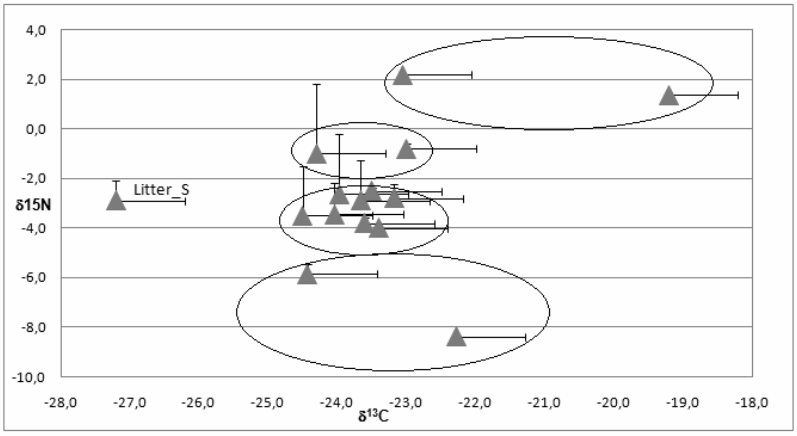


Figure 3. Variation in δ ^{15}N and δ^{13}C values (± SD, n= 1-3) of Collembola taxa from two Romanian beech forests, showing the feeding guilds. Data are calibrated to the litter layer of Sinaia forest.

Table 3. Schematic representation of the trophic structure (feeding guilds 1–4) and possible food resources of springtails from two Romanian beech forests, as indicated by their ^{15}N/^{14}N and ^{13}C/^{12}C ratios.

Species	δ^{13}C	δ^{15}N	Feeding guilds	Trophic resources
Kalaphorura paradoxa	-23.1	2.2	carnivores/omnivores	living and dead animals (nematodes, tardigrades, rotifers) and fungi
Thaumanura caroli	-19.2	1.4		
Protaphorura fimata	-24.3 ± 2.8	-1.0 ± 0.8	secondary decomposers	predominantly feeding on microorganisms, in particular fungi
Folsomia ksenemani	-23 ± 0.2	-0.8 ± 0.5		
Isotomiella minor	-23.5 ± 0.1	-2.5 ± 0.5	primary decomposers	litter/detritus with adhering fungi and bacteria
Folsomia quadrioculata	-24.5 ± 2.0	-3.5 ± 0.4		
Hymenaphorura sp.	-24 ± 2.4	-2.6 ± 0.3		
Isotoma anglicana	-23.2 ± 0.6	-2.81 ± 0.6		
Tetrodontophora bielanensis	-23.7 ± 1.6	-2.9 ± 1.2		
Parisotoma notabilis	-23.6 ± 0.2	-3.8 ± 0.9		
Seira domestica	-24 ± 1.3	-3.4 ± 0.4		
Morulina verrucosa	-23.4	-4		
Pogonognathellus flavescens	-24.4 ± 0.4	-5.8 ± 0.9	phytophages	lichens and algae
Ceratophysella silvatica	-22.3	-8.4		

hyphae juices. Therefore, we assigned these species to the carnivores/omnivores feeding guild. However, the ^{15}N/^{15}N ratios do not separate them distinctly as carnivores, but indicate that they also feed on fungi. Besides, *Kalaphorura paradoxa* belongs to Onychiuridae, living in mountains forests, but the ecology and food preferences of this species remains poorly known. Edaphic Onychiuridae species are considered to be primarily fungivorous (Hopkin 1997, Rusek 1998).

Based on a shift of 3 ‰ per trophic level we distinguished other three feeding guilds: (i) secondary decomposers (fungivores) predominantly feeding on microorganisms, in particular fungi; (ii) primary decomposers (detritivores), feeding on litter/detritus with adhering fungi and bacteria; (iii) phytophages: feeding mainly on plant tissues (algae, lichens, mosses and high plants).

<u>*Secondary decomposers or fungivores*</u>

Secondary decomposers consisted of two species with δ^{15}N values spanning from -1.5‰ to -0.5‰, and d^{13}C values spanning from -25.4‰ to -23.3‰. Previously, *Protaphorura fimata* has been assigned to different feeding guilds. It was found that this species lives on plant resources, presumably fine roots or root hairs and it is phytophagous in the presence of plants but being able to switch to fungal/litter diet if plants are absent (Endlweber et al. 2009). However, if both resources are present, Collembola preferentially feed on roots, thereby incorporating both nitrogen and carbon from plants. This suggests that in the rhizosphere of plants euedaphic Collembola species such as *P. fimata*

and potentially a variety of other species, being almost uniformly classified as decomposers, and in fact predominantly incorporate root resources by directly feeding on fine roots and/or root hairs (Endlweber et al. 2009). Collembola may eat fungus hyphae and spores and could change their food requirements (pollen, algae, plant tissues) throughout the year. *F. quadrioculata,* assigned as primary decomposers, had $\delta^{15}N$ values ranging between -2.9‰ and -2.6‰, whereas *F. ksenemani* had values between -1.4‰ and -0.5‰, when assuming a difference of ^{13}C and $\delta^{15}N$ between trophic levels (Minagawa & Wada 1984), which suggests *F. ksenemani* to be at a higher trophic position than *F. quadrioculata*. The switch of *F. ksenemani* to a different food resource could be due to indirect interactions in the food web as the two collembolan species were positioned on different trophic positions, according to different $\delta^{15}N$ values.

Primary decomposers or detritivores

We assign the following species *Morulina verrucosa, Seira domestica, Parisotoma notabilis, Tetrodontophora bielanensis, Folsomia quadrioculata, Isotoma anglicana, Hymenaphorura* sp., *Isotomiella minor* to this group. These Collembola with $\delta^{15}N$ and $\delta^{13}C$ values from -4‰ to -2.4‰ and -23.4‰ to -23.6‰, respectivly, are near or similar to the signature of the L/F layer. This suggested to function as primary decomposers, which mechanically degrade and shred dead organic matter, including litter, feeding on it, and the adhering fungi and bacteria. *Tetrodontophora bielanensis,* feed on a wider spectrum of fungi during the year according to fungus availability in soil, but they do not feed on bacteria, actinomycetes and other groups of soil microorganisms (Matic & Koledin 1985). Some species skeletonize the leaves just between the veins, while other species eat partly degraded pieces of organic matter or excrements of other soil fauna (Rusek 1998). In a small area of Scots pine litter (Ponge 1991) found that *Parisotoma notabilis* and *Isotomiella minor* seemed to be mainly coprophagous. Few years later, *I. minor and Folsomia quadrioculata* was observed to have nearly exclusively holorganic humus as gut content, probably derived from holorganic faeces found in the immediate environment (Ponge 2000). A smaller content of fungal material was also noticed in the last species. He showed that food resources are vertically distributed and there is a good correlation between the gut contents of springtails and the composition of their immediate environment.

Comparable $\delta^{15}N$ and $\delta^{13}C$ values for *Folsomia quadrioculata* and *Isotomiella minor* were observed by Chahartaghi et al. (2005) in three German forests, who similarly assigned these species as primary decomposers. In contrast, $\delta^{15}N$ and $\delta^{13}C$ values for *Parisotoma notabilis* measured in our study were not in line with the results of Chahartaghi et al. (2005); they included this species in the secondary decomposer group.

Collembola have their own enzymes (amylase, laminarinase, xylanase, lichenase, cellulase complex and trehalase) to utilise diferent constituents of organic matter as food. The presence of trehalase activity indicates that *Folsomia quadrioculata* has the ability to digest the cell contents but not cell walls of lichens and microbes, especially of fungi (Berg et al. 2004). Analysis of the enzymatic capabilities in other two species of Collembola (*Seira domestica* and *Parisotoma notabilis*) showed that chitinase activity was very high compared to trehalase and cellulase activity. Chitin is a major component of fungal cell walls and more chitinase than trehalase and cellulase activity suggests these species as fungivorous grazers, but also they have the ability to digest algae and plant materials (Berg et al. 2004).

Phytophages

Ceratophysella silvatica was the most depleted in ^{15}N and ^{13}C with -8.4‰ and -22.3‰, respectivly, suggesting algae and lichens as major food resources: $\delta^{15}N$= -10.4‰ (algae) and $\delta^{15}N$= -12.8‰ (lichens) (Chahartaghi et al. 2005). This species lives in litter of mountain forest and has strong chewing mouthparts with a molar plate, which is adapted for feeding on the solid tissue of lichens or algae. *Pogonognathellus flavescens* is hemiedaphic species or epigeic and may feed on mosses ($\delta^{15}N$: -6.5‰ to -6.2‰) or plant derived tissues ($\delta^{15}N$: -6.7‰ to –5.8‰) (Chahartaghi et al. 2005). Previous studies on izotope analysis (Scheu & Falcă 2000, Chahartaghi et al. 2005) assigned *Pogonognathellus flavescens* as as primary decomposers, which is in contrast with our observed isotope ratios.

Collembola are plastic in their diet choice, which implies that changes in carbon turnover rates, might well be due to diet shifts of the present decomposer community rather than by changes in species composition as indicated by Krab et al. 2013.

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