Stable isotopes of amino acids indicate that soil decomposer microarthropods predominantly feed on saprotrophic fungi

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Abstract. Soil microarthropods are essential for nutrient cycling in forest ecosystems as they are integral components of decomposer food webs. They channel carbon and nutrients from leaf litter and roots to higher trophic levels; however, knowledge on the relative importance of different channels and on their variation with forest type is lacking. Although the importance of root-derived inputs for sustaining soil food webs is increasingly recognized, the pathways by which they are channeled to higher trophic levels are little understood. For the channeling, ectomycorrhizal fungi may play a significant role, but until now methods allowing to separate the contribution of ectomycorrhizal and saprotrophic fungi to the nutrition of soil animal communities are lacking. Using dual analysis of 15N and 13C in amino acids (AAs), we investigated trophic positions and basal resources of two major groups of soil microarthropods, Collembola and Oribatida, in beech and spruce forests in Germany. By applying a 13C fingerprinting approach and Bayesian mixing models, we separated in a first step the relative contribution of bacteria, fungi, and plants to the nutrition of soil microarthropods. As fungi were identified as the major food source, in a second step we attempted to separate the contribution of ectomycorrhizal vs. saprotrophic fungi. For the first time, we provide direct evidence that soil microarthropods mainly rely on saprotrophic fungi, whereas ectomycorrhizal fungi are consumed by only few species. While trophic niches of Collembola and Oribatida species generally varied little between beech and spruce forests, plant detritus as basal resource of soil microarthropods was somewhat more important in beech forests, whereas in spruce forests microbial resources dominated. Overall, the dual analysis of carbon and nitrogen in AAs provided insight into food web structure of soil microarthropods in unprecedented detail, and for the first time allowed to estimate the relative importance of mycorrhizal and saprotrophic fungi for soil food web nutrition, a long-standing riddle in soil food web ecology. The technique provides the perspective for a comprehensive understanding of the trophic structure and energy channeling in soil food webs.

Key words: amino acid 13C fingerprinting; amino acid CSIA; Collembola; ectomycorrhizal fungi; energy channels; forest type; fungal feeding; Oribatida; soil food web; soil fungi; soil mesofauna; trophic position.

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INTRODUCTION

Together with soil microbes, microarthropod decomposers in forest soils are responsible for the major share of leaf litter decomposition, nutrient cycling, and carbon sequestration. They are linked to energy channels originating directly from plants or from bacteria and fungi utilizing plant-derived resources (Moore and Hunt 1988, Moore et al. 2005). The relative importance of these different energy channels may shift with land-use intensity (Moore 1994, Bardgett et al.
It is a long-standing riddle whether food sources and therefore trophic niches of soil animals are conserved across different habitats and to which extend soil animals can adapt and change their diet depending on available food resources (Chen et al. 2017, Potapov et al. 2019a). Both Collembola and Oribatida species occupy a gradient of trophic niches spanning several trophic levels according to bulk isotope analyses (Schneider et al. 2004, Chahartaghi et al. 2005, Maraun et al. 2011, Potapov et al. 2016a). In feeding studies, both Collembola and Oribatida can survive, grow, and reproduce on a variety of food sources (Ruess et al. 2005, Endlweber et al. 2009, Heethoff and Scheu 2016), and Oribatida therefore have been termed “choisy generalists” (Schneider and Maraun 2005). In natural habitats, trophic niches of Collembola and Oribatida are assumed to be rather conserved among species (Schneider et al. 2004, Chahartaghi et al. 2005, Ferlian et al. 2015, Potapov et al. 2016a), but may also shift, for example, in different cropping systems (Li et al. 2020) and forest types (Krause et al. 2019, Maraun et al. 2020). In part, contrasting findings may be attributed to the use of different methods; while bulk stable isotope analyses mainly identify trophic positions of species, they only provide limited insight into the basal resource utilized, and fatty acid analyses only allow characterizing relative differences in basal resources (Ruess et al. 2005, Ruess and Chamberlain 2010, Kühn et al. 2019). In addition, storage compounds for carbon such as lipids may derive from different food components/sources than proteins containing the majority of tissue nitrogen.

Compound-specific isotopic analysis of nitrogen and carbon in amino acids (AAs) of soil animals allows to simultaneously analyze trophic position and basal resource, thereby delineating trophic niches of animals in unprecedented detail (Pollierer et al. 2019). Calculation of trophic position using δ¹⁵N signatures of the trophic and source AAs glutamic acid (Glu) and phenylalanine (Phe), respectively (Chikaraishi et al. 2009, 2014, Steffan et al. 2013), is more precise than using bulk stable isotope measurements, since the baseline isotopic signature is conserved in Phe, thereby circumventing the problem of baseline identification (Whiteman et al. 2019). Using δ¹³C signatures of essential AAs (eAAs), a
fingerprinting approach allows to distinguish between fungi, bacteria, and plant litter as basal resources of soil animals (Larsen et al. 2009, 2013). Mixing models can be used to calculate relative contributions of eAAs from these resources to animal eAAs (Larsen et al. 2016, Potapov et al. 2019b). A recent study suggests that $^{13}$C fingerprints may even be used to distinguish between ectomycorrhizal and saprotrophic fungi and their consumers, disclosing the riddle to distinguish feeding of soil animals on these two fungal functional groups (Pollierer et al. 2020).

Here, we analyzed compound-specific AA carbon and nitrogen isotope signatures (CSIA) of Collembola and Oribatida as abundant microarthropod decomposers in old-growth beech and coniferous forests. We compared trophic structure, that is, trophic position and basal resource, between the same species inhabiting the two forest types. When inferring basal resources, we not only separated fungi, bacteria, and plant litter, but also attempted to separate feeding on different fungal functional groups, that is, saprotrophic vs. ectomycorrhizal fungi. We hypothesized that (1) Collembola and Oribatida species occupy distinct trophic positions, which are similar in beech and spruce forests, but that (2) the utilization of basal resources differs between these two forest types, with the bacterial energy channel being more important in spruce forests and the plant/fungal energy channels being more important in beech forests, and that (3) saprotrophic fungi are more important for the nutrition of microarthropod decomposers than ectomycorrhizal fungi.

**Materials and Methods**

**Study site and sampling**

Samples were taken in the Hainich forest in the framework of the Biodiversity Exploratories, a long-term research project in Germany (www.biodiversity-exploraties.de). The Hainich is a hilly region in central Germany (285–550 m a.s.l.); parent rock is mainly Triassic limestone. Luvisol is the main soil type; Cambisols and Stagnosols also occur. Soil pH is on average 4.59 ± 0.67 (mean ± SD), annual precipitation is 500–800 mm, and the mean annual temperature is 6.5°–8.0°C (Fischer et al. 2010). About 1 m$^2$ of leaf litter was collected in four ~700-yr-old coniferous forest sites (Norway spruce, *Picea abies*), and in four ~120- to 150-yr-old deciduous forest sites (European beech, *Fagus sylvatica*) in September/October 2016. Animals were extracted by heat (Kempson et al. 1963) and stored in 70% ethanol until determination using Weigmann (2006) and Hopkin (2007). For analyses, we chose taxa of Collembola and Oribatida with the highest abundance/biomass. For a list of analyzed species, numbers of pooled individuals and replicates, see Appendix S1: Table S1.

**Extraction and derivatization of amino acids**

For CSIA, dried samples were transferred to Pyrex culture tubes and flushed with N$_2$, sealed and hydrolyzed in 6 N HCl at 110°C in a heating block for 20 h (Larsen et al. 2013). After hydrolysis, lipophilic compounds were removed by adding n-hexane/DCM to the Pyrex tubes that were flushed briefly with N$_2$ and sealed before they were vortexed for 30 s. The aqueous phase was then filtered through a Pasteur pipette lined with glass wool that had been pretreated at 450°C. All samples were transferred into 4 mL dram vials before evaporating the samples to dryness under a stream of N$_2$ at 110°C in a heating block for 30 min. The samples were then stored at −18°C. To volatize the AAs, we followed the derivatization procedure of Corr et al. (2007), methylating the dried samples with acidified methanol and subsequently acetylating them with a mixture of acetic anhydride, trimethylaniline, and acetone (N-acetyl methyl ester derivatives). To reduce oxidation of AAs during derivatization, reaction vials were flushed and sealed with N$_2$ prior to the methylation and acetylation reactions.

**Compound-specific measurements of amino acids**

AA derivatives were injected into a Thermo Trace GC coupled via a GP interface to a Delta Plus mass spectrometer (Thermo, Bremen, Germany). The GC was equipped with an Agilent J&W VF-35ms GC column (30 m × 0.32 mm × 1.00 µm). The temperature program started with 80°C held for 1 min, increased by 20°C per minute to 135°C, then by 5°C per minute to 160°C and held for 3 min, then increased again by 8°C per minute to 300°C and held for 3 min. The injection temperature was 280°C, and helium was used as carrier gas. The flow rate of helium...
was 2 mL/min. All samples were analyzed in triplicate. A standard mixture of pure AAs with known δ\(^{13}\)C and δ\(^{15}\)N values was derivatized and analyzed along with batches of 10–20 samples to check for reproducibility of derivatization. In addition, two of these derivatized standard mixtures were measured in triplicate after every 5 samples to check for reproducibility of GC-C-IRMS measurements. For means and standard deviations of derivatized standard mixtures, see Appendix S1: Table S2. Nor-leucine was added to each sample after hydrolysis but before derivatization, thereby serving as internal reference to check for possible errors during derivatization. The N isotope composition of AAs in samples was expressed relative to atmospheric N by normalizing measured values (vs. reference gas) using scales derived from known δ\(^{15}\)N values of the standard mixtures mentioned above. In detail, we plotted the measured AA δ\(^{15}\)N values (vs. reference gas) of standard mixtures against the known AA δ\(^{15}\)N values (vs. atmospheric N). As the slope of the resulting linear regression line was not equal to zero, we used the equation derived from the regression line for linear normalization of AA δ\(^{15}\)N values in samples to atmospheric N. The C isotopic composition was corrected for carbon added during derivatization following O’Brien et al. (2002) and expressed relative to Vienna PD Belemnite.

Statistical analyses

AA-based trophic position of Collembola and Oribatida (TP\(_{\text{CSIA}}\)) was calculated using the following equation (Chikaraishi et al. 2009, 2014):

\[
\text{TP}_{\text{CSIA}} = 1 + \left( \frac{\delta^{15}\text{N}_{\text{Glu}} - \delta^{15}\text{N}_{\text{Phe}} + \beta}{\text{TDF}_{\text{Glu-Phe}}} \right)
\] (1)

The equation takes into account the difference in δ\(^{15}\)N values between Glu and Phe in the primary producer (β), which is +8.4 ± 1.6‰ for terrestrial C\(_3\) plants (Chikaraishi et al. 2010, 2014). We adopted a value of 7.6 ± 1.2‰ for the trophic discrimination factor (TDF\(_{\text{Glu-Phe}}\)) as proposed by Chikaraishi et al. (2009, 2014) to calculate TP\(_{\text{CSIA}}\). When calculating TP\(_{\text{CSIA}}\), we propagated variance by using the differential solution of eq. (1) according to Blum et al. (2013) and Ohiokuchi et al. (2017). Variations in trophic positions of Collembola and Oribatida species with forest type were analyzed separately using a two-way ANOVA, with species and forest type as independent variables and trophic position as dependent variable.

To predict the biosynthetic origin of AAs in Collembola and Oribatida (the fingerprinting approach), we performed linear discriminant function analysis (LDA, R package MASS; Venables and Ripley 2002) with δ\(^{13}\)C values of the eAAs isoleucine (Ile), leucine (Leu), methionine (Met), phenylalanine (Phe), threonine (Thr), and valine (Val). In a first step, we used training data including fungi, bacteria, and plants obtained from Larsen et al. (2013, 2016) as classifier variables to identify the contribution of these resource groups to the diet of Collembola and Oribatida. As fungi were identified as the main food source of most species, in a second step we performed a LDA with training data from Pollierer et al. (2020) to separate Collembola and Oribatida consuming saprotrophic and ectomycorrhizal fungi. For calculating the probability of group membership of the classifier samples, we used a leave-one-out cross-validation approach.

Relative contributions of eAAs from diets to consumers were estimated in the software FRUITS version 2.1 (Fernandes et al. 2014) with δ\(^{13}\)C values normalized to the mean value of all the eAAs. FRUITS is executed with BUGS, which is a software package for performing Bayesian inference Using Gibbs Sampling that includes an expert system for determining an appropriate Markov chain Monte Carlo scheme based on the Gibbs sampling.

RESULTS

Trophic position as indicated by δ\(^{15}\)N values of glutamic acid and phenylalanine

Trophic position of Collembola differed significantly between species with differences depending on forest type (ANOVA species × forest type 3.15 = 4.7, \(P = 0.017\); Fig. 1). In general, TP of Collembola was higher in spruce than beech forests; differences were most pronounced in Protaphorura armata and Parisotoma notabilis, with TP
Collembola species were all close to TP 3 (feeding on microbial or animal biomass), with the exception of \textit{P. armata} which had a significantly lower TP in beech forests (2.86 ± 0.04; one-sample \textit{t}-test, \( P = 0.006 \)), whereas it was significantly higher than 3 in spruce forests (3.44 ± 0.01; one-sample \textit{t}-test, \( P = 0.001 \)). Oribatida species differed significantly in TP irrespective of forest type (ANOVA species \( F_{12,31} = 40.3, P < 0.0001 \)), with TP ranging from below 2 (phytophages) to ~3. TP of \textit{S. magnus} was significantly lower than 2 (1.50 ± 0.17; one-sample \textit{t}-test, \( P = 0.003 \)). \textit{Phthiracarus} sp., \textit{Eupelops plicatus}, \textit{Eupelops hirtus}, and \textit{Euzetes globulus} occupied a mean TP between 2.33 and 2.88, whereas TP of the other oribatid mite species was close to 3. TP of Oribatida also differed significantly between forest types (ANOVA forest type \( F_{1,31} = 8.3, P = 0.007 \)), mainly owing to low values of \textit{S. magnus} and \textit{Phthiracarus} sp. in beech forests. For average TPs of Collembola and Oribatida also refer to Appendix S1: Table S3.

**Basal resources as indicated by δ\(^{13}\)C values of essential amino acids**

Linear discriminant function analysis (LDA; for group means, coefficients, and proportion of trace see Appendix S1: Table S4) placed Collembola close to fungi (Fig. 2A). However, the position varied between species with, for example, \textit{P. armata} and \textit{P. notabilis} from beech forests being shifted toward plant resources, whereas \textit{Folsomia quadrioculata} from both forest types was shifted toward bacterial resources. In fact, the relative contribution of basal resources as inferred from linear discriminants of LDA differed significantly between Collembola species (MANOVA species \( F_{3,13} = 2.7, P = 0.04 \)), but did not differ...
significantly between Collembola from beech and spruce forests (MANOVA forest type $F_{1,13} = 1.8$, $P = 0.21$). We estimated the relative contribution of bacterial-, plant-, and fungal-derived eAA to the diet of consumers using the $\delta^{13}C$ signatures of the three most informative eAA according to LDA (Leu, Thr, Val). In line with the results from the fingerprinting analysis, the fungal contribution to eAAs was highest in Collembola species, with the exception of $P$. armata, which had equally high contributions (44%) from plant and fungal eAAs in beech forests (Fig. 3). In the other species, the proportion of fungal eAAs ranged between 52% and 65%, whereas the contribution of plant eAAs ranged from 16% to 37%. $Folsomia quadrioculata$ had the
The highest proportion of bacterial eAAs among the studied Collembola species (21 ± 12% in both beech and spruce forests); in the other species, the contribution of bacterial eAAs ranged between 11% and 14%, but was slightly higher in Lepidocyrtus sp. in spruce forests (19 ± 13%). For estimated relative contributions of plants, fungi, and bacteria to Collembola nutrition, see also Appendix S1: Table S5a.

Most Oribatida species were positioned within the fungal training data in LDA (Fig. 2B). Only Phthiracarus sp. and S. magnus from beech forests and Platynothrus peltifer from both beech and spruce forests were shifted toward plant resources. As for Collembola, the relative contribution of basal resources as inferred from linear discriminants of LDA differed significantly between Oribatida species (MANOVA species $F_{11,33} = 2.3$, $P = 0.005$), but did not differ significantly between Oribatida from beech and spruce forests (MANOVA forest type $F_{1,33} = 0.1$, $P = 0.92$). As estimated in a mixing model from the $\delta^{13}C$ signatures of Leu, Thr, and Val, fungi contributed the majority of eAAs to most species of Oribatida, with highest fungal contributions to eAAs of Damaeus onustus in both forest types (almost 80%) and to eAAs of E. plicatus in spruce (>90%; Fig. 4). In some Oribatida species, however, the contribution of plant-derived eAAs as estimated by FRUITS was equally high or higher than that of fungal eAAs. In particular, this was true for Phthiracarus sp., Damaeus clavipes and

Fig. 3. Proportional contribution ± SD of the essential amino acids leucine, threonine, and valine (the three most informative essential amino acids according to the linear discriminant function analysis) from bacteria, fungi, and plants to the nutrition of Collembola species (F. quadri.—Folsomia quadrioculata, Lepido. sp.—Lepidocyrtus sp., P. notabi.—Parasotoma notabilis, and P. armata—Protaphorura armata) based on the Bayesian mixing model FRUITS. Bacterial contributions decrease from left to right, whereas plant contributions increase from left to right.

Fig. 4. Proportional contribution ± SD of the essential amino acids leucine, threonine, and valine (the three most informative essential amino acids according to the linear discriminant function analysis) from bacteria, fungi, and plants to the nutrition of oribatid mite species (Phthir.—Phthiracarus sp., D. cla.—Damaeus clavipes, P. pelti.—Platynothrus peltifer, S. mag.—Steganacarus magnus, D. grac.—Damaeus gracilipes, A. coleopt.—Achipteria coleoptera, E. glo.—Euzetes globulus, E. hirtus—Eupelops hirtus, D. ripa.—Damaeus riparius, O. qua.—Oribatella quadricornuta, E. plic.—Eupelops plicatus, and D. onustus—Damaeus onustus) based on the Bayesian mixing model FRUITS. Mean fungal contributions in species increase from left to right.
Damaeus gracilipes from beech forests, and for P. peltifer and E. hirtus from spruce forests, where plant-derived eAAs ranged from 46 ± 24% to 55 ± 24%. The contribution of bacterial eAAs in general was lower than that of plant and fungal eAAs. In most Oribatida species, the bacterial contribution was slightly higher in spruce forests compared to beech forests, with the exception of E. plicatus, where it was higher in beech forests. The highest estimated bacterial contribution (>20%) occurred in Achipteria coleoptrata, Phthira-carus sp. and Oribatella quadricornuta from spruce forests, and in S. magnus from beech forests. For estimated relative contributions of plants, fungi, and bacteria to Oribatida nutrition also see Appendix S1: Table S5b.

Separation of saprotrophic and mycorrhizal contributions to essential amino acids of soil microarthropods

As all investigated soil animals had significant fungal contributions to their eAA, we employed a training data set consisting of eAA \(^{13}\)C signatures of leaf litter, saprotrophic, and ectomycorrhizal fungi (Pollierer et al. 2020), to separate contributions of the two fungal functional groups. In the LDA with these potential resources (for group means, coefficients, and proportion of trace, see Appendix S1: Table S6), the majority of Collembola and Oribatida grouped close to saprotrophic fungi (Fig. 5); however, some species also had mycorrhizal contributions to their eAAs. In Collembola, the utilization of different fungal functional groups and leaf litter as inferred from linear discriminants of LDA differed significantly between species (MANOVA species \(F_{3,13} = 4.4, P = 0.02\)), but not between forest types (MANOVA forest type \(F_{1,13} = 0.1, P = 0.76\), with the interaction also being non-significant (MANOVA species × forest type \(F_{3,13} = 2.0, P = 0.17\)). Two out of three samples of \(F. quadriculata\) from beech forests were classified as feeding on ectomycorrhizal fungi; in addition, two samples of Lepidocyrtus sp. (one from beech and spruce forests) and one sample of \(P. armata\) from beech forests were also classified as ectomycorrhizal fungi feeders. As in Collembola, in Oribatida the utilization of different fungal functional groups and leaf litter as inferred from linear discriminants of LDA differed significantly between Oribatida species (MANOVA species \(F_{11,32} = 3.5, P < 0.001\)), but not between forest types (MANOVA forest type \(F_{1,32} = 1.6, P = 0.22\), with the interaction also being non-significant (MANOVA species × forest type \(F_{0,32} = 1.3, P = 0.25\). The species classified as feeding (in part) on ectomycorrhizal fungi were \(A. coleoptrata\), \(D. onustus\), and \(Damaeus riparius\) in both forest types, and the genus \(Eupelops\) in beech forests. Notably, only very few samples (one \(Lepidocyrtus\) sp. from beech forests out of 21 Collembola samples, and one sample each of \(O. quadricornuta\), \(P. peltifer\) and \(S. magnus\) out of 51 in Oribatida samples) were classified as having leaf litter as basal resource (for LDA classification of Collembola and Oribatida species, see Appendix S1: Table S7).

Discussion

Using compound-specific isotope values of N and C in amino acids to identify trophic position and basal resource of Collembola and Oribatida provided detailed insights into the trophic structure of these important microarthropod decomposers. Here, we compared species living in beech and spruce forests to investigate whether forest type influences trophic niches and energy fluxes from resources to microarthropod consumers. In general, the results document the major importance of fungal resources for the nutrition of soil microarthropod decomposers. For the first time, our results provide direct evidence that major soil microarthropod taxa predominantly rely on saprotrophic fungi, whereas ectomycorrhizal fungi play a minor role for most species. Irrespective of forest type, Collembola and Oribatida species occupied distinct trophic niches; however, direct energy fluxes from plants to soil animals were slightly more pronounced in beech forests, whereas in spruce forests microbial resources dominated.

Trophic position and basal resources of Collembola in beech and spruce forests

The trophic position of Collembola was generally close to 3, suggesting that they mainly rely on microbial diets. However, the trophic position differed significantly between Collembola species, with these differences depending on the forest type, beech, and spruce, they were sampled from. As microbial diets lead to trophic level
inflation of decomposer animals in brown food webs (Steffan et al. 2017), the generally higher trophic position of Collembola in spruce compared to beech forests may be attributed to a higher contribution of microbial resources to the diet in the former. By contrast, plant-derived resources presumably are more important for the nutrition of Collembola in beech forests. The difference between beech and spruce forests was most pronounced in *P. armata* and *P. notabilis*, with the former being more than half a trophic position higher in spruce compared to beech forests. Indeed, the fingerprinting approach and the mixing model suggested higher plant contributions to eAAs in these species in beech than spruce forests. *P. armata* has been suggested to

Fig. 5. Linear discriminant analysis (LDA) for separating (A) Collembola and (B) Oribatida species feeding on different fungal functional groups (saprotrophic vs. ectomycorrhizal fungi). Data points represent mean scores of Oribatida species (± SD) in beech (gray symbols) and spruce (black symbols) forests, and mean scores of resource groups (Litter, Saprotrophs, and Ectomycorrhiza). The same species from different forest types are connected by dotted lines. For full species names, refer to Appendix S1: Table S1. Training data including litter (green), saprotrophic (pink), and ectomycorrhizal (purple) fungi were used as endmembers to predict biosynthetic origin of essential amino acids in consumers. LDA is based on eAAs isoleucine, leucine, phenylalanine, threonine, valine, and methionine. Ellipses around resource groups are 75% confidence intervals.
rely on root-derived carbon (Scheunemann et al. 2015, Pausch et al. 2016, Potapov et al. 2016b), which may be attributed to feeding on roots and on fungi receiving root-derived carbon (Endlweber et al. 2009, Ferlian et al. 2015). Proportions of these resources may shift depending on availability/palatability, for example, in different cropping systems (Li et al. 2020); this presumably is also the case in beech compared to spruce forests, as in the other forest-derived resources are particularly important (Cesarz et al. 2013, Zieger et al. 2017). Bulk stable isotope values of enriched these species in bulk 13C and 15N suggest that this species mainly consumes fungi, potentially mixed with litter material (Charhartagh et al. 2005, Potapov et al. 2016a). Different trophic positions in beech and spruce forests may also indicate different proportions of litter and fungi depending on litter quality. Trophic positions of F. quadrioculata and Lepidocyrtus sp. were similar in beech and spruce forests; F. quadrioculata from both forest types and Lepidocyrtus sp. from spruce forests had the highest contributions of bacterial eAAs among Collembola (~20%), suggesting that they are more closely linked to the bacterial energy channel. Low enrichment of these species in bulk 13C and 15N compared to leaf litter (Ferlian et al. 2015) and high incorporation of root-derived carbon (Li et al. 2020) suggest that they may in part rely on root-associated bacteria. Interestingly, these two species (along with one sample of P. armata) were the only Collembola species classified (in part) as feeding on ectomycorrhizal fungi in LDA, corroborating the assumption that they feed on microorganisms closely associated with root-derived carbon.

**Trophic position and basal resources of Oribatida in beech and spruce forests**

In Oribatida, the range of trophic positions was broader compared to Collembola, with a gradient from plant feeders/primary decomposers to fungal feeders/secondary decomposers. This is in line with findings of Schneider et al. (2004) who showed that Oribatida communities can span a gradient of three to four trophic levels using bulk isotope measurements. Trophic positions of Oribatida differed significantly between species, and this was independent of forest type, suggesting that the trophic niche of Oribatida species is relatively constant even among different forest types. Fingerprinting suggested that all Oribatida species studied in large rely on fungal resources. Some species were shifted toward plant resources, for instance the ptyctinomous S. magnus and Phthiracarus sp., which are endophagous in leaves and needles as juveniles, as well as P. peltifer, especially in spruce forests. These species have bulk isotope δ15N values close to litter and have been classified as primary decomposers (Schneider et al. 2004, Pollierer et al. 2009) or as herbivorous grazers due to their gut-enzymatic capabilities (Siepel and Ruiter-Dijkman 1993), suggesting that they feed on litter and associated fungi. The mixing model suggested that D. clavipes and D. gracilipes from beech forests also belong to this group, although their trophic position as calculated from AA δ15N values was close to 3 indicating that their diet predominantly comprises microorganisms. Several Oribatida species had high fungal contributions and intermediate contributions from plant eAA, suggesting that they mainly feed on fungi and to some extent also on leaf litter. This group included A. coleoptrata, E. globulus, E. hirtus, and D. riparius. Finally, O. quadricornuta, E. picatus, and D. onustus had very high fungal eAA contributions, suggesting that they are almost pure fungal feeders. Interestingly, D. onustus, which had the highest fungal contributions to their eAAs, was classified as feeding on ectomycorrhizal fungi in most cases. Also, A. coleoptrata in many cases was classified as feeding on ectomycorrhizal fungi, in particular in spruce forests. Indeed, in a labeling study the fungal marker fatty acid was labeled via the root channel in A. coleoptrata (Polrier et al. 2012), suggesting that they incorporate root-derived carbon via feeding on ectomycorrhizal fungi. In the other species, only few samples were classified as feeding on ectomycorrhizal fungi, suggesting that most Oribatida species preferentially consume saprotrophic fungi. This may be due to the habitat they colonize, that is, the leaf litter layer where saprotrophic fungi dominate (Klironomos and Kendrick 1996), but also to effective defense mechanisms in ectomycorrhizal fungi (Böllmann et al. 2010). Our findings confirm earlier notions that ectomycorrhizal fungi are little consumed by most decomposer soil animals (Potapov and Tiunov 2015, Bluhm et al. 2019).
The bacterial contribution to eAAs of Oribatida was generally lower than that of plant-derived resources and fungi, suggesting that bacteria play a subordinate role for their nutrition. However, the estimated bacterial contribution was above 20% in *A. coleoptrata*, *O. quadricornuta*, and *Phthiracarus* sp. in spruce forests, and in *S. magnus* in beech forests, suggesting that the bacterial energy channel plays a significant role for some Oribatida species, as also indicated by the analyses of neutral lipid marker fatty acids before (Crotty et al. 2011, Pollierer et al. 2012, Ceszar et al. 2013). In most species, the contribution of bacterial eAAs was higher in spruce than in beech forests, although differences in the use of basal resources between forest types were not significant. A similar study investigating differences in trophic niches of Oribatida between beech and spruce forests using the neutral lipid fatty acid composition also found a shift of many Oribatida species toward the bacterial energy channel in spruce forests (Maraun et al. 2020). They concluded that Oribatida may shift trophic niches according to resource availability and that this trophic plasticity may facilitate coping with changing environmental conditions. However, in our study trophic positions of Oribatida species as inferred from δ¹⁵N values of trophic and source AAs were surprisingly constant even among forest types, suggesting that the variation in the use of basal resources is limited and that the trophic niche of most species is rather constant even if they colonize different forests. Discrepancies between conclusions drawn from fatty acid and AA analyses may also arise from different dietary sources of storage vs. structural compounds.

Calculated trophic positions of Oribatida did not consistently correlate with the relative contribution of plant/microbial eAAs as calculated by mixing models. Potentially, the trophic discrimination factor used to calculate trophic positions varies between Oribatida species; leaf litter feeders may have depleted Phe signatures leading to an overestimation of their trophic position (Pollierer et al. 2019). In addition, different fungal species may occupy different trophic positions depending on whether they utilize organic nitrogen from dead organic matter or inorganic nitrogen from soil (Pollierer et al. 2020) leading to variable trophic positions of their consumers. Another source of bias in estimation may stem from the fact that in the mixing model only ¹³C eAA values of the three most informative eAAs were used, not covering the total variance of ¹³C eAAs. Finally, the metabolism of carbon and nitrogen need not be linked closely as the two elements may originate from different sources leading to differential conclusions on trophic position and basal resources. The dual analysis of nitrogen and carbon in AAs may be used to disentangle this differential utilization of resources in future studies under controlled laboratory conditions.

**Conclusions**

The trophic structure of soil micorarthropods in beech and spruce forests suggests that Collembola and Oribatida in both systems mainly rely on fungi as food source, whereas bacteria and plant leaf litter play subordinate roles for their nutrition. Fingerprinting of ¹³C in eAAs allowed to separate the relative contribution of saprotrophic and mycorrhizal fungi to microarthropod nutrition. The results suggest that most species preferentially feed on saprotrophic fungi, whereas ectomycorrhizal fungi were only used by few species. Trophic niches of species were surprisingly constant among forest types; however, slightly higher trophic positions in spruce compared to beech forests indicate higher microbial contributions to the diet in the former. The dual analysis of nitrogen and carbon in AAs allows to delineate trophic structure and energy channels in soil food webs in unprecedented detail, for the first time providing direct evidence on the differential utilization of saprotrophic vs. ectomycorrhizal fungi by soil animals. It opens the perspective to detect even slight variations in food web structure as caused by different management practices, tree species, and changing environmental conditions.

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**Supporting Information**

Additional Supporting Information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/ecs2.3425/full