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The Effect of Diplopoda on the Dynamics of Amino Acids in the Soil

by

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Abstract: Composition and dynamics of amino acids in leaf litter and faeces of Diplopoda in the course of decomposition was investigated. *Pachyiulus flavipes* KOCH collected in the South Crimea was used as a model subject for laboratory experiments. The total amount of protein amino acids in animal faeces was found to be considerably higher than that in undigested litter. The content of protein amino acids tended to increase in aged faeces exposed in the leaf litter. A decrease in the amino nitrogen was recorded in litter during the same period. A high content of free amino acids as well as intense ammonia efflux were recorded in faeces indicating an active protein hydrolysis. It is suggested that the increase in content of protein amino acids is due to the microbial activity which is known to be higher in faeces than that in litter. The impact of Diplopoda on humus dynamics is discussed.

1. Introduction:

Diplopoda are widely used as a model subjects for investigating the role of soil saprophages in the destruction and humification of leaf litter. However, in most investigations the role of these saprophages was considered in terms of their effects in comminuting plant remains. Little attention has been paid to the impact of Diplopoda in the transformation of organic matter.

Diplopoda are known to be numerous in many types of natural ecosystems in the temperate, tropical and subarid zones. They are mainly associated with tree and shrub vegetation. In a wet and humid climate, millipedes also attain high population densities in grass habitats (meadow steppe, high-mountain meadows).

The feeding activity of Diplopoda plays an important role in the litter dynamics and also in the formation of humus profile (SOKOLOV 1955, GHILAROV 1957, STRIGANOVA 1969). Hence it is of interest to study the contribution of these phytosaprophages to the processes of chemical transformation of organic matter in the soil. The present communication considers the effect of diplopod feeding activity on the amino acid content in the decaying litter.

2. Material and Methods:

The experimental studies were made on *Pachyiulus flavipes* KOCH, which is one of the largest representatives of the diplopod fauna of the USSR. *P. flavipes* is distributed in the Crimea (DIRSCH 1937). It is numerous on the southern slopes of the Crimean Mountains in open woodlands of the East-Mediterranean type, where animals occur under the canopy of arboreous vegetation (*Carpinus orientalis, Quercus pubescens, Juniperus* sp.) and also under stones on mountain screes, feeding on remains of herbs and shrubs. Young individuals often feed on living plant tissues. Faeces of *P. flavipes* constitute the bulk of the organic particles on the soil surface.

For the experiments animals were collected in open woodland habitats at the spring peak of their activity in April, maintained singly in glass jars and were fed on overwintered oak litter collected at their natural habitats.

The feeding activity of *P. flavipes* was measured gravimetrically (STRIGANOVA 198O). For chemical analyses the faeces were collected in the following way: the animals were placed in groups in vessels containing oak litter. To maintain a close-to-natural humidity the litter in the vessels was regularly wetted. A part of the faeces were sampled after 24 hours, air-dried and used for the tests. The other part was left in the vessels with leaf litter for 100 -110 days, and then dried.

Estimation of the amino acid composition was made of the leaf litter and as well as of 1st-day faeces and of faeces exposed in the litter during the experiment. The determination of amino acids was performed on dry material (dried at 50 - 60° C). From the samples (2 - 3 g dry mass), proteins were progressively extracted with water, 70 % ethanol, 0.2 and 2,0 % sodium hydroxide (PLESHKOV 1976). Protein was precipitated with 50 % trichloracetic acid, over a water bath (KRISZHENKO 1978). The protein precipitate was separated by centrifugation, washed and dried, and subjected to acid hydrolysis. The resulting precipitate was dried and the protein amino acids were determined. Free amino acids were extracted from the original centrifugate before further treatment (washing, drying, hydrolysis). The amino acid composition was determined in a sodium nitrate buffer (pH 2,2) in an Automatic Amino Acid Analyzer (AAA-88I) (STRIGANOVA et al. 1989).

3. Results

The daily amount of food consumed and assimilated was found to vary widely in different size groups (Table 1). The feeding activity was determined in individuals weighing between 35 mg (a month after hatching) and 3700 mg (old females). During the activity period in spring the majority of the population of *P. flavipes* was represented by animals with a body mass between 500 and 2500 mg. The relationship between live and dry weights in *P.flavipes* was derived from measurements of 2O specimens, which were weighed live individually, killed and dried at 1OO° C then reweighed. The mean percentage dry matter was 35.3. The dry mass of specimens used for feeding experiments was calculated on the basis of this value. The daily food consumption per individual ranged from 3,8 to 141,8 mg dry mass. The realtionship between the rate of food consumption and the body dry mass is expressed by the regression equation:

$$C = 2{,}0701 \pm 0.44\ W^{0.63 \pm 0.11}$$ (Fig. 1).

Table 1: Feeding activity of *Pachyiulus flavipes* KOCH.

n	mean dry mass [mg ± S.E.]	daily food consumption [mg/Ind. ± S.E.]	Food assimilation [mg/Ind. ± S.E.]	Assimilation efficiency A/C
20	11,1 ± 0,9	3,8 ± 0,2	2,1 ± 0,2	0,55
20	26,5 ± 2,5	19,3 ± 1,9	13,3 ± 1,5	0,67
20	38,8 ± 1,2	13,4 ± 0,6	6,6 ± 0,4	0,49
20	190,9 ± 20,5	88,5 ± 1,1	21,0 ± 1,9	0,24
20	623,3 ± 53,5	96,6 ± 0,4	18,7 ± 1,2	0,19
12	930,0 ± 17,9	141,8 ± 0,4	37,2 ± 2,2	0,26

This relationships can be traced only in the middle and the oldest age groups. In the smallest specimens the rate of litter consumption was half than that expected. Presumably the leaf litter was not their preferred food source. Analysis of the intestinal content revealed that in nature they fed on live roots of grasses or organic detritus.

The assimilation efficiency ranged from 67 % (body mass 140 - 17O mg) to 19 % (largest females). In the smallest animals assimilation efficiency varied at the level of about 50 - 60 %. In individuals with a body mass of over 500 mg assimilation declines below 30 %. The relationship between the value of the food assimilated and the body mass is expressed by the equation:

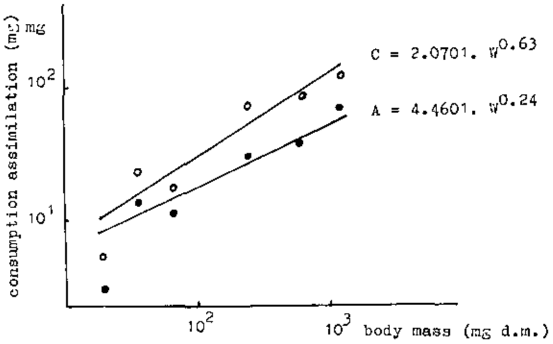

Fig. 1. Relations of rates of the food consumption (C) and assimilation (A) to the body mass of *Pachyiulus flavipes.*

$A = 4{,}4601\ W^{0{,}24}$ (Fig. 1).

The considerable individual fluctuations in assimilation efficiency should be noted. The actual food rations of the smallest and largest specimens were higher than expected. Diplopoda compensate for the low rates of food uptake by augumented assimilation. This suggests the existence of symbiotroph relations with microflora, the composition and activity of these intestinal microbial communities vary with the age of the animals. For 1 g of litter consumed the animals produce on an average about 600 mg of faeces.

The content of protein amino acids in the leaf litter and in the faeces of *P. flavipes* is given in Table 2. At the beginning of the experiment 17 amino acids were recorded in the litter. After 3,5 months cystine, which from the very outset was presented in only trace amounts was no longer revealed. A similar composition of amino acids was noted in both "fresh" and aged faeces.

In the litter the total amount of amino acids declined during the experiment. In the experimental vessels they were obviously consumed by microorganisms. In nature, utilization of the amino acids released in the course of decomposition proceeds much more rapidly, their main consumers being plant roots. By contrast, in faeces the total content of amino acids increases, which is suggestive of intensive metabolic processes taking place between the dead plant remains and the microflora concentrated in the faeces.

Of some interest is the analysis of the quantitative ratios of amino acids in certain individual fractions i.e. water-soluble and weakly- or strong alkaline (Fig. 2). The highest amino acid content of all the variants was noted in the weakly alkaline fraction. In the litter the ratio between the individual fractions was nearly maintained despite the total decline of the amino acid pool. Only the percentage of the water-soluble fraction was considerably lower at the end of the experiment. This fraction includes the most mobile forms, primarily those used by microorganisms.

In the faeces these ratios changed sharply. Following the evacuation of faeces from the intestine the water-soluble fraction of protein amino acids was completely absent since it had been utilized in the gut. Almost 90 % of protein amino acids were concentrated in the weakly-alkaline fraction. As products of the symbiotic microflora mainly transport and reserve forms were present.

In aged faeces in which the most stable compounds are formed the proportion of the strongly-alkaline fraction increases. Among protein amino acids the most important and abundant were asparatic and glutamic acids, alanine, threonine, proline and glycine.

Phenylalanine showed a tendency to accumulate in the litter and in the faeces. In aged faeces there was an accumulation of glutamine, alanine, threonine, serine, leucine and valine. The absolute content of these amino acids was far higher than that in the litter. Thus, Diplopoda enrich the litter with amino acids. With ageing of the faeces redistribution of the pool of amino acids takes place.

Table 2: Content of protein amino acids in oak litter and faeces of *P. flavipes* (μg g^{-1}).

	Content of amino acids [μg/g]			
	oak litter		faeces	
Protein amino acids	I*)	F*)	I	F
Lysine	0,17	0,08	0,15	0,16
Histidine	0,07	0,02	0,04	0,11
Arginine	0,12	0,07	0,10	0,14
Asparatic acid	0,80	0,37	1,88	1,13
Threonine	0,33	0,16	0,35	0,47
Serine	0,24	0,17	0,22	0,60
Glutamine	0,64	0,26	0,70	0,91
Proline	0,37	0,12	0,18	0,35
Glycine	0,19	0,19	0,46	0,44
Alanine	0,40	0,19	0,43	0,57
Cysteine	0,05	-	-	-
Valine	0,36	0,13	0,27	0,44
Methionine	0,08	traces	0,03	0,08
Isoleucine	0,08	0,09	0,20	0,08
Leucine	0,21	0,16	0,33	0,28
Tyrosine	0,16	0,06	0,10	0,17
Phenylalanine	0,16	0,25	0,13	0,20
Total pool:	4,44	2,34	5,57	6,13

*) I - initial content; F - final content.

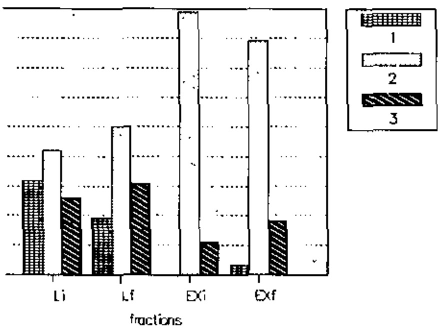

Fig. 2. Distribution (%) of protein amino acids between water-soluble, weakly-, or strong alkaline-soluble fractions in the litter and faeces of Diplopoda. L_i - Initial content of amino acids in the litter at the beginning of the experiment. L_f - Final content of amino acids in the litter at the end of the experiment. Ex_i - Initial content of amino acids in faeces. Ex_f - Final content of amino acids in faeces. 1 - water-soluble amino acids fraction; 2 - fraction of amino acids extracted by 0,2 % sodium hydroxide solution; 3 - fraction of amino acids extracted by 2,0 % sodium hydroxide solution.

Under experimental conditions the group of easily accessible forms increased, while in nature they do not accumulate since they are used by plant roots. At the same time an increase of amino acids fixed in complex compounds (strongly-alkaline fraction) took place.

Comparison of free amino acids is shown in Table 3. In faeces amino acids accumulated and their diversity increased. At the beginning of the experiment 9 amino acids were recorded in the litter, and 15 at the end. In all, 16 forms were found in faeces. The total of free amino acids in faeces was 7-times than that in the litter. The presence of free amino acids in the absence of roots coincided with accumulation of available nitrogen in the faeces.

Table 3: Content of free amino acids in oak litter and faeces of *P. flavipes* (μg g^{-1}).

Free amino acids	Content of free amino acids [μg/g]			
	Litter		Faeces	
	I*	F*	I	F
Lysine	*0,0005*	*0,0038*	*0,0147*	*0,0029*
Histidine	-	-	0,0019	-
Arginine	0,0031	0,0151	-	0,0104
Aspararticacid	-	0,0287	0,0168	0,0211
Threonine	-	0,0229	0,0456	0,0345
Serine	-	0,0154	0,0351	0,0209
Glutamic acid	-	0,0663	0,0656	0,0707
Glycine	-	0,0113	0,0237	0,0196
Alanine	-	*0,0480*	*0,1061*	*0,0963*
Valine	0,0228	0,0295	0,0509	0,0422
Isoleucine	0,0159	0,0206	0,0350	0,0279
Leucine	0,0204	0,0316	0,0541	0,0485
Tyrosine	0,0071	0,0041	0,0117	0,0060
Phenylalanine	0,0051	0,0068	0,0176	0,0099
Phenylalanine-aminobutiric acid	0,0020	0,0061	0,0083	0,0122
Ethanolamine	0,0003	0,0014	0,0166	0,0038
Ornitine	-	-	*0,0004*	-
Total pool:	0,0772	0,3116	0,5041	0,4269

*) I - initial content; F - final content.

Figure 3 demonstrates the ammonia dynamics in litter and faeces characterizing the activity of the ammonification processes. In the faeces the flow of ammonia was by an order higher thay that in the litter. In aged faeces the content of ammonia somewhat declined while in the exposed litter it increased. *Thus, with time, the rate of ammonification processes became similar.*

4. Discussion:

Millipedes are known to be the active mineralizers of plant remains (STRIGANOVA, 1971). The degradation of cellulose proceeds actively in their intestine, the initial stages of this process being carried by symbiotic microorganisms. Cellulose and other carbohydrates are utilized by Diplopoda as their main energy sources. The content of cellulose in the faeces of *P. flavipes* was 30 % lower than that in the diet (KOZLOVSKAJA et al., 1983). In our experiments the animals utilized *up to 50 % of the cellulose contained in their diet. They also used microbial populations consumed* with the litter as nitrogen sources. Symbiotic microorganisms actively consume accessible nitrogen from metabolic products and hence, the most accessible watersoluble fraction of the protein amino

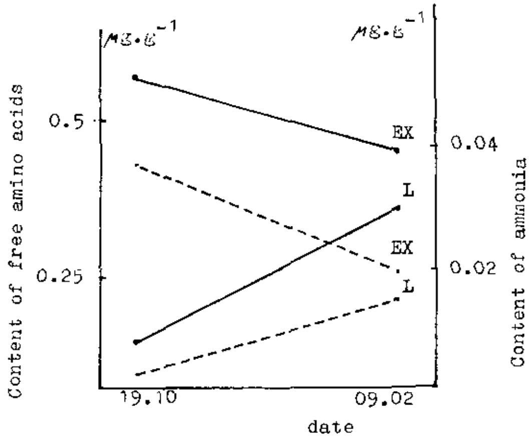

Fig. 3. Dynamics of free amino acids and ammonia in the litter (L) and faeces (Ex) during the experiment. ---------- free amino acids; - - - - - - ammonia.

acids disappears in the gut. At the same time, the nitrogen of mobile humus compounds is utilized and its total content declines (STRIGANOVA 1980).

It has long been thought that nitrogen compounds are only slightly transformed in the millipede intestine. This view was based on the fact that the humus compounds in the faeces changed negligibly. However, the intensive flow of ammonia and drastic re-arrangement in the composition of free amino acids suggest that the processes of hydrolysis of protein and ammonification are very active. These processes also occur in the litter. But with ageing of the faeces ammonification progresses much more rapidly. The ammonia released is involved in resynthesis reactions. It has been demonstrated for other Diplopod species that humification occurs in the faeces in the soil (KOZLOVSKAJA et al., 1983). This is in agreement with own data obtained on the fixation of the amino nitrogen in the most stable fraction of protein amino acids.

Thus the effect of Diplopoda on the dynamics of organic material is achieved in two stages:

(1) the intestinal processes responsible for the chemical composition of the newly formed faeces, e.g. decomposition of cellulose, acceleration of mineralization processes and partial hydrolysis of humic compounds;

(2) the processes in the faeces released into the soil, following the end of the cellulolytic stage the transformation of organic material is shifted towards humus formation. These processes are more active in the faeces than in the leaf litter.

This problem calls for further study, in particular the investigation of microbial successions in faeces released into the soil, as well as research into the dynamics of individual fractions of humus compounds in the soil and excrements.

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