Biol Fertil Soils (1998) 27:79–84

ORIGINAL PAPER

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Significance of earthworms in stimulating soil microbial activity

Received: 26 August 1997

Abstract The stimulatory effect of earthworms (*Lumbricus terrestris* L.) on soil microbial activity was studied under microcosm-controlled conditions. The hypothesis was tested that microbial stimulation observed in the presence of a soil invertebrate would be due to the utilization of additional nutritive substances (secretion and excretion products) that it provides. Changes in microbial activity were monitored by measuring simultaneously CO_2 release and protozoan population density. The increase in CO_2 released in the presence of earthworms was found to result from both earthworm respiration and enhanced microbial respiration. The stimulation of microbial activity was confirmed by a significant increase in protozoan population density, which was 3–19 times greater in the presence of earthworms. The respiratory rate of *L. terrestris* was estimated to be 53 $\mu l\ O_2\ g^{-1}\ h^{-1}$. Earthworm respiration significantly correlated with individual earthworm weight, but there was no correlation between the increase in microbial respiration and earthworm weight. This finding does not support the hypothesis given above that enhanced microbial respiration is due to utilization of earthworm excreta. A new hypothesis that relationships between microbial activity and earthworms are not based on trophic links alone but also on catalytic mechanisms is proposed and discussed.

Key words Soil microflora · Earthworm · Soil biotic interactions · CO_2 release · Protozoa

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Introduction

It is a well established fact that earthworms, like other representatives of soil-inhabiting fauna, modify the biological activity of soils (Satchell 1983; Daniel and Anderson 1992; Edwards and Bohlen 1996). This phenomenon is observed in casts (epigeic feces) and/or burrows, which are lined with mucus and other deposits like those found in endogeic feces; it has especially been demonstrated for anecic species of the genus *Aporrectodea* (Kretzschmar 1987) and the burrowing species *Lumbricus terrestris* (Jeanson 1979; Binet and Curmi 1992). Earthworm deposits have a localized effect on microbial behavior. In particular, they induce a population increase in the main functional groups (Loquet et al. 1977).

However, the basic mechanisms of how earthworms affect microbial populations are not yet well understood. One of the main hypotheses reported in the literature (Lee 1985) is that microbial stimulation observed in the presence of a soil invertebrate might be due to the utilization of additional nutritive substances (secretion and excretion products) that the invertebrate provides. It should be possible to verify this assumption by testing for a relationship between microbial CO_2 output and the individual weight of the worms responsible for the additional C resource supply. The work reported here was a preliminary attempt to quantify the stimulatory effect of earthworms on microbial activity.

To ensure that the enhanced activity of microbes was largely dependent on the utilization of substances released by the worms, highly simplified microcosm experiments were set up. Earthworms were forced to continuously burrow and crawl over a small quantity of soil, thus maximizing the earthworm's deposits, i.e. intestinal mucus and especially cutaneous mucus, within a small volume of soil. The experiments were also designed so that worm respiration could be distinguished from increased microbial respiration in the presence of earthworms. Changes in microbial activity were moni-

tored by measuring CO_2 release and protozoan population density.

Materials and methods

Microcosms

The earthworms were collected by the formalin method from a permanent pasture (I.N.R.A. Centre, Dijon), and were then maintained in culture on the parent soil at 17 °C for 1 month. Prior to the start of the study, the worms were individually placed on the soil utilized for the study and, 4 days later, were transferred to a fresh batch of the same type of soil.

Soil for the study was taken from a depth of 0—30 cm in a plot cultivated with maize since 1975, located on the Rheu experimental site near Rennes (Brittany, France). This soil consists mainly of fine particles (75% silt and 13% clay), is very poor in organic matter (1.7%) and slightly acid (pH_{H_2O} 5.5). It has a total N content of 0.1%, which, although low, is typical of intensively cultivated soils (Table 1). The soil was air-dried and sieved through a 2-mm screen. Each dish (Petri dish) received 10 g soil (dry basis) moistened to 23% dry weight, and one earthworm of the species *L. terrestris*. Mean individual earthworm weight was 2.9 ± 1.1 g and 2.9 ± 1.4 g for test 1 and test 2, respectively. The covered Petri dishes were then incubated in a darkened room at 20 °C. Both CO_2 release and the number of protozoa were monitored every 2 days for 16 days.

Table 1 Texture and chemical characteristicsof the soil (silty soil from the Rennes basin, France). Results expressed as percentages of air-dried soil

Texture		Soil organic matter	1.7
Clay	13.0	Organic carbon (Anne)	1.0
Fine silt	25.0	Total Nitrogen (Kjeldahl)	0.1
Coarse silt	50.0	Total calcium	0.34
Fine sand	7.0	Totl potassium	1.40
Coarse sand	5.0	Total phosphoric acid	0.16
$pH_{(H_2O)}$	5.5		
$pH_{(KCl)}$	5.0		

Experimental design

Two different tests were run. Test 1, which yielded cumulative measurements, consisted of incubating the microcosms in CO_2 collection jars and changing the vials of NaOH every 2 days. Under these conditions, the same microcosms and worms were monitored over time (continuous measurements). In test 2, the microcosms were incubated in the open air and were only enclosed in jars for the 48 h of CO_2 monitoring. In this case, successive measurements were effected on different microcosms and worms. Test 1 included only two treatments: soil with earthworms (SW) and soil without earthworms (S). Test 2 included a third treatment, in which the worm was removed prior to placing the microcosm in the respirometry jar (SWR). This was done to distinguish CO_2 released during the earthworm's respiration (SW–SWR) from that released due to the stimulation of microbial respiration (SWR–S) with respect to the overall respiratory activity in the presence of earthworms (SW–S). The microcosms utilized for counting the number of protozoa were incubated in the open air. The tests were run five times for each treatment.

CO_2 measurements and protozoan counts

CO_2 release was monitored by enclosing the microcosms in hermetically sealed, 1-l jars containing a vial of 0.2 N NaOH. The trapped CO_2 was colorimetrically assayed in a continuous-flow autoanalyzer (Technicon). The amoebae were counted following the method of Darbyshire et al. (1974).

Analyses of variance followed by a Newman-Keuls test were run to assess the effects of worm treatments on CO_2 output. Statistical differences for protozoa were analyzed according to Alabouvette et al. (1981), i.e. two results differ significantly at the 95% confidence level if their ratio is greater than 2. A regression test was done to analyze the relationship between CO_2 release and individual earthworm weight (log-transformed data).

Results

Soil respiration and protozoan density

Earthworm weight remained stable during the study, with an overall mean loss of around 3% in the first test and a gain of 1.8% in the second test. Mortality was relatively low, i.e. 2.7% of all worms used in the experiments. Earthworm activity, assessed as a function of cast production rate, was high, since most of the worms transformed all the soil in the dishes into casts over a 48-h period.

The two tests, i.e. closed vessels and open air, yielded very similar results under the same treatments. Comparison of the SW and S treatments revealed that overall CO_2 release from the microcosm was around five times more in the presence of an earthworm (Tables 2, 3). The soil worked by the earthworm in the SWR treatment from which the worm was removed prior to assaying CO_2, released 1.5 times more CO_2 than did the control soil (treatment S). The analysis of variance showed that the SWR results differed significantly from those for S ($F = 106$; $F_{(1,6)}\ 0.01 = 13$). The Newman-Keuls test showed that the mean CO_2 output from SWR was significantly higher than the mean CO_2 releasd from S (Fig. 1). The stimulation of microbial activity in the presence of earthworms was confirmed by a significant increase in the protozoan population densi-

Table 2 Test 1: continuous measurements of CO_2 release. Means expressed as μg CO_2-C per microcosm over 48 h (5 replicates). Mean individual earthworm weight: 2.908 ± 1.108 g

Treatments	Time (days)							
	0–2	2–4	4–6	6–8	8–10	10–12	12–14	14–16
Soil alone	1874 ± 273	1260 ± 37	750 ± 47	1411 ± 120	854 ± 120	594 ± 31	756 ± 190	711 ± 130
Soil + worm	5987 ± 1358	5646 ± 499	5224 ± 1405	4912 ± 1522	5534 ± 1641	4529 ± 1752	4551 ± 1577	5944 ± 1887

Table 3 Test 2: measurements of CO_2 release; mean (±SE) expressed as μg CO_2-C per microcosm over 48 h. Protozoan counts expressed as number per gram of dry soil. Mean individual earthworm weight was of 2.912±1.407 g. The worm effect was separated into two components: worm respiration and microbial stimulation

Treatments	Time (days)							
	0–2	2–4	4–6	6–8	8–10	10–12	12–14	14–16
	CO_2 release (μg CO_2-C microcosm^{-1} 48 h^{-1})							
Soil alone (S)	1874± 273	1072± 70	855± 22	1007± 167	1253± 236	916± 65	970± 101	886± 94
Soil+worm removed (SWR)		1599± 319	1254± 159	1604± 405	1768± 168	1512± 399	1291±220	1603±445
Soil+worm (SW)	5987±1358	4950±1822	4180±1203	4564±1388	5403±1857	4638±1310	4637±644	5470±283
Worm effect (SW–S)	4113	3878	3325	3557	4149	3722	3666	4584
Worm respiration (SW–SWR)		3352	2926	2804	3634	3126	3346	3867
Microbial stimulation (SWR–S)		526	399	752	514	596	320	717
	Protozoa density (no. g^{-1} dry soil)							
S	–	213± 43	480± 124	448± 98	335± 74	387± 72	341± 39	367± 73
SW	–	4047±1416	3488±1680	2213± 578	1110± 376	1056± 138	1119±132	1151±254

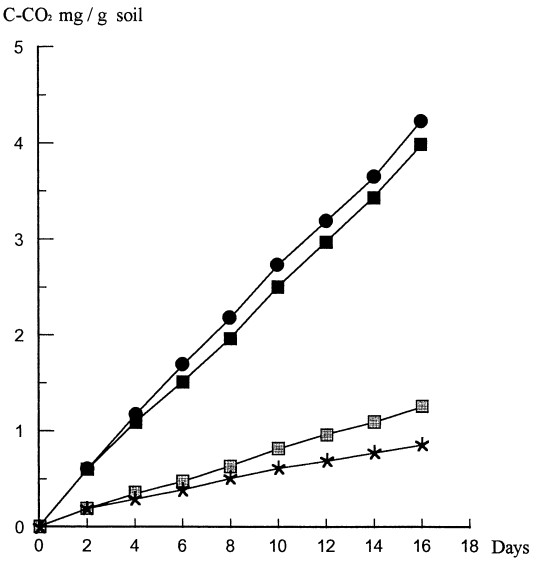

Fig. 1 Cumulative changes in respiration under the different worm treatments for test 1 and test 2. Soil + worm: test 1 (*black circle*), test 2 (*black square*). Soil + worm removed: test 2 (*gray square*). Soil alone: test 1 and test 2 curves combined (*asterisk*)

ty, which was 3–19 times higher in the SW treatment than in the control (Table 3). Both CO_2 release and the protozoan count were found to be highest in the initial measurements. Thereafter, they remained quite stable despite some variability in the data probably related to variations in soil moisture content.

The difference (SW–SWR), showing CO_2 release by the earthworms alone, allowed us to calculate a mean rate of CO_2 output by the earthworms, which was 42.4 μl g^{-1} live worm h^{-1}. The same calculation using SW–S, which corresponded to the overall respiratory activity in the presence of the worm, gave a mean rate 49.4 μl g^{-1}, live worm h^{-1}, i.e. an overestimation of 16.5%.

Hypothesis test

The well-known relationship between respiration and individual weight was expressed by the following equation:

$$R = a P^{1-b},$$

i.e. in the logarithmic form:

$$\log R = (1-b)\log P + \log a,$$

where R is respiration (μl CO_2 g^{-1} live worm h^{-1}), P is worm live weight (mg), and a and b are constants. The value of a depends on the systematic position of the animal studied, and that of b varies little from 0.25 for the entire animal kingdom (Fenchel 1974; Lavigne 1982).

Hence, we attempted to verify the existence of this relationship on the basis of our results. For each of the seven points in time when measurements were taken, the five SWR values were subtracted from the five corresponding SW values (Table 3). Taking the death of two worms into account, 33 R values were thus obtained, associated with 33 P values for the worms in treatment SW; earthworm weight ranged from 1000 mg to 6000 mg (on fresh matter). After logarithmic transformation of all data, analysis of variance showed the existence of a significant relationship between R and P (Fig. 2), with a correlation coefficient of $r = 0.85$ ($F = 86$; $F_{(1,32)}0.01 = 7.5$). The value obtained for b (0.28) was very close to the generally accepted mean value (0.25).

Regarding the respiratory surplus (SWR–S), it may be assumed that it is a measure of the respiration of microflora corresponding to the utilization of carbon-

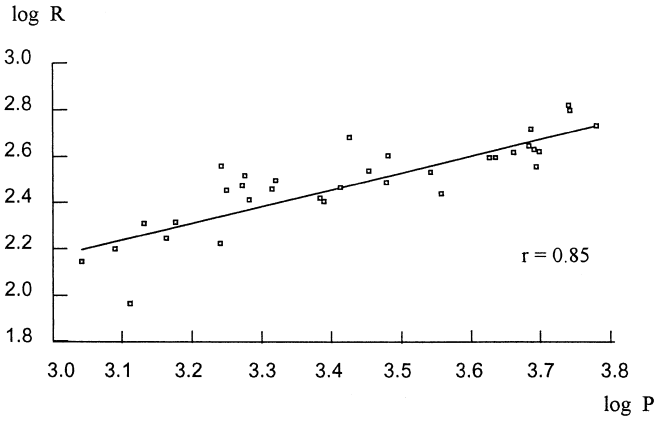

Fig. 2 Logarithmic relationships between amounts of CO_2 released during worm respiration (*log R*) and the individual weight of the worms (*log P*); $r=0.85$, $P<0.01$

eous substances excreted from the gut and body surface of a worm of a given size. So we tested for a relationship between the stimulation of microbial respiration (SWR–S) and weight of the removed worms. Analysis of variance showed no correlation between the logarithms for the two variables ($r=0.053$; $F<1$) (Fig. 3). These results are discussed below.

Discussion

The reason for not providing any food (plant litter) for the worms during the study was to restrict the dependence of increased microbial activity on the utilization of substances released by the worms (secretions and excretions). Under these simplified experimental conditions, the only earthworm activities affecting the soil were locomotion and casting, and the effects of the nutrient-enrichment process defined by Devliegher and Verstraete (1997) on microbial activity could be avoided. Besides secreting mucus from their body surface, the worms ingested soil. Soil ingestion was more rapid than that reported in the literature, i.e. around 0.8 g dry soil g^{-1} dry worm day^{-1}, versus the 0.3 g, 0.3–0.9 g and 0.1 g dry soil g^{-1} dry worm day^{-1} reported by Hartensein and Amico (1983), Shipitalo et al. (1988) and Satchell (1967), respectively. This behavior was probably due to both the absence of plant litter and presence of homogenized, non-compacted soil that was easy to ingest. Considering the stability of the earthworms weight, it is likely that they continuously ingested soil throughout the study. This also suggests that *L. terrestris* could be coprophagous in certain environments, such as our special incubation conditions, which contrasts with the observation of Hartensein and Amico (1983).

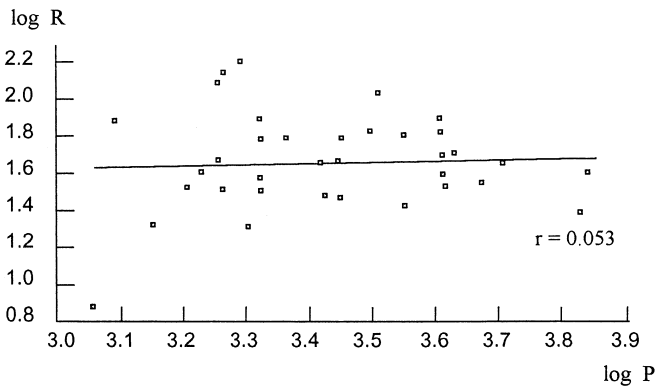

Fig. 3 Logarithmic relationships between amounts of CO_2 released as a consequence of microbial stimulation (*log dR*) and the individual weight of the worms (*log P*); $r=0.053$, $P>0.05$

We showed that the increase in overall biological soil activity observed in the presence of worms was due not only to the latter's metabolic activity, but also to the stimulation of microflora, whose basic activity increased by around 50%. In a study on the tropical worm *Pontoscolex corethrurus,* Barois (1987) noted a stimulation of 30% of the soil microflora above baseline. Many studies report on microbial stimulation in fresh casts, burrow walls and soils burrowed by worms (Parle 1963; Loquet et al. 1977; Satchell and Martin 1984; Shaw and Pawluk 1986; Scheu 1987; Binet 1989; Tiwari et al. 1989; Daniel and Anderson 1992; Hendriksen 1997). In our study, the stimulatory effect of worms on soil microflora was confirmed by the substantial increase in the protozoan population density.

Higher protozoan activity, measured as the number of active protozoa, has been previously observed in urban compost in the presence of *Eisenia fetida* (Rouelle et al. 1985) and within walls of burrows dug by *L. terrestris* in soil columns incubated at 15 °C (Binet 1989). Recently, Winding et al. (1997) reported an increase in protozoan activity caused by a higher number of active bacteria in the presence of the epigeic species, *Lumbricus festivus.* Meanwhile, epigeic and endogeic earthworms have been found to prey on soil protozoa (Miles 1963; Bonkowski and Schaefer 1997). In soils, predation (grazing) of bacteria by protozoa is a well-known food-web interaction (Singh 1946; Pussard 1971; Griffiths 1989; Clarholm 1981; Kuikman et al. 1991). Reviewing soil biotic interactions in agroecosystems, Andren et al. (1988) concluded that bacterivorous organisms (amoebe, nematodes, etc.) are better indicators of bacterial activity than the bacterial biomass. So, in accordance with Winding et al. (1997), the increased numbers of amoebae observed in our study must be related to the marked increase in the bacterial population in the soil burrowed by *L. terrestris.* The experimental procedure of the second test enabled evaluation of the respiratory activity of *L. terrestris*. Assuming a respiratory quotient of 0.8 (Konopacki 1907; Phillipson and Bolton 1976), it was calculated that the mean release of CO_2 g^{-1} h^{-1} corresponded to a mean O_2 intake of 53 μl g^{-1} live worm h^{-1} at 20 °C. This intake was higher than the value of 41 μl O_2 g^{-1} live worm h^{-1} found for *Octalasium lacteum* at 15 °C (Scheu 1991) but lower

than those reported by various authors: 74 μl O_2 g^{-1} live worm h^{-1} at 19 °C for *L. terrestris* (Byzova 1965); 75 μl O_2 g^{-1} live worm h^{-1} at 15 °C for *Allolobophora caliginosa* Sav (Barley and Jennings 1959); 156 μl O_2 g^{-1} live worm h^{-1} at 10 °C for *L. castaneus* (Phillipson and Bolton 1976). However, if we base the calculation on SW–S, rather than SW–SWR as most authors have done, the value obtained (62 μl O_2 g^{-1} live worm h^{-1}) is closer to that reported for the same species at the same temperature by Byzova (1965).

Our results also allowed us to verify that there is a positive correlation between the quantities of CO_2 produced by worms and their individual weight. Given this satisfactory correlation, it may be concluded that experimental errors (variations in water content and, especially, intestinal soil content; error in weighing worms) were low and acceptable.

We further attempted to test whether the microbial stimulation observed in the presence of an invertebrate is essentially due to the utilization of additional nutritive substrates that it provides (secretion and excretion products). To test this hypothesis, we examined whether there was a relationship between additional microbial CO_2 output and the individual weight of the removed worms responsible for the additional food supply (mucus, excreta).

Two initial assumptions were made:

1. Given that casts are N- and P-rich it may be assumed that all carbonaceous substances excreted by the worms were utilized by the microflora.
2. With regard to the relative stability of the respiratory surplus over time (SWR–S), it may be considered that the worms released carbonaceous substances at a constant rate and that the microflora decomposed them at the same rate.

Following from this, it may be concluded that the respiratory surplus, dR, corresponds to the utilization of the quantity, dS, of substances excreted by a worm with weight P, i.e

$$dR = k\,dS$$

Moreover, since dS is produced by earthworm metabolic activity, the following relationships may be established:

$$dS = a\,P^{1-b},$$

hence:

$$dR = k\,a\,P^{1-b},$$

i.e. $dR = K\,P^{1-b}$.

If the microbial respiratory surplus in the presence of an earthworm is due only to the utilization of substrates excreted by the worm, there should be a verifiable correlation between dR and P.

No correlation between the experimental values for these two parameters was demonstrated. However, this result does not enable us to total exclude the hypothesis tested, since certain relationships on which the hypothesis is based may be erroneous, i.e the amount of earthworm deposits (mucus, urine) may not be proportional to the size of the individual worm. As there is no doubt that a relationship between microbial activity and earthworm products exist, it is likely that the relationship is more complex. It is also possible that microbial utilization of earthworm excreta is masked by other phenomena such as:

1. Soil bioturbation by worms that enhances the utilization of soil organic matter and favors soil aeration.
2. Stimulating effects of worms on protozoa which in turn may induce an increase in bacterial activity.
3. Existence of chemical mediators released by earthworms which act at low concentrations on microbial metabolism, as already suggested by Pussard (1991).

The last hypothesis is supported by at least three arguments. The production of stimulating or catalytic substances has already been reported by Levrat et al. (1992), who demonstrated the enhancement of bacterial metabolic activity due to the production of stimulatory factors by amoebae. Secondly, this would be in line with the priming effect related to the secretion of intestinal mucus by earthworms observed by Martin et al. (1987) and Barois (1987). The great activation of bacterial activity during the transit of ingesta through the gut might be due to the secretion of stimulatory substances in addition to carbonaceous substances such as mucus and urine. Thirdly, a poor correlation between microbial abundance and microbial metabolic activity (CO_2 output) has often been observed (Scheu 1987; Asmar et al. 1992). So, all these observations suggest that relationships between microbial activity and earthworms are not based on trophic links alone but also on catalytic mechanisms. We propose the hypothesis that earthworms release chemical mediators along their gut and from their body surface, or indirectly through protozoa that they activate, which act at low concentrations on microbial metabolism, as vitamins or chemical catalysts do.

In conclusion, additional microcosm experiments involving various earthworm species are required to provide quantitative evidence for the stimulatory effect of earthworms on microbial activity. Further studies based on the use of axenic earthworms are also needed to test our hypothesis. In accordance with the findings of Binet and Trehen (1992), the results obtained in the present study showed that the difference between the respiratory activity of microbes in the presence and absence of worms could not be attributed solely to the latter, as has been suggested by some authors (Cortez and Hamed 1988; Cortez et al. 1989). This finding suggests that either microbial activity is not affected by animals – a now untenable assumption – or that the term "animal" in fact includes the animal's microbial environment – a confusing definition which may hamper further understanding of faunal-microflora interactions.

Acknowledgements The authors wish to thank Mme Delay for technical assistance with the counts of protozoa.

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