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Stable Isotope Trophic Fractionation ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) in Mycophagous Diptera Larvae

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Abstract—The use of isotopic analysis for reconstructing the structure of food webs requires determination of the trophic fractionation of carbon and nitrogen stable isotopes ($\Delta^{13}C$ and $\Delta^{15}N$). Fungi and mycophagous animals play a key role in soil communities, but there are very limited field data on the degree of isotope fractionation in animals that feed on fungi. We studied the bulk isotopic composition of mycetophagous Diptera larvae inhabiting fruit bodies of saprotrophic and mycorrhizal macromycetes, as well as larva feeding on parasitic rust fungi. Trophic enrichment in ^{13}C and ^{15}N was at the minimum (0.0 and 0.9‰, respectively) in the larvae of gall midges *Mycodiplosis* sp. feeding on rust fungi (Pucciniales). In the larvae of dipterans inhabiting fruiting bodies of saprotrophic and mycorrhizal macromycetes, the $\Delta^{13}C$ and $\Delta^{15}N$ values averaged 0.9 and 3.4‰, respectively. This corresponds to the values usually observed in grazing food chains. The accumulation of ^{15}N was more pronounced in the larvae that fed on saprotrophic fungi, but no clear relationship was found between the degree of trophic fractionation and the taxonomic affiliation of animals or fungi. As suggested by our data and the analysis of published studies, the variations in the trophic fractionation in mycophages are strong, but they are not likely to impede the identification of the "mycorrhizal" and "saprotrophic" energy channels in the soil food webs.

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INTRODUCTION

Measurement of the ratio of stable isotopes of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) is widely used as a tool for studying the trophic structure of soil communities (Scheu and Falca, 2000; Tiunov, 2007). The use of isotopic analysis to reconstruct the structure of food webs is based on two main principles: the isotopic composition of consumers reflects the isotopic composition of their diet integrated over time and the consumers are slightly enriched in heavy nitrogen (^{15}N) and to a lesser extent in heavy carbon (^{13}C) compared to their food. The latter phenomenon is called "trophic fractionation" (usually denoted by Δ) and averages 2–4‰ for nitrogen ($\Delta^{15}N$) and 0.5–1.0‰ for carbon ($\Delta^{13}C$) in grazing food chains (Post, 2002; Martínez del Rio et al., 2009). At basal levels of the detrital food chains, the accumulation of a heavy carbon may be higher, while the accumulation heavy nitrogen may be lower than in grazing food chains (Pollierer et al., 2009; Potapov et al., 2013, 2019).

The success of the reconstruction of specific trophic relationships, as well as the accuracy of estimating key quantitative parameters of the entire food web, such as the average length of food chains and the number of trophic levels, depends on the correct determination of the trophic fractionation of nitrogen and carbon isotopes. When reconstructing the structure of detrital (soil) food webs "bacterial" and "fungal" energy channels are usually recognized (Wardle, 2002; Goncharov and Tiunov, 2013). There is no doubt that mycophagous invertebrates (collembolans, oribatid mites, nematodes, many insects) constitute one of the most important functional groups of soil organisms. To decipher the structure of food webs in forest soils, trophic links of soil mycophages with saprotrophic and mycorrhizal fungi should be distinguished. In the first case, we are talking about detrital food chains sensu stricto, since saprotrophic fungi assimilate the energy of dead organic matter. In the case of feeding on mycorrhizal fungi, energy (carbon) comes mainly from the living plant roots. The significance of this energy flow remains a matter of debate (Pollierer et al., 2007; Goncharov et al., 2016; Potapov and Tiunov, 2016).

The identification of the trophic relationships of soil animals with mycorrhizal and saprotrophic fungi is facilitated by a consistent difference in the isotopic composition of their fruit bodies and, probably, vege-

tative mycelium (Gebauer and Taylor, 1999; Mayor et al., 2009). However, we do not have reliable data on the nature of trophic fractionation of nitrogen and carbon isotopes when feeding on mycorrhizal and saprotrophic fungi. The degree of trophic fractionation in mycophages was evaluated in a number of laboratory experiments, primarily using collembolans, and showed significant variability in specific food–consumer pairs (Haubert et al., 2005; Staaden et al., 2010; Semenina and Tiunov, 2011). However, laboratory models, as a rule, use cultures of saprotrophic micromycetes on artificial substrates and may not completely reflect the natural situation.

Strangely enough, the data on the isotopic composition of specialized mycetophagous larvae of Diptera inhabiting the fruit bodies of macromycetes, were not used to assess the trophic fractionation of isotopes in mycophages. We know of only one study (Remen, 2010), in which the difference in the isotopic composition of Anthomyiidae and Mycetophilidae larvae and their fungal substrates was measured. Another possible model for assessing the extent of trophic fractionation in specialized mycetophages may be the Cecidomyiidae larvae that consume rust fungi.

The goal of this study was to assess the degree of fractionation of carbon and nitrogen isotopes in the trophic chain consisting of fungi and mycetophages under natural conditions.

MATERALS AND METHODS

Collection of fruit bodies of saprotrophic and mycorrhizal fungi, inhibited by mycophages, as well as of plant litter was carried out during the growing season (from May to October 2016) at the Malinki Biogeocenological Station of the Severtsov Institute of Ecology and Evolution RAS (55.4595° N, 37.1794° E). The territory adjacent to the station is covered with over-ripe coniferous 150- to 160-year-old forests dominated by Norway spruce and Scots pine.

Rust fungi and *Mycodiplosis* sp. larvae feeding on them were collected in June and July 2016 on the territory of the Zvenigorod Biological Station of the Moscow State University (55.6996° N, 36.7227° E). Samples of *Puccinia bromina, Coleosporium tussilaginis, Puccinia caricina* and *Melampsora salicina* (sensu lato) rust fungi were collected along with the green leaves of the plants on which they developed (*Pulmonaria officinalis, Tussilago farfara, Urtica dioica, Salix* spp.).

In total, 92 fruit bodies of mycorrhizal fungi, 57 fruit bodies of saprotrophic fungi, and 20 samples of rust parasitic fungi were collected. Mycorrhizal fungi were represented by 11 species from seven genera; saprotrophic fungi by ten species from eight genera; rust fungi by four species from three genera (Table 1).

When processing the fruit bodies of saprotrophic and mycorrhizal fungi, tissue samples were taken separately from the stipe (sterile middle plectenchyma) and in the hymenophore (mid-radius of the cap). However, we did collect pairwise samples of fungal tissues, the material was collected only if mycetophagous larvae were present. Unless otherwise indicated, the isotopic composition of the hymenophore is given for macromycetes in the text. For rust fungi, the sample consisted of scrapings of spores with mycelium. The parts of the fruit bodies intended for isotope analysis were dried at 50°C for three days. Mycetophagous larvae were fixed with 70% ethanol, identified and dried at 50°C.

Dried samples of fungal fruit bodies and litter were ground in a Retsch MM200 ball mill (Retsch GMBH, Germany). Whenever possible, larvae were analyzed individually. The approximate weight of samples for isotope analysis was 30–500 μg of fungal material, 50–550 μg of animals, and 1500 μg of plant material.

The isotopic composition of carbon and nitrogen ($^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios) was measured using a Flash 1112 elemental analyzer and a Thermo Delta V Plus isotope mass spectrometer (Thermo Scientific, United States) at the Joint Usage Center, Institute of Ecology and Evolution. The isotopic composition of nitrogen and carbon was expressed in per mil (δ, ‰) deviations from the international standard (N_2 of the atmosphere and VPDB). The analytical accuracy of determining the isotopic composition was <0.15‰ for both $\delta^{15}N$ and $\delta^{13}C$. Together with the determination of the isotopic composition, the total content of carbon and nitrogen (% N, % C) was determined in all samples.

In order to obtain comparable data from different collection points, the data on the isotopic composition of fungi collected at the Zvenigorod Biological station were corrected:

$$\delta = \delta_{\text{measured}} + (\delta_{\text{Malinki litter}} - \delta_{\text{Zvenigorod plant}}).$$

The average values of $\delta^{13}C$ and $\delta^{15}N$ of fresh litter in the vicinity of the Malinki Biological station averaged −30.1 ± 0.3‰ and −0.5 ± 0.3‰, respectively ($n = 20$).

Trophic fractionation (Δ) was determined as the difference between the isotopic composition of animals and the substrate from which they were extracted:

$$\Delta^{13}C = \delta^{13}C_{\text{mycetophage}} - \delta^{13}C_{\text{substrate}},$$

$$\Delta^{15}N = \delta^{15}N_{\text{mycetophage}} - \delta^{15}N_{\text{substrate}}.$$

Results were analyzed using linear regression (Pearson correlation) and analysis of variance (ANOVA). Checking for the normal distribution and homogeneity of variation did not reveal the need for preliminary data transformation. Comparison of the means was performed using Tukey's honestly significant difference (Tukey HSD), pairwise comparison was conducted using Student's *t*-test. The calculations were performed in the STATISTICA 8 package. The isotopic composition of individual groups of fungi and mycetophages in the plots were compared using stan-

Table 1. Isotopic composition ($\delta^{13}C$ and SD; $\delta^{15}N$ and SD) of mycorrhizal, saprotrophic, and parasitic fungi (hymenophores or sporangia)

Fungal species	*n*	$\delta^{13}C$	SD	$\delta^{15}N$	SD
Mycorrhizal					
Amanita muscaria (L.) Lam.	13	–26.3	0.2	9.1	1.4
A. pantherina (DC.) Krombh.	10	–25	0.4	6.6	2.4
Cortinarius triumphans Fr.	3	–26.1	0.1	6.9	1.1
Gomphidius glutinosus (Schaeff.) Fr.	4	–28	0.8	5.8	2.4
Lactarius flexuosus Pers.	16	–27.4	0.2	7.4	1.2
L. pergamenus (Sw.) Fr.	10	–25.9	0.2	7.1	0.5
Leccinum scabrum (Bull.) Gray	11	–28.3	0.5	8.3	1.6
Russula decolorans (Fr.) Fr.	10	–24.5	0.7	6.3	2.6
R. vesca Fr.	1	–25.9	–	4.2	–
R. virescens (Schaeff.) Fr.	11	–24.8	0.7	4.7	1.8
Tricholoma album (Schaeff.) Quél.	2	–25.1	–	5.6	–
Saprotrophic					
Agaricus arvensis Schaeff.	12	–22.5	0.2	4.4	1.3
Hypholoma fasciculare (Huds.:Fr.) P. Kumm.	3	–24.5	0.8	1.3	1.8
H. lateritium (Schaeff.) P. Kumm.	2	–24.7	0.2	1.8	0.5
Lycoperdon perlatum Pers.	4	–24	0.5	–0.2	0.5
Macrolepiota procera (Scop.) Singer	3	–25.1	0.2	4.2	0.5
Megacollybia platyphylla (Pers.) Kotl. & Pouzar	7	–24.4	1.3	–0.2	2
Mycena pura (Pers.) P. Kumm.	7	–23.9	1	2.1	0.7
Mycena sp.	3	–24.3	0.6	1.5	0.3
Pluteus cervinus (Schäffer:Fr) P. Kumm.	11	–24.7	1	1.9	1.3
Strobilurus esculentus (Wulfen) Singer	5	–24.8	0.2	2.3	0.2
Parasitic					
Puccinia bromine Erikss.	3	–29.3	0.8	1	0.7
P. caricina DC.	6	–29.4	2.3	0.8	0.9
Coleosporium tussilaginis (Pers.) Lév.	4	–28.3	0.2	0.9	0.2
Melampsora salicina (Moug. & Nestl. ex DC.) Desm.	7	–30.2	0.5	0.1	0.4

n is the number of measurements; for Tables 1–3.

dard ellipses that bound the area of the 95% confidence interval (Jackson et al., 2011). Calculations of the area and overlap of ellipses were performed in the SIBER R package (R Core Team, 2014). The mean values ± 1 standard deviation (SD) are presented in the text.

RESULTS

The isotopic composition of the hymenophores of individual types of macromycetes varied quite strongly (Table 1); however, it was consistently different in fungi belonging to different functional groups (Fig. 1). The mean $\delta^{13}C$ values were –25.8 ± 1.4‰ in mycorrhizal, –24.3 ± 0.7‰ in saprotrophic, and –29.4 ± 1.5‰ in rust fungi. Saprotrophic fungi were, on average, more enriched in ^{13}C relative to litter compared to other fungi (HSD; $P < 0.001$). Rust fungi did not differ in their isotopic composition from the plants they parasitized.

The mean $\delta^{15}N$ values were 7.4 ± 2.3‰ in mycorrhizal, 2.1 ± 1.5‰ in saprotrophic, and 0.6 ± 0.7‰ in rust fungi. Mycorrhizal fungi were significantly more ^{15}N-enriched relative to litter compared to other fungi (HSD; $P < 0.05$). The overlap of the Jackson's ellipses was 22.3% for mycorrhizal and saprotrophic fungi and only 0.2% for rust and saprotrophic fungi (Fig. 1).

In saprotrophic fungi, the hymenophore tissue was enriched in ^{13}C and ^{15}N relative to the stipes; the differences were statistically significant for $\delta^{15}N$. In mycorrhizal fungi, the values of $\delta^{13}C$ and $\delta^{15}N$ in hymenophores and stipes did not differ (Fig. 2).

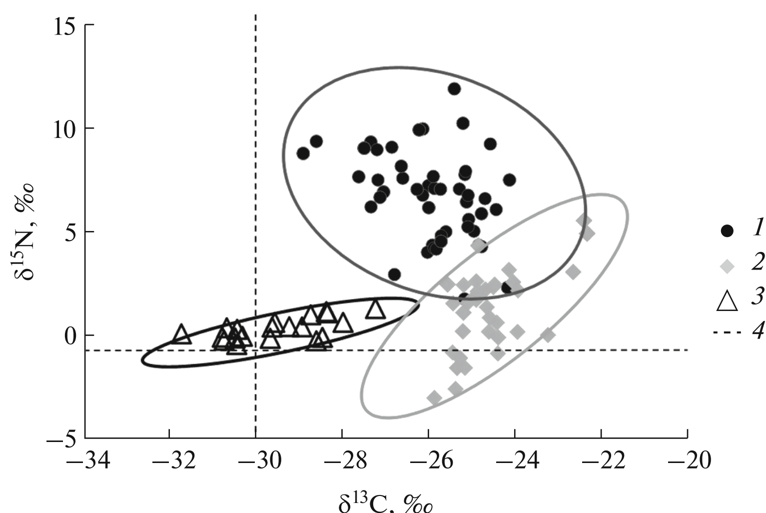

Fig. 1. The isotopic composition ($\delta^{13}C$ and $\delta^{15}N$ values) of (*1*) mycorrhizal, (*2*) saprotrophic, and (*3*) parasitic fungi; (*4*) mean isotopic composition of plant tissues. Each point reflects the results of the analysis of one sample; for higher fungi—hymenophores; for rust fungi—spores and mycelium. Ellipses limit the range of the 95% confidence interval.

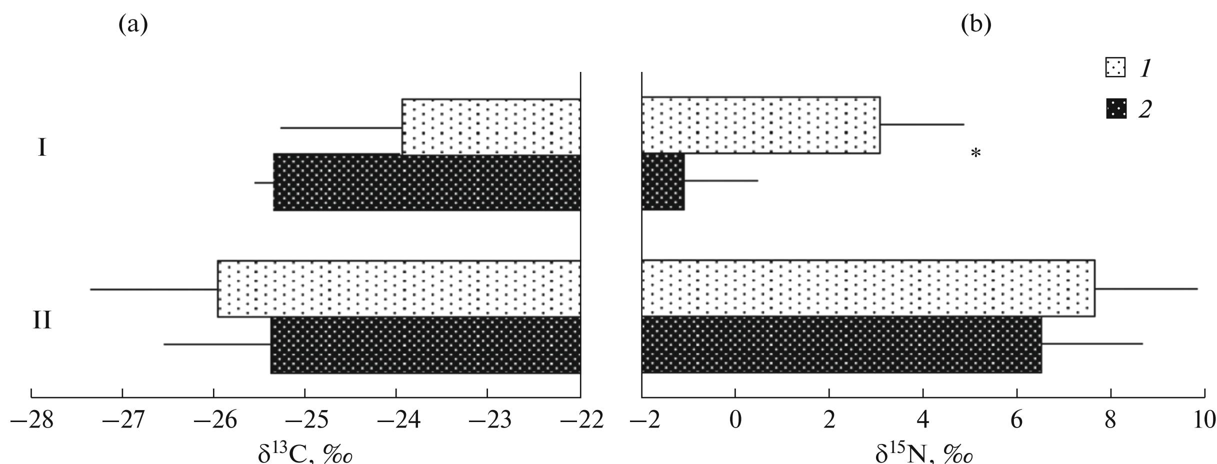

Fig. 2. The isotopic composition ($\delta^{13}C$ (a) and $\delta^{15}N$ (b), mean ± SD) of hymenophores (*1*) and stipes (*2*) of (I) saprotrophic and (II) mycorrhizal fungi. * Means statistically significant differences (HSD test, $P < 0.05$).

The isotopic composition of mycetophagous larvae correlated with the isotopic composition of fungal substrates ($R = 0.79$ for $\delta^{13}C$ and 0.82 for $\delta^{15}N$) and was significantly different in the larvae that fed on fungi of different functional groups ($\delta^{13}C$: $F = 179.4$, $\delta^{15}N$: $F = 97.6$, $P < 0.001$ (Table 2)).

The $\Delta^{13}C$ and $\Delta^{15}N$ values also differed in mycetophages that consumed fungi of different functional groups ($F = 9.8$ and 41.1, respectively, $P < 0.001$). Trophic fractionation was insignificant in larvae of gall midges feeding on rust fungi, $\Delta^{13}C = 0 \pm 1.2$‰ and $\Delta^{15}N = 0.9 \pm 1.0$‰. In different species of dipterans feeding on saprotrophic and mycorrhizal fungi, trophic fractionation varied widely: from −1.4 to 2.5‰ for $\Delta^{13}C$ and from 0.7 to 8.5‰ for $\Delta^{15}N$ (Fig. 3).

The trophic fractionation of nitrogen isotopes was, on average, stronger, and the fractionation of carbon isotopes was weaker in larvae feeding on saprotrophic fungi than in larvae feeding on mycorrhizal fungi. However, the difference was only significant in a few taxa (*Pegomya* sp., *Mycetophila* sp., *Megaselia* sp., *Metalimnobia quadrimaculata,* Table 3). In the entire data set, mean $\Delta^{13}C$ and $\Delta^{15}N$ values in Diptera larvae feeding on macromycetes were 0.9 ± 0.2‰ and 3.4 ± 0.4‰, respectively (each dipteran species feeding either on mycorrhizal or saprotrophic fungi is taken as one replication, $n = 23$).

The isotopic signature of mycophages and the degree of trophic fractionation did not depend on the location (cap or stipe), despite the significant (in the

Table 2. The isotopic composition of the fruit bodies of the fungi belonging to three functional groups and the mycetophages feeding on them

Fungi, mycetophages	$\delta^{13}C$, ‰		$\delta^{15}N$, ‰		*n*
	mean	SD	mean	SD	
Fungi					
mycorrhizal	–25.8	1.4	7.4	2.3	92
saprotrophic	–24.3	0.7	2.1	1.5	57
parasitic	–29.4	1.5	0.6	0.7	20
Mycetophages on fungi					
mycorrhizal	–24.9	1.1	9.7	2.7	92
saprotrophic	–24	1.3	7	3.5	57
parasitic	–29.4	1.3	1.5	1.3	20

The differences in the $\delta^{15}N$ values of the three groups were statistically significant in both fungi and larvae (Tukey HSD, $P < 0.05$).

case of saprotrophic fungi) differences in the isotopic composition of different parts of the fruit bodies. We found no significant correlations between the C/N ratio in the tissues of fungi or mycophages and the $\delta^{15}N$ values of fungi or larvae, or $\Delta^{15}N$ values. The $\delta^{13}C$ values of mushrooms and mycetophages negatively correlated with the C/N ratio in the fruit bodies and in the tissues of mycetophages ($R = -0.610$ and -0.727, respectively, $P < 0.05$). The $\delta^{13}C$ value slightly but significantly decreased with an increase in the C/N value in the tissues of mycetophages ($R = -0.434$, $P < 0.05$).

DISCUSSION

The variability in the degree of trophic fractionation remains "a bottleneck of isotope ecology." Since it is impossible to estimate the $\Delta^{15}N$ value in each food–consumer pair experimentally, the average value (3.4‰ per trophic level) is usually used in the reconstruction of trophic links, although there is no doubt that in fact it varies widely. In particular, in the detrital food webs, there is a very small enrichment of primary decomposers in ^{15}N compared with plant litter, but an unexpectedly large accumulation of ^{13}C (the so-called detrital shift) (Korobushkin et al., 2014; Kudrin et al., 2015; Potapov et al., 2019).

The food of soil saprophages is predominantly saprotrophic microorganisms (Striganova, 1980). Laboratory experiments show that the peculiarities of the isotopic composition of saprophages are primarily determined by the fractionation of isotopes in the plant residue–microorganisms system (Potapov et al., 2013), but this notion needs to be confirmed in field experiments. Obtaining information on the isotopic

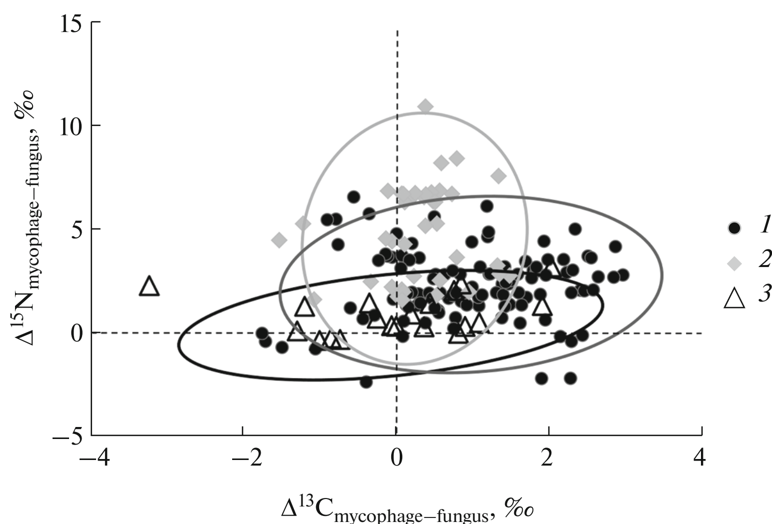

Fig. 3. Trophic fractionation ($\Delta^{13}C$ and $\Delta^{15}N$ values) of mycophages feeding on (*1*) mycorrhizal, (*2*) saprotrophic, and (*3*) parasitic fungi. Each point reflects the results of the analysis of one pair of samples (fungal tissue and mycetophage). Ellipses limit the range of the 95% confidence interval.

Table 3. Trophic fractionation ($\Delta^{13}C$ and $\Delta^{15}N$ values, ‰) of individual taxonomic groups of mycetophages collected in saprotrophic and mycorrhizal fungi; mean and standard deviation (SD)

Diptera larvae	*n*		$\Delta^{13}C$, ‰				$\Delta^{15}N$, ‰			
	mycorrhizal	saprotrophic	mycorrhizal		saprotrophic		mycorrhizal		saprotrophic	
Anthomyiidae										
Anthomyia pluvialis L.	–	4	–	–	0.2	(0.3)	–	–	6.7	(0.2)
Anthomyia sp.	2	–	2.5	(0.0)	–	–	3.7	(0.1)	–	–
Pegomya sp.	10	6	1.6	(1.8)	0.5	(0.2)	1.4	(2.3)	7.2	(0.9)*
Drosophilidae										
Drosophilidae gen. sp.	–	2	–	–	–1.4	(0.2)	–	–	4.9	(0.6)
Scaptomyza sp.	42	8	0.3	(1.0)	0.1	(0.6)	2.4	(1.8)	3.0	(1.8)
Heleomyzidae										
Heleomyzidae gen. sp.	2	–	2.1	(0.2)	–	–	2.9	(0.1)	–	–
Limoniidae										
Metalimnobia quadrimaculata L.	8	3	1.4	(0.5)	0.6	(0.7)	3.7	(1.6)	8.5	(2.2)*
Mycetophilidae										
Mycetophilidae gen. sp.	1	1	0.3	–	1.3	–	1.9	–	3.3	–
Mycetophila fungorum De Geer	14	4	1.2	(0.7)	0.1	(0.1)*	2.1	(1.0)	2.1	(0.5)
Mycetophila sp.	10	2	1.2	(0.6)	1.4	(0.1)	0.7	(1.1)	2.8	(0.1)*
Rymosia sp.	4	–	1.0	(1.1)	–	–	1.8	(1.5)	–	–
Trichonta sp.	4	2	2.0	(1.0)	0.7	(0.2)	3.7	(1.1)	2.7	(1.3)
Phoridae										
Megaselia scalaris Loew	2	–	0.4	(0.1)	–	–	1.3	(0.2)	–	–
Megaselia sp.	7	7	0.4	(0.6)	0.2	(0.2)	2.8	(1.5)	5.6	(1.0)*
Sciaridae										
Sciara sp.	5	–	2.2	(0.7)	–	–	3.0	(0.8)	–	–
Mean	13	10	1.3	(0.8)	0.4	(0.8)	2.4	(1.0)	4.7	(2.2)
Median			1.2		0.4		2.4		4.1	

* Is statistically significant differences between larvae from saprotrophic and mycorrhizal fungi (*t*-test, $P < 0.05$). "–" means a lack of data.

composition of soil microorganisms meets considerable methodological difficulties (Tiunov et al., 2015). However, the fruit bodies of macromycetes and the larvae of Diptera associated with them can be a convenient model for assessing the degree of trophic fractionation in mycophages. This is especially important in connection with the pronounced difference in the isotopic composition of saprotrophic and mycorrhizal fungi. It is well known that the fruit bodies of mycorrhizal fungi are, on average, enriched in ^{15}N and depleted in ^{13}C compared with the fruit bodies of saprotrophic macromycetes (Henn and Chapela, 2001; Mayor et al., 2009). Our data confirm this phenomenon, although the difference in the $\delta^{13}C$ values of fruit bodies of saprotrophic and mycorrhizal fungi was small (Fig. 1). The steady and significant difference between the $\delta^{15}N$ values of saprotrophic and mycorrhizal fungi (~5‰, Table 2) suggests that bulk isotopic analysis allows for a relatively clear separation of "mycorrhizal" and "saprotrophic" flows of matter and energy in soil food webs.

Contrary to expectations, the isotopic composition of soil mycetophages does not give indications of active feeding on mycorrhizal fungi (Kudrin et al., 2015; Potapov and Tiunov, 2016). These data partly contradict the results of experimental studies (Pollierer et al., 2007) and require verification. The absence of a "mycorrhizal signal" could be an artifact explained by the incomplete correspondence of the isotopic composition of fruit bodies and the vegetative mycelium (Wallander et al., 2004), or by the features

of trophic fractionation when feeding on fungi of different functional groups.

Evaluation of the isotopic composition of the fruit bodies of macromycetes is complicated by their chemical and isotopic heterogeneity. As a rule, hymenophores (or caps) are enriched in ^{15}N and ^{13}C compared to sterile tissues (or stipes). This is apparently due to the accumulation of proteinaceous compounds in the generative tissues, which are enriched in ^{13}C and ^{15}N as compared with storing (lipids) and structural (chitin) compounds (Hobbie et al., 2012). We did not set out systematically to investigate variations in the isotopic composition of individual parts of fruit bodies; in our sample, a marked enrichment in ^{13}C and ^{15}N of hymenophores was observed in saprotrophic, but not in mycorrhizal fungi (Fig. 2).

The isotopic composition of insect tissues is also heterogeneous. The detected decrease in δ^{13}C values of larval tissues with an increase in the C/N ratio, i.e., the fat content, is consistent with the well-known phenomenon of the ^{13}C depletion in lipids (Tsurikov et al., 2015). This can also explain the decrease in δ^{13}C with an increase in the C/N ratio in the tissues of the larvae. In addition, we used samples that included the whole body of larvae. The presence of the intestinal contents could have introduced an error in the determination of the isotopic composition of animals. Such a mistake seems to be inevitable when working with small soil saprophages (collembolans, mites, nematodes), and we did not try to avoid it in our model system. The C/N mass ratio was significantly wider in the tissues of fungi (on average, 11.2 ± 0.4) than in the tissues of mycetophages (5.1 ± 0.1). Thus, an increase in the C/N ratio in larval samples could reflect an increased contribution of the intestinal contents to the isotopic signature.

The magnitudes of the trophic fractionation of nitrogen isotopes varied significantly in individual food–consumer pairs. In most cases, the δ^{15}N value was higher in larvae feeding on saprotrophic fungi than in larvae feeding on mycorrhizal fungi (Table 3), but this trend was not always observed. We also failed to find a clear relationship between the magnitude of trophic fractionation and the taxonomic affiliation of animals or fungi. Noteworthy, in our study the δ^{15}N value was on average higher in of Anthomyiidae than in Mycetophilidae, although an inverse relationship was found in another experiment (Remen, 2010).

A strong variation in δ^{15}N and δ^{13}C values within one taxonomic group of mycophages was noted also in other studies, including laboratory experiments (Fig. 4). Our estimates of δ^{15}N and δ^{13}C are approximately in the middle of the range of values obtained earlier. Moreover, the average trophic fractionation of nitrogen (3.4‰) and carbon (0.9‰) in larvae of Diptera feeding on macromycetes is close to the values obtained by averaging the data of many experimental works with different types of consumers, mainly in grazing food chains (Post, 2002; Vanderklift and Ponsard, 2003; Tiunov, 2007).

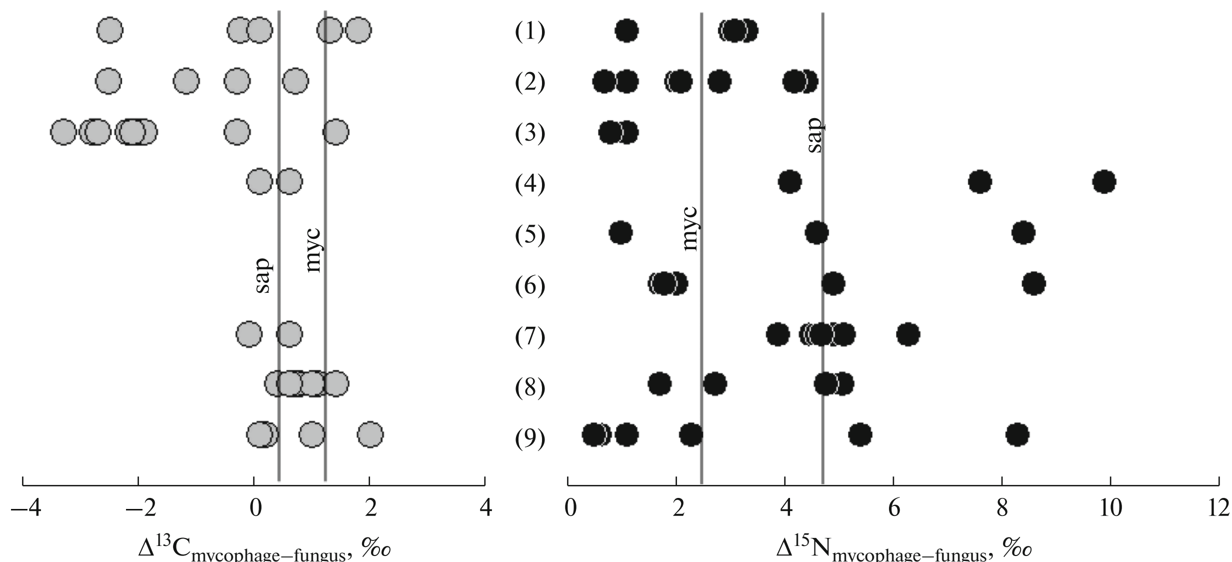

Fig. 4. Trophic fractionation of carbon and nitrogen isotopes (Δ^{13}C and Δ^{15}N) in mycophages (previously published studies). Field data, Diptera larvae: Anthomyiidae (1) and Mycetophilidae (2) on mycorrhizal macromycetes, Mycetophilidae (3) larvae on saprotrophic macromycetes. Collembolans on saprotrophic micromycetes: *Xenylla grisea* (4), *Vertagopus pseudocinereus* (5), *Sinella tenebricosa* (6), *Protaphorura fimata* (7), *Heteromurus nitidus* (8), *Folsomia candida* (9). The vertical lines show the average trophic fractionation by mycetophages on saprotrophic (sap) and mycorrhizal (myc) fungi in our experiments (Table 3). Previously published data (Scheu and Folger, 2004; Haubert et al., 2005; Ruess et al., 2005; Larsen et al., 2009; Remen, 2010; Staaden et al., 2010; Semenina and Tiunov, 2011; Potapov et al., 2013) were used.

The larvae of gall midges of *Mycodiplosis* sp., feeding on parasitic fungi, fall out of the general pattern. In this system, the minimum degree of consumer enrichment with heavy isotopes was noted. Rust fungi almost did not differ in their isotopic composition from the green leaves they ate (Fig. 1), while the larvae of gall midges were only 0.9‰ enriched in ^{15}N compared with fungi (Fig. 3). The causes of this phenomenon are not fully understood, but in general it corresponds to the situation that is observed in food chains with the participation of parasites. Parasites are often depleted in ^{15}N compared to hosts, which is uncharacteristic of "normal" food relationships (Pinnegar et al., 2001; Navarro et al., 2014). However, this feature is not manifested in all cases, and the mechanisms behind it have not yet been deciphered (Lafferty et al., 2008).

Thus, the most important result of our work is the assessment of the degree of trophic fractionation of nitrogen and carbon isotopes in mycophages under natural conditions. The estimates obtained for $\delta^{15}N$ and $\delta^{13}C$ values correspond to the values observed in grazing food chains. The fractionation of nitrogen isotopes was slightly stronger in mycetophages that feed on saprotrophic fungi, but the difference with mycetophages feeding on mycorrhizal fungi was small and, on average, did not reach the level of statistical significance. Consequently, variations in the degree of trophic isotope fractionation should not impede the effective identification of "mycorrhizal" and "saprotrophic" energy channels in soil food webs. Our results are based on a study of the fungal fruit bodies; a detailed study of the isotopic composition of the vegetative mycelium of fungi of different functional groups should be the next step in the effective use of isotope analysis in the reconstruction of the trophic links of soil fungi and associated mycetophages.

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COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflict of interest. This article does not contain any studies involving animals or human participants performed by any of the authors.

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