PERGAMON

Soil Biology & Biochemistry 32 (2000) 1191–1196

Soil Biology & Biochemistry

www.elsevier.com/locate/soilbio

Importance of food quality on selected enzyme activities in earthworm casts (*Dendrobaena octaedra*, Lumbricidae)

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Received 11 November 1999; received in revised form 24 January 2000; accepted 27 February 2000

Abstract

Dehydrogenase (DHA), acid (SPA) and alkaline (APA) phosphomonesterase activities in the casts of *Dendrobaena octaedra* were analysed while the animals were offered one of six different food sources. The food consisted of air dried leaves of dandelion, lupin, rye, alder, beech and larch. The food consumption rate of the earthworms lay between 11.1 mg (dandelion) and 0.3 mg d·w· g^{-1} f·w· d^{-1} (larch) while the cast production varied between 252 mg (dandelion) and 835 mg d·w· g^{-1} f·w· d^{-1} (beech). A deterioration of the food quality caused a reduction of the food consumption rate, but simultaneously the mean turnover rate of soil aggregates was enhanced. The enzyme activities were influenced by the food which affected the specific nutrient state of the casts. DHA, SPA and APA were significantly correlated with the organic *C* and the total *N* content of the casts. Interenzymatic correlations illustrated the applicability of the three enzyme activities as useful indicators of the influence of earthworms and their food on soil quality.

Keywords: Earthworms; Soil quality; Food consumption; Cast production; Enzyme activity; Nitrogen; Organic carbon

1. Introduction

The biochemical breakdown of plant residues caused by soil animals and microorganisms is the main process of soil humus development (Haider, 1996). Earthworms are one of the most important groups of soil animals involved in decomposing litter and in incorporating plant residues in the upper soil horizons. Their great importance in the nutrient cycle is uncontested. The burrowing and casting activities of earthworms contribute to the activity of soil microorganisms (Edwards and Bohlen, 1996) and nutrient-enriched earthworm casts are good media supporting microbial growth (Lee, 1985). To evaluate microbial biomass and activity, the determination of selected enzyme activities becomes more and more attractive (Frankenberger and Dick, 1983). In this connection, the applicability of dehydrogenase activity has been investigated by many authors, because dehydrogenase plays a significant role in the oxidation of soil organic matter. According to Tabatabai (1984) dehydrogenase activity can be considered as a major indicator of the total microbial activity in soils. Phosphatases may also be used for biochemical soil characterization. Of the five major groups of phosphatases, the phosphomonesterases have been studied most intensively (Tabatabai, 1984). Nannipieri et al. (1979) showed analogies between phosphomonesterase activities and the bacterial biomass in soil. Syers and Springett (1984) found an increase in the number of bacteria and actinomycetes with enhanced phosphomonesterase activity in earthworm casts. In contrast to the acid phosphomonesterase, the origin of the alkaline phosphomonesterase is limited to the synthesis of animal and microbial cells (Juma and Tabatabai, 1988). Satchell and Martin (1984) suggested that the increase in the alkaline phosphomonesterase activity in earthworm casts may be derived from the earthworms' own

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0038-0717/00/$ - see front matter
PII: S0038-0717(00)00035-3

enzymes and stimulation of the microflora. Lavelle et al. (1995) summarized the mutualistic relationships between earthworms and microorganisms.

Our aim was to measure dehydrogenase and acid and alkaline phosphomonesterase activities in the casts of *Dendrobaena octaedra*. Living in the litter layer, *D. octaedra* is classified as an epigeic earthworm, although the animals create a system of horizontal and vertical burrows in the upper mineral soil layer (Bouché, 1977). Lee (1985) classifies *D. octaedra* as detritivorous and according to Graff (1953), it is the most important litter-decomposing earthworm in temperate forests. We investigated and measured the role of different food sources on enzyme activities in the casts, as well as the rates of food consumption and cast production by the earthworms. Under standard laboratory conditions, the earthworms were offered food of varying nutrient constitution and secondary plant compounds.

2. Material and methods

2.1. Experimental conditions

A laboratory investigation was made to obtain the casts of adult individuals of *D. octaedra* fed on various food sources. The experiments were carried out with the leaves of six plant species with a wide range of C–N ratios. Fresh leaves of dandelion (*Taraxacum officinale*, N: 3.59%, C–N: 11), lupin (*Lupinus polyphyllus*, N: 4.50%, C–N: 9), rye (*Secale cereale*, N: 1.23%, C–N: 31) and alder (*Alnus glutinosa*, N: 2.98%, C–N: 15) and half-year old fallen leaves from the litter layer of beech (*Fagus sylvatica*, N: 0.91%, C–N: 48) and larch (*Larix decidua*, N: 1.01%, C–N: 42) were collected in the area surrounding Braunschweig (Lower Saxony, Germany). The leaves were air-dried and cut into pieces between 1 and 2 cm^2. For each food treatment, 56 earthworm individuals were kept for 6 weeks in special microcosms (eight earthworms per microcosm) with a constant temperature of 11°C. The microcosms consisted of two parallel glass sheets (Evans, 1947) with a distance of 5 mm filled with soil aggregates between 0.5 and 1 mm diameter and a moisture content of 18% (w/w). The dried plant material was remoistened and offered on the soil surface.

The soil used was Gleyic Luvisol (FAO) from the Ap-horizon without plant debris of a arable loam near Neuenkirchen (Lower Saxony, Germany). The soil contained an organic-C (Corg) of 1.79% and a total N content (Nt) of 0.165% (C–N = 10.85). Soil texture was as follows: 2.8% sand, 80.2% silt and 17.0% clay. After the experiments, the microcosms were air dried and cast aggregates as well as unconsumed plant materials were separated from the soil aggregates. For each food treatment, the food consumption (FC) rate and the cast production (CP) of the animals were estimated in mg d.w. g^{-1} f.w. d^{-1}. The value for FC and CP is the arithmetic mean of the values analysed for each microcosm containing eight earthworms.

2.2. Chemical and biochemical measurements

Nitrogen and carbon content of the leaves was analysed by the macro-element analyser LECO CHN-1000 (Leco, Kirchheim, Germany). The Kjeldahl method (Kandeler, 1993) was used to estimate the Nt of the soil and casts. In order to include nitrate, nitrite, nitro and nitroso groups, 25 g of salicylic acid was added to 1 l of the sulphuric acid. The Corg of the soil and casts was determined by a macro-carbon analyser (Leco Carbon Determinator IR 12, Leco, MI, USA).

Enzyme activities were determined in fresh surface casts collected at the end of the experiment. Dehydrogenase activity (DHA) was determined by the standard method of Malkomes (1993) at 30°C and was calculated as μg triphenylformazan g^{-1} dry mass d^{-1} (μg TPF g^{-1} d^{-1}). Acid (SPA) and alkaline (APA) phosphomonesterase activities (buffer-pH: 6.5 and 11.0, respectively) were measured by the method of Tabatabai and Bremner (1969), modified by Margesin (1993) at 37°C. The phosphomonesterase activities were calculated as μg nitrophenol g^{-1} dry mass h^{-1} (μg NP g^{-1} h^{-1}).

3. Results

3.1. Food consumption (FC) and cast production (CP)

D. octaedra showed the highest FC when offered dandelion (11.1 mg g^{-1} d^{-1}), while the FC was lowest when the animals were fed on larch (0.3 mg g^{-1} d^{-1}) (Fig. 1). The C–N ratio of the food had a significant influence on the consumption rate of *D. octaedra* and was negatively correlated with the FC ($R = -0.97$, $P = 0.013^*$) (Fig. 1). The extrapolation showed that the ingestion limiting C–N ratio is about 49.

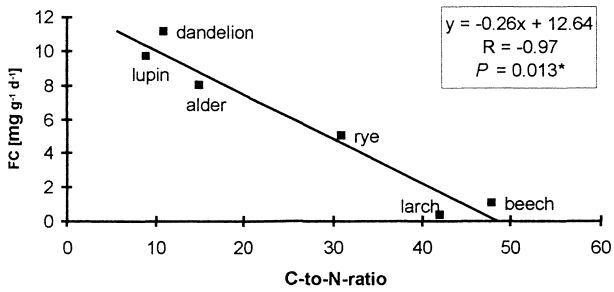

Fig. 1. Correlation between the food consumption (FC [mg d.w. g^{-1} f.w. d^{-1}]) of *D. octaedra* and the C–N ratio of the food sources.

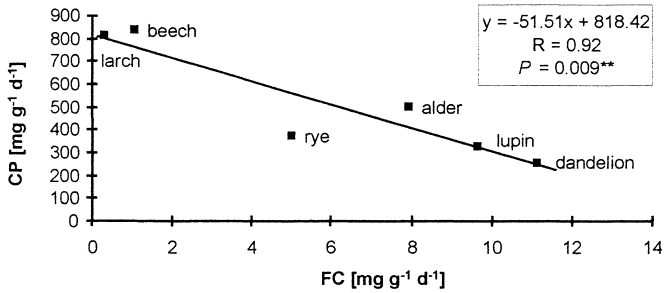

Fig. 2. Correlation between the cast production (CP [mg d·w· g^{-1} f·w· d^{-1}]) and the food consumption (FC [mg d.w. g^{-1} f.w. d^{-1}]) of *D. octaedra*.

The CP of *D. octaedra* was negatively correlated with the FC ($R = 0.92$, $P = 0.009^{**}$) (Fig. 2). With 835 mg g^{-1} d^{-1}, the CP was the highest when the animals fed on beech leaves and with 252 mg g^{-1} d^{-1}, lowest when offered dandelion. These observations made clear that a decrease in food quality (less decomposable plant material) provokes an increase in the mean turn-over rate of soil aggregates. We found that when offered a lower food quality, *D. octaedra* ingested higher rates of soil aggregates in order to satisfy its nutrient demand with soil inhabited by microorganisms. A high soil consumption rate provoked a greater amount of casts. Figs. 1 and 2 illustrate this phenomenon. Measurements of the earthworm fresh weight before and after the experiment showed that *D. octaedra* lost 0.03% weight d^{-1} feeding on alder, 0.09% weight d^{-1} feeding on rye, 0.10% weight d^{-1} feeding on beech and 0.12% weight d^{-1} feeding on larch (dandelion: +0.03% d^{-1}, lupin: +0.01% d^{-1}). Correlations between the percentage change of weight d^{-1} and the C–N ratio of the food ($R = -0.94$ and $P = 0.006^{**}$) and the percentage change of weight d^{-1} and the Nt of the leaves ($R = 0.95$ and $P = 0.003^{**}$), showed that food with a C–N ratio higher than 11 and a Nt lower than 3.5% is not acceptable to guarantee the long-term survival of *D. octaedra* under the described laboratory conditions.

3.2. Chemical and biochemical measurements in earthworm casts

The contents of Corg and Nt in the earthworm casts were different depending on the food (Table 1). Dandelion as food caused the highest contents of Corg (1.87%) and Nt (0.262%), resulting in the lowest C–N ratio (7.14) in the casts. The lowest contents of Corg and Nt were detected in cast material after feeding the earthworms on larch (Table 1). Only in this case, Nt in the soil (0.165%) was higher than in the casts (0.162%). The Corg contents of casts from feeding on rye, beech, alder and larch were lower than in the soil Corg (1.79%).

Table 1
Organic carbon (Corg), total nitrogene (Nt) and C–N ratios of the casts of *D. octaedra* in relation to different food sources

Food	Dandelion	Lupin	Alder	Rye	Beech	Larch
Corg (%)	1.87	1.85	1.63	1.68	1.64	1.56
Nt (%)	0.262	0.233	0.169	0.181	0.167	0.162
C–N ratio	7.14	7.94	9.64	9.28	9.82	9.63

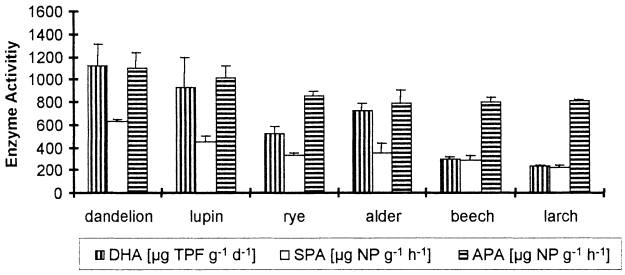

Fig. 3. Enzyme activities of dehydrogenase (DHA [µg TPF g^{-1} d^{-1}]), acid (SPA [µg NP g^{-1} h^{-1}]) and alkaline (APA [µg NP g^{-1} h^{-1}]) phosphomonesterase in the casts of *D. octaedra*.

In our study, the enzyme activities in the casts were highly influenced by the different food sources. The DHA, SPA and APA in the casts were highest when the animals were feeding on dandelion and lupin (Fig. 3). The lowest DHA and SPA were found when the earthworms were offered larch, while the lowest APA was detected when the earthworms were fed on alder (Fig. 3). Compared with the enzyme activities in the soil of 291 µg TPF g^{-1} d^{-1} (DHA), 190 µg NP g^{-1} h^{-1} (SPA) and 429 µg NP g^{-1} h^{-1} (APA), only the DHA in the casts resulting from feeding on larch was lower than the enzyme activities in the soil.

The correlations of the enzyme activities in the earthworm casts with their Corg and Nt (DHA: $R = 0.90$ and $P = 0.016^{*}$; APA: $R = 0.95$ and $P = 0.004^{**}$, SPA: $R = 0.92$ and $P = 0.009^{**}$) were significant for all three analysed enzyme activities (Fig. 4). Furthermore, DHA was significantly correlated with the APA and the SPA in the casts (Fig. 5).

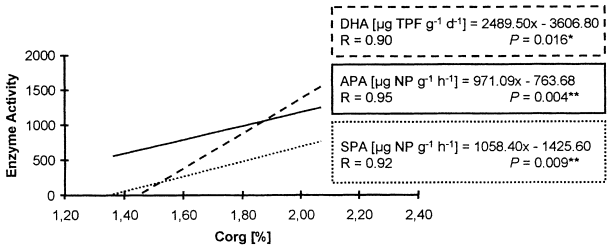

Fig. 4. Correlation between the enzyme activities of dehydrogenase (DHA [µg TPF g^{-1} d^{-1}]), acid (SPA [µg NP g^{-1} h^{-1}]) and alkaline (APA [µg NP g^{-1} h^{-1}]) phosphomonesterase and the Corg contents [%] of the casts of *D. octaedra*.

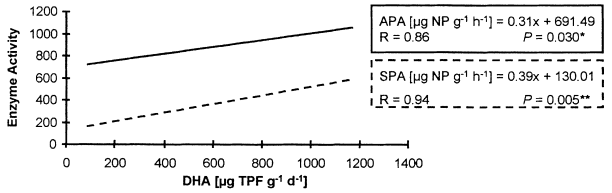

Fig. 5. Interenzymatic correlations between the activities of dehydrogenase (DHA [µg TPF g^{-1} d^{-1}]) and the acid (SPA [µg NP g^{-1} h^{-1}]) and alkaline (APA [µg NP g^{-1} h^{-1}]) phosphomonesterase in the casts of *D. octaedra*.

4. Discussion

The food consumption (FC) rate of earthworms is the result of a complex interaction between the chemical composition and the durability of the food. Examination of the gut content of *Lumbricus terrestris* living in a beechwood forest showed that earthworms are selective litter feeders (Judas, 1992). According to Satchell (1967), food palatability is the most important factor influencing the FC rate of earthworms. Satchell and Lowe (1967) reported that the FC of *L. terrestris* is elevated with an increasing N content of the food because protein N raises the food palatability. Although having the highest N content, lupin was not the food ingested most in our study. Since the investigations of Mangold (1951), it is known that lupanin represses the FC of earthworms. With a lupanin content of 0.6% (Wink et al., 1980), lupin therefore is less attractive than dandelion. The FC is also influenced by the durability of the food particles (Boström and Lofs-Holmin, 1986). It is likely that in spite of a higher N content, the hard needles of larch were less digestible than the beech leaves. In the present study, we found a negative correlation between the C–N ratio and the food consumption. This result agrees with the investigations of Wittich (1953) who reported that the C–N ratio is one of the most important factors limiting food ingestion of earthworms.

A high C–N ratio indicates low food quality and in consequence, provokes a lower FC but a higher rate of soil ingestion, resulting in a higher cast production (CP). With a declining FC rate, soil aggregates were chosen as an alternative food source containing microorganisms. Experiments of Scheu and Parkinson (1994) showed that *D. octaedra* consumes high rates of soil microorganisms. Lavelle et al. (1995) pointed out the mutualistic relationships between endogeic earthworms and the ingested microflora. It can be assumed that such relationships may also exist for the epigeic *D. octaedra* but limited by the C–N ratio of food. We conclude that *D. octaedra* is able to change its feeding behaviour from "mainly detritivorous" to "mainly geophagous". It must be considered that laboratory conditions led to this result.

In general, earthworm intestines contain the same microorganisms as the surrounding soil (Satchell, 1983). During the passage through the earthworm intestines, some kinds of microorganisms may increase rapidly in numbers. While the investigations of Parle (1963) showed that bacterial growth is increased in the earthworm intestines, Kristufek et al. (1992) could also prove the enrichment of fungal hyphae. These relationships are also valid for earthworm casts. Tiwari et al. (1989) suggested that an increased bacterial and fungal biomass in earthworm casts is responsible for higher enzyme activities.

Tiwari et al. (1989) and Mulongoy and Bedoret (1989) also found higher dehydrogenase and phosphomonesterase activities in nutrient-enriched earthworm casts. Because of difficult digestive and less palatable food, the earthworm casts in our study contained a smaller Corg than the soil (soil Corg: 1.79%) when the earthworms were fed alder, rye, beech or larch. This observation confirms that not only the amount but also the quality of the organic matter is responsible for microbial activity. Inside the intestine, the high mucus content and the partially decomposed organic matter enable a higher microbial biomass enrichment than the soil organic matter (Barois and Lavelle, 1986).

The higher nutrient enrichment of the casts supported higher enzyme activities. Juma and Tabatabai (1978) and Pang and Kolenko (1986) also investigated positive correlations between the Corg and Nt of soils and their acid and alkaline phosphomonesterase activities. Considering different mulch types as food for earthworms, Buck et al. (1999) found the C–N ratio positively correlated with SPA in their casts. Similar to our study, Domsch et al. (1979) and Beck (1984) analysed the same relationship between the DHA and the APA in several soils. Both authors also found significant correlations between the microbial biomass and the DHA as well as the APA. Obviously, the food-induced nutrient status of the casts directly influenced the enzyme activities of the microorganisms. Ponge (1988) concluded from his results of studies on casts of *D. octaedra* after it consumed pine needles that microbial activity is only promoted when the casts are squashed by the animal movements, enhancing the surface. In some cases, unfavourable food may therefore lead to a decline in the enzyme activity under the soil standard, but in general, earthworms contribute to higher enzyme activities. This is not only restricted to their casts, but also in the burrow walls, higher enzyme activities can be detected. Tiunov and Scheu (1999) detected an increase in basal respiration, microbial biomass and bacterial volume in those walls.

Measuring the dehydrogenase and the phosphomonesterase activities in earthworm casts is a practical way to estimate the effects of earthworms on the soil microflora according to their food. Regarding the soil

structure, Flegel et al. (1998) found phosphomonesterase activities, in particular, showed significant correlations with the water stability of earthworm casts. Our investigations suggest that the relationship between the nutrient state and the enzyme activities in earthworm casts is similar to the conditions existing in soil aggregates. Nevertheless, better microbial conditions caused by the quality and quantity of organic matter in earthworm casts has to be taken into account.

Acknowledgements

Especially, we are grateful to the Editor-in-Chief Prof. Waid for his valuable comments. Prof. O. Larink is acknowledged for his advice. We also thank K. Brinkmann, K. Steffens, M. Kondermann and G. Schnelle for technical assistance.

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