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MICROFLORAL ABUNDANCE AND ENZYMATIC ACTIVITY OF *LUMBRICUS RUBELLUS* HOFFM. (OLIGOCHAETA: LUMBRICIDAE) CASTS IN PEAT MEADOW SOILS

ABSTRACT: The abundance of the main microfloral groups, and dehydrogenase and urease activity, in the casts of *Lumbricus rubellus* Hoffm. and in the 0–5 cm and 5–10 cm soil layers of drained peaty meadows were compared. The abundance of microorganisms in casts was significantly higher than in the soil, as was the level of enzyme activity. The difference was particularly marked in comparisons with the 5–10 cm soil layer. In the cases of fungi, bacteria and actinomycetes, the degree to which abundance was greater in casts was highest at sites where the abundance of microflora in the soil was low. The activity of the two enzymes was always higher in casts than in soil, and the difference was especially marked for the meadow on alder peat.

KEY WORDS: *Lumbricus rubellus,* microflora, enzymes, casts, peat meadows

1. INTRODUCTION

The role of earthworms in the development of the physico-chemical and biological properties of soil is considerable and relatively well-known, as documented in reviews and monographs (Edwards and Lofty 1977, Lee 1985, Edwards and Bohlen 1996). One of the important effects of earthworms on soil habitats is their production of large amounts of faeces. Over the season they excrete from several to over 200 tons ha^{-1} of dry weight casts (Lee 1985). To date, the majority of studies into the role and properties of these characteristic soil forms have considered mineral soils (Brown 1995). Only a few papers have been concerned with organic soils (Kozlovskaya 1969, 1976, Chmielewski and Makulec 1993; Makulec and Chmielewski 1994). The high organic matter, moisture contents, and microfloral activity of these soils, can create relationships between microorganisms and earthworms that are different from those in mineral soils.

This paper therefore compares the abundance of the main microfloral groups, and enzymatic activity, in casts of *Lumbricus rubellus* Hoffm. from several drained peaty meadows, and in soil samples from those meadows.

2. STUDY AREA AND METHODS

The study was conducted on fens drained about 30 years ago in the lower basin of the river Biebrza, Poland (53° 10' N, 22° 30' E). Recently these areas have been used as hay meadows. Three meadows developed on peats of different origin were studied: A) a meadow on sedge-moss peat, B) a meadow on tall-sedge peat, and C) a meadow on alder peat.

The type of peat largely determines the physico-chemical properties of soils, with sedge-moss peats being characterized by low porosity, a high water table, and a high soil moisture content. In turn, alder peats have high porosity, as well as low soil moisture and a low water table, while tall-sedge peats occupy an intermediate position along this gradient (Table 1).

Table 1. Physical soil properties in the different sites

Soil properties	A sedge-moss peat	B tall-sedge peat	C alder peat
Bulk density (g cm^{-3})	0.233	0.249	0.291
Moisture (%)	72.2	56.8	44.3
pH in KCl	5.18	5.49	5.36

The abundance of earthworms was estimated by the formalin extraction method (Raw 1959). Surface areas of 0.3 m × 0.3 m were flooded with 0.3% formaldehyde, and the emerging earthworms were collected. At each site, 10 such samples were taken at monthly intervals between May and October 1995.

Fresh casts were collected on August 28th 1995, the vegetation on several delineated plots was cut and all the casts produced earlier removed, the following day, fresh casts were collected from the surface in quantities necessary for microbiological analysis. The casts of *L. rubellus* contained visible fragments of poorly-decomposed plant material and it was therefore possible to distinguish them from those of the other earthworm species, including the similarly-sized *Octolasion lacteum* (Oerley). At the same time, control soil samples 10 cm deep were taken and divided into two layers corresponding to depths of 0–5 cm and 5–10 cm. It is in this part of the soil profile that *L. rubellus* – the dominant species on peaty meadows – occurs.

The abundance of microbial cells was determined by the plate method and expressed per gram of soil. The total number of bacteria was determined on agar culture with soil extract. The abundance of fungal colonies was estimated on Martin synthetic medium (1950). Actinomycetes were estimated on Kuster-Williams medium (1964) and cellulolytic microorganisms on Omelianski's medium (Rodina 1968). For counts of ammonifying bacteria, we used nutrient agar with meat broth. The abundance of microorganisms utilizing mineral forms of nitrogen was determined on nitrate starch agar.

The plate method determines not only the abundance of living microorganisms but also that of forms remaining as spores. For this reason, the research also considered soil urease and dehydrogenase, whose activity depends first and foremost on living microorganisms. Urease activity was determined using the Hoffmann and Teicher method (1961), while dehydrogenase activity was estimated by employing the method developed by Casida *et al.* (1964).

Two-factor ANOVA and t-test were used for statistical analyses of results.

3. RESULTS

3.1. MEAN ABUNDANCE AND BIOMASS OF LUMBRICIDAE IN THE SEASON

Among the three study sites, it was the meadow with tall-sedge peat (B) that had the highest mean density of earthworms, with almost 250 indiv. m^{-2} $season^{-1}$ (Table 2). Lumbricidae were also relatively abundant in the meadow with sedge-moss peat. The lowest mean density – of only 27 indiv.m^{-2} $season^{-1}$ was noted in the meadow with alder peat. Mean biomass showed similar trends, and ranged from 11 g m^{-2} $season^{-1}$ (C) to 62 g m^{-2} $season^{-1}$ (B).

Table 2. Mean (n = 10) density and biomass of earthworms in 1995 growth season (May–October)

	A sedge-moss peat	B tall-sedge peat	C alder peat
indiv. m^{-2}	183.3	249.6	27.0
g fresh wt m^{-2}	54.9	62.1	11.1

3.2. SPECIES COMPOSITION, NUMBERS AND BIOMASS OF LUMBRICIDAE AT THE TIME OF CAST COLLECTION

In the period of cast collection, the numbers and biomass of earthworms formed a clear gradient from the highest values in site A to the lowest values in site C, in agreement with the gradient of soil moisture (Table 3).

Table 3. Numbers, biomass and earthworm species composition on time at cast collection (28th Aug. 1995)

Species		A sedge-moss peat			B tall-sedge peat			C alder peat		
			S.E.	%		S.E.	%		S.E.	%
Lumbricus rubellus	N	220.8	(26.4)	67.4	101.4	(11.7)	62.4	17.8	(8.3)	100
Hoffm.	B	75.4	(10.5)	84.3	66.5	(10.8)	91.2	13.7	(6.4)	100
Octolasion lacteum	N	62.5	(12.2)	19.1	1.4	(1.1)	0.8			
(Oerley)	B	10.4	(2.0)	11.7	1.7	(1.4)	2.3			
Dendrobaena octaedra	N	29.2	(6.8)	8.9	45.8	(8.5)	28.2			
(Sav.)	B	2.3	(0.6)	2.6	3.0	(0.5)	4.1			
Dendrodrilus rubidus	N	15.3	(7.2)	4.6	13.9	(6.0)	8.5			
(Sav.)	B	1.3	(0.5)	1.4	1.7	(0.8)	2.4			
Total	N	327.8			162.5			17.8		
	B	89.4			73.0			13.7		

N – number of indiv. m^{-2}, B – biomass, g fresh wt m^{-2}, S.E. – standard error.

Four earthworm species were recorded. The clear dominant was *L. rubellus*, and this was the only species occurring in the meadow with alder peat. At the two remaining sites, *L. rubellus* accounted for more than 60% of the total abundance of Lumbricidae, and for 84% (A) and 91% (B) of the total biomass. It can thus be assumed that the casts collected were produced primarily by this species.

3.3. ABUNDANCE AND ENZYMATIC ACTIVITY OF THE MICROFLORA OF CASTS AND SOIL

In most cases, microbial counts were higher in casts than soils and generally the lowest values were observed in the deepest soil layer (Table 4). Only in the case of soil bacteria was there an exceptionally small rise or even a fall in abundance in casts compared with soil. In the case of actinomycetes, abundance in casts was considerably greater than in soil, and the cast/soil ratio was greatest at sites where this group was less abundant in the soil. Cellulolytic organisms showed similar trends. Fungi were always more numerous in casts, and their abundance was lower in line with the soil moisture gradient of the sites, and the situation was thus the converse of that in soil. An interesting comparison between soils and casts was that involving the ratio between bacterial and fungal number. In the upper soil layer, the ratio for sites A, B and C were 139, 104 and 66 respectively. In contrast, the values taken by this ratio in casts were markedly lower, and more uniform, at 25, 22 and 29 respectively.

Microorganisms engaged in ammonification and using mineral nitrogen were particularly numerous in casts and the soil under meadow on tall-sedge peat (B). This attests to the intensive processes of transformation of nitrogenous substances ongoing at this site.

Two-factor analyses of variance showed the significant influence of casts and soil layer on the abundance of all of the microfloral groups (Table 6). The F values for this factor were much higher than those for peat origin (sites). The interactions are also statistically significant.

Dehydrogenase activity in casts was similar at all sites and was always higher than in soil, especially in the case of alder peat site (Table 5).

Urease activity was 2–4 times as great in casts as in soil. The highest level of activity was noted for meadow B – a finding clearly linked with the great abundance there of microorganisms responsible for ammonification or the utilization of mineral nitrogen. As in the case of microfloral abundance, enzymatic activity reflected more closely the influence of casts or soil than site (Table 6).

Table 4. Microfloral abundance (n = 10) in earthworm casts (a) and in peat soil: 0–5 cm (b), 5–10 cm (c) and casts soil ratio

		A sedge-moss peat			B tall-sedge peat			C alder peat		
		mean	S.E.	casts/soil ratio	mean	S.E.	casts/soil ratio	mean	S.E.	casts/soil ratio
Bacteria (10^7 g^{-1})	a	22.0	(1.8)		16.9	(1.0)		19.8	(1.8)	
	b	20.9	(1.7)	1.0	19.7	(1.1)	0.8	15.3	(1.1)	1.3
	c	12.5*	(1.5)	1.8	22.8*	(1.6)	0.7	14.1*	(1.3)	1.4
Actinomycetes (10^6 g^{-1})	a	24.0	(2.9)		9.2	(0.8)		9.4	(1.0)	
	b	2.7*	(0.5)	8.9	8.6	(1.7)	1.1	2.8*	(0.6)	3.3
	c	0.2*	(0.1)	120	11.1	(1.3)	0.8	3.6*	(0.4)	2.6
Fungi (10^6 g^{-1})	a	8.8	(1.2)		7.8	(0.6)		6.9	(0.6)	
	b	1.5*	(0.3)	5.8	1.9*	(0.3)	4.1	2.3*	(0.3)	3.0
	c	0*	(0.0)	100	1.3*	(0.3)	6.0	0.4*	(0.1)	17.2
Ammonifying microorganisms (10^7 g^{-1})	a	21.3	(1.7)		58.9	(4.9)		16.6	(2.7)	
	b	13.9*	(1.9)	1.5	10.3*	(1.4)	5.7	9.9*	(0.9)	1.7
	c	7.9*	(0.7)	2.7	20.6*	(2.3)	2.8	10.4	(1.4)	1.6
Microorganisms utilizing mineral nitrogen (10^7 g^{-1})	a	44.8	(4.5)		53.1	(3.3)		34.1	(4.2)	
	b	16.8*	(1.0)	2.6	29.1*	(2.9)	1.8	15.3*	(1.3)	2.2
	c	3.6*	(0.7)	12.4	39.4*	(2.8)	1.3	23.4*	(1.3)	1.4
Cellulolytical microorganisms (10^4 g^{-1})	a	14.2	(2.5)		7.4	(1.1)		8.0	(1.2)	
	b	8.3*	(0.8)	1.7	8.8	(0.9)	0.8	5.0*	(0.3)	1.6
	c	1.0*	(0.1)	14.0	4.0*	(0.3)	1.8	1.9*	(0.3)	4.2

*significant difference ($P \leq 0.05$, t-test) relative to earthworm casts; S.E. – standard error.

Table 5. Enzymatic activity (n = 5) in earthworm casts (a) and in peat soil: 0–5 cm (b) and 5–10 cm (c)

		A sedge-moss peat			B tall-sedge peat			C alder peat		
		mean	S.E.	casts/soil ratio	mean	S.E.	casts/soil ratio	mean	S.E.	casts/soil ratio
Dehydrogenase (μl H_2 g^{-1} $24h^{-1}$)	a	107.8	(3.1)		112.2	(6.4)		110.8	(3.0)	
	b	105.9	(2.8)	1.0	82.5*	(0.8)	1.4	5.2*	(0.2)	21.3
	c	38.4*	(0.7)	2.8	50.6*	(1.8)	2.2	4.5*	(0.1)	24.6
Urease (ppm NH_4-N $18h^{-1}$)	a	154.7	(5.5)		196.2	(4.7)		150.7	(3.3)	
	b	71.1*	(1.1)	2.2	61.9*	(1.9)	3.2	44.5*	(1.1)	3.4
	c	54.4*	(2.2)	2.8	47.4*	(2.2)	4.1	39.1*	(0.8)	3.8

*significant difference ($P \leq 0.05$, t-test) relative to earthworm casts; S.E. – standard error.

Table 6. Results of two-factor ANOVA analysis of microfloral abundance and enzymatic activity in casts and soils differing by peat origin. F values and levels of significance are shown

	Peat origin (sites) (d.f. = 2)	Place (casts, soil layers) (d.f. = 2)	Interaction (d.f. = 4)
Microflora:			
Bacteria	3.96*	3.35*	8.43***
Actinomycetes	9.51***	51.56***	28.71***
Fungi	0.52n.s.	115.11***	2.44*
Ammonifying microorganisms	52.78***	76.89***	29.37***
Microorganisms utilizing mineral nitrogen	39.75***	66.44***	10.05***
Cellulolytical microorganisms	5.19**	36.66***	6.12***
Enzymatic activity:			
Dehydrogenase	267.34***	549.12***	79.88***
Urease	48.70***	1000.00***	27.14***

d.f. – degree of freedom, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, n.s. – not significant.

4. DISCUSSION

L. rubellus, the dominant species in the soil of the studied meadows, is a litter-feeder living mainly in the upper soil layer. Piearce (1978) analysed the gut contents of this species and found large amounts of poorly-decomposed plant material and a small admixture of mineral soil fraction. Under laboratory conditions, earthworms of this species excrete 1.6–3.6 g d.w. of casts per gram biomass per day (Martin 1982). The transit time for food is relatively long, 6–8 hours (Daniel and Anderson 1992). *L. rubellus* has well-developed calciferous glands which neutralize acid components of the gut contents (Piearce 1972). All of these features favour the mass appearance and development of microflora. It is known that in the alimentary canal of earthworms there occur the same species of microflora as in the soil (Satchell 1983). An increase in their numbers in fresh earthworm casts as compared with the soil, is largely an effect of their food preference. *L. rubellus* consume large amounts of plant remains abundantly colonized by microorganisms. It is also known that in the hindgut, the overall abundance of bacteria, actinomycetes and fungi is several times higher than in the foregut or the soil (Kozlovskaya 1976, Krištůfek *et al.* 1992). According to Kozlovskaya (1969, 1976), the relative rise in the abundance of microflora in the gut and casts of *L. rubellus* is dependent on the availability of "new" plant litter. In summer, when there are few such fragments, the rise in question is only a 2–3 fold one. In contrast, it may be as great as 10–40 fold in autumn. This may also account for the relatively limited increase in the abundance of microflora noted in this study.

It is notable that the greatest relative difference between microfloral abundance in casts and that in the nearby soil was found in sites where abundance in the soil was low. This was true for bacteria, fungi and actinomycetes, as well as for soil enzymes, particularly dehydrogenase (Tables 4 and 5). Such a regulatory function has already been attributed to *L. rubellus* on similar meadows (Chmielewski and Makulec 1993).

There are also differences between casts and the soil in the relative representation of different microfloral groups. While the abundance of bacteria is either little higher or even lower in casts, there is an increase in fungi and actinomycetes – groups less sensitive to variations in soil temperature and humidity and capable of breaking down the complex organic substances of peaty mucks. This may enhance the unfavourable phenomenon of the disappearance of drained peats, especially where the influx of fresh plant residues is limited (Makulec 1991).

The level of activity of soil enzymes reflected well the state of the microflora at a

given time. In the same way that the microflora is more abundant in casts than in soil, the level of activity of dehydrogenase and urease was several times greater in casts. The high level of urease in faeces from meadow B correlates clearly with the great abundance there of organisms engaged in ammonification or using mineral nitrogen. With the highest mean abundance and biomass of Lumbricidae also, it is this site which has exceptionally intensive processes of nitrogen transformation.

Comparisons of the results from peaty soils with those from mineral soils do not reveal more significant differences where the microflora of the soil and of *L. rubellus* casts is concerned (Satchell 1983, Daniel and Anderson 1992, Krištůfek *et al.* 1992, Brown 1995). However, the relative rise in the abundance of most groups – and particularly of bacteria – was much more marked in the above studies than in the present one. This is probably a result of seasonal differences rather than of the specifics of peaty soils (Kozlovskaya 1976).

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5. SUMMARY

The abundance of the main microfloral groups, and dehydrogenase and urease activity, in the casts of *Lumbricus rubellus* Hoffm. and in the 0–5 cm and 5–10 cm soil layers of drained peaty meadows were compared. The study was conducted on three meadows developed on peats of different origin: A) a meadow on sedge-moss peat, B) a meadow on tall-sedge peat, and C) a meadow on alder peat (Table 1).

Among the three study sites, it was the meadow with tall-sedge peat that had the highest mean density and biomass of earthworms (Table 2). Lumbricidae were also relatively abundant in the meadow with sedge-moss peat. The lowest mean density and biomass was noted in the meadow with alder peat.

In the period of cast collection, the numbers and biomass of earthworms formed a clear gradient from the highest values in site A to the lowest values in site C, in agreement with the gradient of soil moisture (Table 3). Four earthworm species were recorded. The clear dominant was *L. rubellus*, and this was the only species occurring in the meadow with alder peat.

In most cases, microbial counts were higher in casts than soils and generally the lowest values were observed in the deepest soil layer (Tables 4 and 6). In the cases of fungi, bacteria and actinomycetes, the degree to which abundance was greater in casts was highest at sites where the abundance of microflora in the soil was low. Dehydrogenase activity in casts was similar at all sites and was always higher than in soil, especially in the case of alder peat site (Table 5). Urease activity was 2–4 times as great in casts as in soil. The highest level of activity was noted for meadow B (Tables 5 and 6).

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