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Nitrogen turnover of *Sinella coeca* (Collembola: Entomobryidae)

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Abstract. Adult collembolans were fed with two different food types. Leaves of *Taraxacum officinale* and *Dactylis glomerata* were given as food sources in two physical forms: either as intact leaves or as powder. Foods were labelled with ^{15}N. The ^{15}N uptake curves were measured. Turnover rates and turnover times were calculated. Results show that *Taraxacum* leaves are preferred to *Dactylis* leaves. The physical condition of the leaves influenced the nitrogen turnover of the animals to a large extent. Fragmentation of *Dactylis* leaves enhanced nitrogen utilization by roughly two-fold and decreased turnover time by nearly half. Consumption of *Taraxacum* leaves has synergic effect on the nitrogen utilization of *Dactylis* leaves. Simultaneous consumption of *Taraxacum* and *Dactylis* enhanced the nitrogen uptake rate from *Dactylis* leaves compared to the treatment where *Dactylis* was the only food source.

INTRODUCTION

Sinella coeca (Schött, 1896) (Collembola: Entomobryidae) is a widely distributed collembolan species in Europe. It is found frequently in glasshouses, compost and in soils rich in organic matter (Palissa, 1964). Food composition of *S. coeca* under natural conditions has not been investigated. It is known, however, that in the laboratory food choice experiments with this collembolan species shows that it fed selectively on *Actinomycetes* species, such as *A. cf. atroolivaceus* and *A. olivaceus* (Törne, 1964), vesicular-arbuscular mycorrhiza fungi (Thimm & Larink, 1995), the bacterium *Micrococcus luteus* and the fungus *Trichoderma viride* (Bakonyi et al., 1995), and the leaves and bark of beech, red-fir, maple, poplar, sycamore, hornbeam and oak (Tosi et al., 1977). This species consumed large amount of entomopathogenic nematodes in laboratory experiments (Gilmore & Potter, 1993). In our laboratory, *Dactylis glomerata*, *Festuca pratensis*, *Taraxacum officinale* and *Trifolium repens* leaves were consumed easily by *S. coeca*. There is no doubt that *S. coeca* is an omnivorous species. In our food preference tests, *T. officinale* leaves proved to be most preferred and *D. glomerata* leaves the most rejected food in respect to the four above-mentioned plant species (unpublished results).

Collembolans are able to regulate several processes of the soil nitrogen cycle (e.g. Verhoef & Brussaard, 1990). There is, however, limited information available about their own contribution to the quantity of nitrogen flows. In models constructed for the soil biogeochemical cycles of nitrogen, the estimation of rates and rate constants are based mostly on mass balance equations (e.g. Paustian et al., 1990). It is known, however, that the isotope dilution method may give different and often more accurate results than the mass balance equations (Bartholomew, 1971). Therefore, this method gives a powerful technique to construct a more reliable model than those based on mass-balance equations.

There are few data available relating to the nitrogen budget of a collembolan species. Verhoef et al. (1988) provide results on the nitrogen nutrition of *Orchesella cincta* and *Tomocerus minor* and Wolters (1985) for *Tomocerus flavescens*. Nitrogen uptake of *Sinella coeca* from microbial food sources has already been studied (Bakonyi et al., 1995). Therefore, in this model experiment, plant leaves were chosen as food sources. The aim of the experiment was to study differences in nitrogen uptake from preferred and not preferred plant leaves, which are physically and chemically different from each other, and to show the effect of the physical breakdown of leaves on the same parameter. The ^{15}N isotope uptake of the collembolans from different food sources was measured in order to gain quantitative data for calculating nitrogen isotope turnover parameters. These parameters are necessary to solve nitrogen budget models of collembolans, which are in progress at our Department.

MATERIAL AND METHODS

Adults of *Sinella coeca* were used in the experiment. However, it must be noted that the taxonomic situation of the species is not clear (P. Bellinger, pers. commun.). The collembolans have been reared in the laboratory for approximately 10 years. The experimental setup and the nitrogen isotope analysis were the same as in Bakonyi et al. (1995). About 300 adult collembolans were put into a freshly prepared rearing box (17 × 9 × 7 cm). The animals were fed with leaves of *Taraxacum officinale* and *Dactylis glomerata* plants, which have a considerably different structure and nutritional character. *D. glomerata* has much tougher leaves than *T. officinale*. Nitrogen content of leaves was determined by the Kjeldahl method. The nitrogen content of *D. glomerata* leaves was lower ($x = 2.64\%$, $SD = 0.92$, $n = 5$), than that of *T. officinale* ($x = 3.85\%$, $SD = 0.66$, $n = 5$) at $P < 0.05$ level of significance calculated by two-sample t-test.

The plants were labelled in the field, injecting nitrogen isotope as carbamide (labelled with ^{15}N at 49.8 atom percent excess) into the soil. Whole senescent leaves were cut from the plants and dried at laboratory temperature. ^{15}N atom % values of *D. glomerata* and *T. officinale* leaves did not differ significantly from each other ($x = 4.68\%$, $SD = 0.71$, $n = 5$ and $x = 4.43\%$, $SD = 1.31$, $n = 5$, respectively) at $P < 0.05$ calculated by two-sample t-test. Before use, the leaves (intact and ground) were wetted for 24 h. Plants were given either solely or simultaneously. If both plant species were offered at the same time, only one of them was labelled with the isotope. Theoretically, a similar method was used by Gere & Gyurján (1972) investigating the food specialization of different collembolan species. Plants were given as intact and as ground leaves. In the later case the leaves were ground to 0.5–1 mm pieces. They were placed on filter paper discs with a dimension of 2 × 2 cm. Two such filter papers were added to each rearing box in each treatment. Food was offered in excess. Each treatment was set up in five replicates.

The following eight treatments were applied:

Labelled *Dactylis* leaf, alone (DL1).
Labelled *Dactylis* ground leaf, alone (DG1).
Labelled *Taraxacum* leaf, alone (TL1).
Labelled *Taraxacum* ground leaf, alone (TG1).
Labelled *Dactylis* leaf, with unlabelled *Taraxacum* leaf (DL2).
Labelled *Dactylis* ground leaf, with unlabelled *Taraxacum* ground leaf (DG2).
Labelled *Taraxacum* leaf, with unlabelled *Dactylis* leaf (TL2).
Labelled *Taraxacum* ground leaf, with unlabelled *Dactylis* ground leaf (TG2).

Boxes were kept at 20°C in continuous darkness. The collembolans were sampled after 0, 1, 2, 6, 13 and 21 days. Five individuals were sampled on all sampling days from each box for further isotope analysis. Before ^{15}N analysis, the collembolans were kept in tubes with unlabelled rearing food for 24 h to clear the gut from labelled food material. The mean ^{15}N atom % excess of the bodies of five individuals collected at the same time and from the same box was measured. ^{15}N analyses were made by a NOI-5 emissions spectrophotometer (Faust, 1981). The Verhulst-Brody type of curves were fitted to this data by the statistical program CurveExpert 1.3. (made by D. Hyams, 112B Crossgate St., Starkville, MS 39759, USA). The program uses the Marquardt-Levenberg method to solve nonlinear regression. The equation of the curves is as follows: $y = a \cdot (b - e^{-cx})$, where y is the ^{15}N atomic % of the sample, x is the sampling day, and a, b and c are parameters of the curve. ^{15}N uptake curves of the different replicates of the same treatments were compared after linear transformation by covariance analysis (MINITAB Statistical Software, ANCOVA method).

To compare the ^{15}N uptake in the different treatments, the curves were fitted to the mean values of the five replicates. For calculating the nitrogen uptake, nitrogen utilization and turnover parameters, the ^{15}N uptake curves of all the five replicates were used. The method of the curve parameter analysis can be found in detail in Bakonyi (1987). Measurement of the ^{15}N uptake rate (UR) is the differential quotient of the first degree of the x values at $y = 0$. Turnover time (TT) was calculated by dividing the saturation level value of the ^{15}N uptake curve with the uptake rate (UR). ^{15}N turnover rate (TR) is given as a reciprocal of the turnover time and multiplied by 100, i.e. $TR = (1/TT) \cdot 100$. The nitrogen utilization rate (NUR) was calculated by dividing the $^{15}N\%$ in collembolan by the $^{15}N\%$ in the labelled food and multiplying by 100 (Faust, 1981). Differences between the UR, TT, TR and NUR means were calculated by Mann-Whitney nonparametric U-test (MINITAB Statistical Software). Differences between means were inspected at $P < 0.05$ level in all cases.

RESULTS

Parameters of the nitrogen isotope uptake curves by *Sinella coeca* are given in Table 1. Since no statistical differences were found between the replicates, the uptake curves were fitted to the average values of the replicates at each sampling day. The correlation coefficients were high (0.87–0.99). Therefore, it is supposed that the nitrogen isotope uptake curves describe the nitrogen uptake dynamics in a statistically reliable way.

TABLE 1. Parameters of the ^{15}N uptake curves. Curve parameters, correlation coefficients and standard deviations of the Verhulst-Brody type uptake curves in different treatments. Curves were fitted to the mean values of five replicates.

^{15}N-labelled food source	Form	Treatment	Curve parameters			Correlation coefficient	Standard deviation
			a	b	c	r	s
Dactylis	leaf	DL1	1.65	1.16	0.16	0.97	0.21
Dactylis	ground leaf	DP1	2.85	1.02	0.4	0.99	0.15
Taraxacum	leaf	TL1	1.59	1.07	0.63	0.99	0.13
Taraxacum	ground leaf	TP1	1.35	1.07	0.64	0.99	0.07
Dactylis	leaf	DL2	2.06	1.11	0.1	0.95	0.31
Dactylis	ground leaf	DP2	1.2	1.06	0.96	0.99	0.05
Taraxacum	leaf	TL2	1.03	1.07	1.24	0.87	0.3
Taraxacum	ground leaf	TP2	1.43	1.08	0.63	0.95	0.24

Results indicate clearly that the nitrogen isotope uptake rates for intact *Dactylis* leaves (DL1, DL2) are significantly lower than those for any other treatments (Table 2). The speed of the ^{15}N uptake (UR) from *Taraxacum* leaves was significantly higher in all treatments than that from *Dactylis* leaves. The physical status of the plant leaves affected markedly the ^{15}N uptake from *Dactylis*, but was not influenced in the case of *Taraxacum*. Nitrogen isotope was taken up significantly more quickly from ground rather than from intact *Dactylis* leaves. No such difference was found in the case of *Taraxacum*.

Collembolans utilized nitrogen at the highest rate from the ground *Dactylis* leaves. If leaves of a single plant species were presented (DL1 and TL1), the nitrogen utilization rates (NUR) were equal (Table 2). In all other comparisons, the NUR values were significantly different from each other. Grinding enhanced the nitrogen utilization greatly if the leaves were offered alone. In the preference tests, the grinding of leaves decreased the ^{15}N utilization from the *Dactylis*, but enhanced it from the *Taraxacum*. The presence of an alternative food source decreased the NUR in all but one cases: consumption of *Taraxacum* leaves with the *Dactylis* leaves enhanced the NUR from the *Dactylis*.

TABLE 2. ^{15}N turnover parameters of *S. coeca*. Means of five replications (± 1 SD). Parameters were calculated from the ^{15}N uptake curves of each replicates.

^{15}N-labelled food source	Form	Treatment	Uptake rate (%/day)	Utilization rate (%)	Turnover time (days)	Turnover rate (%/day)
Dactylis	leaf	DL1	0.43 (± 0.30)	35.0 (± 4.7)	4.28 (± 2.10)	30 (± 17)
Dactylis	ground leaf	DP1	1.38 (± 0.28)	65.7 (± 1.4)	2.55 (± 0.71)	42 (± 6)
Taraxacum	leaf	TL1	1.17 (± 0.19)	34.8 (± 1.9)	1.48 (± 0.23)	69 (± 11)
Taraxacum	ground leaf	TP1	1.10 (± 0.13)	57.4 (± 1.8)	1.31 (± 0.12)	77 (± 7)
Dactylis	leaf	DL2	0.23 (± 0.06)	46.2 (± 7.7)	11.00 (± 4.20)	10 (± 4)
Dactylis	ground leaf	DP2	1.32 (± 0.18)	34.3 (± 2.5)	0.96 (± 0.19)	107 (± 22)
Taraxacum	leaf	TL2	1.24 (± 0.26)	21.3 (± 1.2)	0.87 (± 0.20)	119 (± 24)
Taraxacum	ground leaf	TP2	1.07 (± 0.41)	28.5 (± 1.6)	1.68 (± 0.82)	70 (± 28)

The TT is significantly longer and the TR is significantly lower in the treatments with intact *Dactylis* leaves (DL1 and DL2) compared to TT and TR parameters of all other treatments (Table 2). In other cases, no significant differences were found between the turnover parameters (TT and TR). In these cases, the turnover times are between 0.87–2.55 days and turnover rates between 42%/day–119%/day. The grinding of leaves decreased TT and increased TR significantly only in the case of *Dactylis*. Alternative food influenced significantly the ^{15}N turnover parameters of a given food type, except in the comparison of treatments TG1 and TG2 (Table 2). Slower turnover of the *Dactylis* leaf nitrogen was found if *Taraxacum* leaves were given simultaneously (DL1 and DL2). *Dactylis* leaves as an alternative food source enhanced the nitrogen turnover from the *Taraxacum* leaves (TL1 and TL2). A similar effect was observed if ground *Taraxacum* was given with the marked *Dactylis* powder (DG1 and DG2).

DISCUSSION

These results demonstrate clearly that *Sinella coeca* is capable to utilize nitrogen from both *Dactylis* and *Taraxacum* leaves, but the nitrogen uptake is dependent on the plant species, physical status of the leaves and the presence of an alternative food source.

S. coeca is able to make fine distinctions between different food types (Thimm & Larink, 1995; Tosi et al., 1977; Törne, 1964). One possible reason of its food preference may be the isoprene content of the food source (Michelozzi et al., 1997). Leonard (1984) found that the feeding preferences of *Folsomia candida* depend significantly on the nitrogen content of the culture medium on which the fungal food sources of the collembolan were grown. Laboratory studies showed that food nitrogen content has critical influence on different parameters of collembolan populations. A lower nitrogen concentration of the same fungal food decreased the egg-production in *S. coeca* (Draheim & Larink, 1995). The *Folsomia candida* population was markedly higher on *Mucor plumbeus* after two months if the fungus was cultivated on a medium containing a higher nitrogen concentration than one that had lower nitrogen level (Leonard & Anderson, 1991). Boot & Anderson (1979) found that moulting and egg-laying rates of *Folsomia candida* increased with the nitrogen available to the fungi given as food, except at the highest nitrogen level. Similarly, Lavy & Verhoef (1996) detected an optimum of food nitrogen content for the growth of *Orchesella cincta*. Moreover, they supposed that at low levels of nitrogen in the food the animals increased their consumption rate. This may be a compensatory mechanism by which the collembolans are able to balance food-nitrogen shortage. In the present experiment, the nitrogen content of *Dactylis* proved to be significantly lower than that of *Taraxacum*. In the case of offering both of the intact leaves, a significantly higher nitrogen uptake was detected from *Taraxacum*. Therefore, it is probable, that *S. coeca* is able to distinguish between the food sources regarding their nitrogen content. In that way, the animals are able to optimize the nitrogen uptake by food selection in order to arrive at optimal growth, development and fertility.

The slower uptake rates, longer turnover times and lower turnover rates in treatments DL1 and DL2 compared to the other treatments indicate that digestion of intact *Dactylis* leaves is more difficult for *S. coeca* than *Taraxacum* leaves. It may be a consequence of the thick cell walls of the *Dactylis* leaves. The importance of the physical structure of the leaf litter on decomposition has already been demonstrated numerously (e.g. Hanlon & Anderson, 1980). In this experiment, collembolans utilized the ^{15}N isotope at much higher level from ground leaves than from intact leaves. An explanation for this finding is that physical destruction of the *Dactylis* leaf structure makes the leaves more digestable for collembolans. Besides, a significantly higher ^{15}N uptake rate as a result of grinding suggests that the palatability of *Dactylis* was affected.

Simultaneous consumption of *Taraxacum* enhanced the nitrogen uptake rate of the collembolans from *Dactylis* leaves. A similar synergistic effect was found in the case of the bacterium *Micrococcus luteus* and the fungus *Trichoderma viride* (Bakonyi et al., 1995). The nitrogen assimilation efficiency of *Tomocerus minor* was enhanced, as well, when a fungus was given as a supplementary food source to a pure algal diet (Verhoef et al., 1988). Synergismus in nitrogen uptake from different food sources may be a possible explanation to the polyphagous feeding habits of several collembolan species.

There is little data available for nitrogen turnover of soil animals. Curry et al. (1995) found a ^{15}N turnover time for the earthworm *Lumbricus terrestris* of about 70 days. The differences in nitrogen turnover times for *Sinella* and *Lumbricus* may be due to differences in the digestibility of their food.

The complex nature of food selection and utilization is also shown by the results of the present experiment. Fragmentation and a presence of an alternative food source modified the nitrogen utilization of the less acceptable food (*Dactylis*) more than that of the more preferred one (*Taraxacum*). On the other hand, simultaneous consumption of *Dactylis* and *Taraxacum* leaves enhances the nitrogen uptake and turnover from *Taraxacum*.

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