Microbial biomass, biovolume and respiration in *Lumbricus terrestris* L. cast material of different age

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Abstract

Effects of the gut passage, the age of cast material and the type of ingested substrate on the microbial community in *Lumbricus terrestris* faeces were studied in laboratory microcosms containing four soil–litter combinations: lime forest soil + lime litter, beech forest soil + lime litter, beech forest soil + beech litter and beech forest soil without litter. Microbial biomass (SIR method), basal respiration and biovolume of bacteria and fungi were measured in earthworm casts and reference material after 1, 5, 10 and 100 d of incubation. To separate effects of mixing of litter and soil in the gut of *L. terrestris* from specific gut or cast associated processes, actual faecal properties were compared with ‘expected’ values, calculated on the basis of corresponding measurements in soil and litter ingested by *L. terrestris*. Microbial respiration, biomass and fungal volume in fresh (1 d old) faeces strongly depended on the type of soil and litter material consumed by *L. terrestris*. The comparison with ‘expected’ values indicated that microbial biomass and volume changed little during the passage through the gut of *L. terrestris*. In contrast, even a short incubation of casts caused marked changes in the microbial properties studied: microbial biomass sharply declined during the first 5 d of incubation; basal respiration which exceeded ‘expected’ values by 30–120% in fresh casts, decreased steadily and was significantly below ‘expected’ values by d 10 of the incubation; biovolume of bacteria and fungi in fresh casts was 20–60% higher than expected and then steadily declined in lime litter but not in beech litter treatments. Generally, the fungal-to-bacterial volume ratio in cast material was not significantly affected by the gut passage nor by the incubation of casts. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Lumbricidae; Earthworms; *Lumbricus terrestris*; Forest; Leaf litter; Microbial biomass; Bacteria; Fungi; Soil fauna; Soil microflora

1. Introduction

In many temperate ecosystems earthworms are important members of the soil fauna. They are involved in the regulation of organic matter decomposition, nutrient cycling processes and in modifications of soil physical properties. Earthworms and soil fauna in general, are suggested to influence the dynamics of soil chemical processes mainly indirectly, by comminuting the litter and affecting the activity of the soil microflora (Petersen and Luxton, 1982; Lee, 1985; Anderson, 1988; Edwards and Bohlen, 1996). However, the nature and mechanisms of these interactions are still poorly understood.

The effect on soil microorganisms of passage through the earthworm gut is a subject of considerable controversy, in part because different counting procedures, earthworm species, soils and food sources have been used. Early studies, based on classical microbiological techniques such as plate counting, indicated a significant increase of microbial colony forming units (CFUs) in earthworm faeces (reviewed by Brown, 1995). It remained unclear, however, whether the differences were caused by the microbial growth during the gut transit, by selective consump-
tion of organic matter rich in microflora, or by differences in the culturability of microorganisms in ingested and egested materials. In contrast to CFU data, quantitative techniques for microbial biomass measurement indicate little or no increase in microbial biomass in earthworm faeces (Scheu, 1987; Daniel and Anderson, 1992), or in worm-worked soil (Wolters and Joergensen, 1992; Zhang and Hendrix, 1995).

The effect of earthworms on soil processes varies between ecological categories and species. *Lumbricus terrestris* L. was chosen in our study because this species dominates earthworm biomass in various temperate ecosystems and strongly affects organic matter transformation and soil development (e.g. Bouché, 1975; Lee, 1985; Schaefer, 1991; Edwards and Bohlen, 1996). *L. terrestris* is a large deep burrowing species (anecic sensu Bouché, 1977), that builds permanent vertical burrows, but feeds mainly on organic materials on the soil surface. Surprisingly, very little information is available on the effects of *L. terrestris* gut passage on microorganisms. The feeding behaviour of *L. terrestris*, which consumes litter and mineral soil in different proportions, compounds a problem of ‘reference material’ in comparing microbial communities in ingested materials and earthworm faeces (Martin and Marinissen, 1993). An attempt to distinguish between nutrient-enrichment processes (NEP) associated with the organic matter incorporation and gut-associated processes (GAP) associated with the passage of soil and organic matter through the gut of *L. terrestris* was made by Devliegher and Verstraete (1995). They concluded that NEP, but not GAP were responsible for the increased microbial biomass and activity reported in the presence of *L. terrestris*. However, Devliegher and Verstraete did not study *L. terrestris* cast material separately, but the whole earthworm-worked soil of experimental containers.

Few attempts have been made to investigate the dynamics of microbial processes associated with ageing of earthworm cast material. As far as we know, no information, except the work of Parle (1963), exists on temporal changes of microbial biomass and activity in cast material of *L. terrestris* or any other anecic earthworm species. Generally, effects of earthworms on microbial biomass and activity have been shown to depend on soil conditions (Shaw and Pawluk, 1986; Wolters and Joergensen, 1992). Therefore, two soil and litter types were used in the present experiment to examine whether any observed effects, if present, are consistent in different soil–litter systems.

Our aim was to evaluate the effects of the gut passage, the age of cast material and the type of ingested substrate on microorganisms in *L. terrestris* faeces in experimental, but resembling natural, conditions. We tried to overcome the problem of ‘reference material’ by comparing cast microflora characteristics with ‘expected’ values calculated on the basis of respective microbial measurements in soil and litter ingested by *L. terrestris*.

2. Materials and methods

2.1. Soil, litter and earthworms

Soil and litter from two different woodland ecosystems were used in the experiment. These were a lime forest in Russia and a beech forest in Germany. The lime forest is located c. 30 km south of Moscow. The forest is an approximately 100–110 yr old pure lime (*Picea abies*) stand. No shrub layer is present, but young spruce trees (*Picea abies*) are scattered. The herb layer is dominated by *Carex pilosa*, additional herbs are *Stellaria holostea*, *Galeobdolon luteum*, *Asarum europaeum*, *Pulmonaria obscura* and *Dryopteris filix-mas*. The soil is silt loam podzolic, formed on glacial moraine material. The beech forest (Göttinger Wald) is situated in southern Niedersachsen about 8 km east of Göttingen. The canopy layer consists almost exclusively of 115–120 yr old beech (*Fagus sylvatica*) trees. No shrub layer is present. Dominant plants in the herb layer are *Allium ursinum*, *Mercurialis perennis* and *Anemone nemorosa*. The soil is shallow and consists of rendzina, terra fusca and brown earth. Further details are given in Schaefer (1991). *L. terrestris* density is high at both locations (more than 30 ind. m⁻² in the lime forest, about 20 ind. m⁻² in the beech forest; A.V. Tiunov, unpublished data; Schaefer, 1991).

Soil materials were taken in May (lime forest) and June (beech forest) 1996 at a depth of 3–10 cm from the mineral soil surface. Overwintered leaf litter was collected from the soil surface at the same time. Adult specimens of *L. terrestris* were obtained by formalin extraction in the beech forest. Earthworms were washed with distilled water and kept for 2 weeks before starting the experiment in containers with appropriate soil–litter combinations at 15°C, a temperature typical for early summer at the study sites.

2.2. Experimental design and cast collection

Four combinations of soil and litter were used in the experiment: lime forest soil + lime litter (LL); beech forest soil + lime litter (BL); beech forest soil + beech litter (BB) and beech forest soil without litter (B0). The experiment was carried out in vertically arranged containers consisting of two perspex sheets (650 mm high, 310 mm wide) separated by plastic strips (10 mm thick) on either side and at the bottom. Containers were filled with sieved (<4 mm) soil at the level of 500 mm. Soil was compacted to the bulk density of 0.95
(lime forest soil) and 0.65 (beech forest soil) kg dry weight $\text{m}^{-1}$. Moisture content of the soil from the lime and beech forest was kept constant throughout the experiment at 34% and 62% (dry wt), respectively. About 25 g of lime or beech litter was added to each of the LL, BL and BB microcosms and replenished periodically as it was consumed by earthworms. Two adult L. terrestris specimens were placed in each microcosm. Average live weight of the earthworms was $6.0 \pm 0.2$ g. Containers were kept at 15°C in permanent darkness. Five replicates were set up per treatment.

Two weeks after the start of the experiment, by which time a burrow system was established by the earthworms, containers were opened and the accumulated faecal material was carefully collected. This cast material was used for preliminary respiratory experiments. After this first sampling, containers were opened every other day and the newly deposited faeces were removed (average age 1 d). Control soil and litter samples were taken from the zones not affected by L. terrestris activity (at least 50 mm away from the nearest burrow). Part of the collected cast materials were left in the same containers for further 4 or 9 d. Loosely arranged casts were wrapped in 0.5 mm gauze and placed in cavities from where control soil samples had been removed. In this way faecal material of an average age of 5 and 10 d was obtained. Faecal material of an age of 100 d was obtained by incubating casts separated by 0.5 mm gauze between two layers of soil from the lime (LL treatment) or beech (BL, BB and B0 treatments) forest. Soil (about 1 kg) and casts were kept in polyethylene bags in a water-saturated atmosphere at 15°C. A plastic tube (inner dia 3 mm) was inserted in each bag to ensure gas exchange.

2.3. Analytical procedures

Microbial biomass was calculated from the maximum initial respiratory response (MIRR) by the substrate-induced respiration method (SIR; Anderson and Domsch, 1978) using an automated respirometer based on electrolytic $\text{O}_2$ microcompensation (Scheu, 1992). Samples were supplemented with 8, 20 or 80 mg glucose $\text{g}^{-1}$ dry weight of soil, cast or litter material, respectively. Glucose was added as an aqueous solution, adjusting the water content to 60% (dry wt) for lime forest soil, 100% for beech forest soil, 80–120% for cast material from different treatments and to 400% for litter. Respiratory responses were found to be at a maximum at these respective glucose concentrations and moisture contents in preliminary experiments. Samples were incubated at 22°C. Oxygen consumption rates ($\mu$O$_2$ $\text{g}^{-1} \text{h}^{-1}$) were measured every 0.5 h. The mean of the five lowest measurements during first 10 h after glucose addition was taken as MIRR. Microbial biomass C ($C_{\text{mic}}, \mu\text{g} \text{g}^{-1}$) was calculated as $38 \times \text{MIRR}$ (Beck et al., 1997). For basal respiration, the average O$_2$ consumption rate of samples not amended with glucose was measured during 15–20 h after attachment of samples to the respirometer. The specific respiration ($\mu$O$_2$, $\mu$O$_2$ $\text{mg}^{-1} C_{\text{mic}} \text{h}^{-1}$) was calculated from data on microbial biomass and basal respiration.

Bacterial and hyphal biovolume was measured using epifluorescence microscopy as outlined by Scheu and Parkinson (1994). Briefly, about 1 g (fresh weight) of soil and cast samples or 0.5 g of litter were blended (Krups, Ireland) for 60 s with 100 ml sterile 1/4 strength Ringer solution. Aliquots from blended solutions were subsequently diluted to obtain c. 200 $\mu$g (dry wt) of soil or cast and 40 $\mu$g of litter material per membrane (201 mm$^2$) for bacterial and 1 or 0.2 mg, respectively, for hyphal volume measurements. Suspensions diluted for bacterial counts were fixed by 2% formaldehyde and passed through 0.2 $\mu$m Nucleopore polycarbonate membranes. Bacteria on membranes were stained by applying 1 ml 0.1 g l$^{-1}$ acridine orange solution in potassium buffer (pH 7.5) for 2 min. Then membranes were destained by 2 ml isopropyl alcohol. Membranes were examined at 1000× magnification with appropriate filter combinations. Twenty fields per membrane were counted. Bacterial cell volume was estimated using a videocamera attached to the microscope and Optima image-analysis software (final magnification ca. 5400×). The mean cell volume ranged between 0.086 and 0.120 $\mu$m$^3$ and did not differ significantly between the materials studied. Therefore, a mean cell volume of 0.1 $\mu$m was taken to convert bacterial numbers to bacterial volume.

Suspensions diluted for hyphal volume measurement were stained prior to filtering by mixing (1:1) with 2.3 g l$^{-1}$ aqueous solution of calcofluor white. After 4 h of staining 2 ml of the suspension were passed through 0.8 $\mu$m Millipore polycarbonate membranes. Membranes were rinsed with 2 ml sterile water. Hyphal length was measured using the grid-intersection method (Olson, 1950) at 400× magnification, 20 fields were inspected per membrane. Hyphal diameters were measured using the Optima software utility (final magnification on the display ca. 2100×). From 30 to 50 hyphae were measured on each membrane. For each sample the average diameter was calculated and used for estimation of the hyphal volume. No attempt was made to distinguish between fungal and actinomycetal mycelium, but only 10.1% of total mycelia measured ($n = 2977$) had a diameter $\leq 1.0$ $\mu$m. Furthermore, very little actinomycetal mycelium had been found during bacterial countings. Thus, we assumed that the majority of the total mycelium in our samples was represented by fungal hyphae.

Total carbon and nitrogen contents in samples were
determined by an elemental analyser (Carlo Erba, Milan).

2.4. Calculation of ‘expected’ values and statistical analyses

Microbial activities in soil and litter differ markedly. *L. terrestris* consumes both soil and litter materials and the faecal microflora consists of a mixture of microorganisms originating from both sources. Therefore, a direct comparison of microbial activity and biomass in *L. terrestris* faeces with ingested substrates is difficult. The ratio of ingested soil and litter varies broadly, but the fraction of litter material in casts can be approximated by using the following equation:

\[ L = \frac{C_{\text{cast}} - C_{\text{soil}}}{C_{\text{litter}} - C_{\text{soil}}} \]

where \( L \) is the fraction of litter material in casts and \( C_{\text{cast}} \), \( C_{\text{soil}} \) and \( C_{\text{litter}} \) the carbon contents in casts, soil and litter, respectively.

The fraction of litter was determined in each sample of cast material and was used for calculating ‘expected’ values of microbial biomass, volume and respiration on the basis of corresponding values in control soil and litter samples. Thus, ‘expected’ values reflect the microbial biomass and activity in a hypothetical mixture of soil and litter with the same proportion of litter as in the particular set of casts. To separate the effect of mixing of litter and soil materials from specific gut or cast associated processes, differences were calculated by subtracting ‘expected’ values from those actually measured in the respective cast samples. These differences were expressed as percentages of the ‘expected’ values.

Data were analysed using two-factor analysis of variance (STATISTICA software package). Prior to ANOVA, data were tested for normality and homogeneity of variance and log-transformed, if required. Differences between means were tested using Tukey’s honestly significant difference (HSD) at the 0.05 probability level.

3. Results

3.1. Soil properties

Beech and lime forest soils used in the experiment differed considerably in pH, carbon and nitrogen content (Table 1). Microbial biomass, basal respiration and bacterial volume were higher in the beech forest.
soil which was richer in organic matter. However, in comparison to beech forest soil, fungal volume was almost double in the acidic soil from the lime forest. Differences between litter types were less pronounced, but both basal respiration and fungal-to-bacterial volume ratios were substantially higher in lime than in beech litter.

### 3.2. Earthworm activity

Out of 40 earthworms 37 survived the first 45 d of the experiment. The earthworms were active in all microcosms and several cocoons were found during cast collection (on average 6.3 per microcosm). In treatments with lime litter the average fresh mass of earthworms increased by 5.5 ± 2.4 (LL) and 9.1 ± 4.2% (BL). In treatments with beech litter (BB) or without litter (B0) earthworm body mass decreased by 12.1 ± 2.4 and 20.0 ± 4.1%, respectively. Preference for lime litter was confirmed by calculation of the litter content in cast material. Mean content of litter in faeces from the LL and BL treatments reached 12–20% and was significantly higher than that in the BB treatment (about 6%; $F_{1,87} = 99.9$, $P < 0.001$).

### 3.3. Fresh cast material

As indicated by significant treatment effects (Table 2), the microbial community in $L.\ terrestris$ faeces was strongly affected by properties of soil and litter materials ingested. Microbial biomass, respiration and volume in 1 d old casts were at a maximum in treatments with lime litter (Table 3; LL and BL) and significantly lower in the B0 treatment, where $L.\ terrestris$ ingested beech forest soil only. Fresh faeces in the B0 treatment had the same carbon content, but increased nitrogen content as compared to control soil. Microbial biomass was not significantly affected by the gut passage (0.9 and 1.0 mg g$^{-1}$ in soil and fresh casts, respectively) but basal respiration was strongly increased, from 1.7 in soil to 7.3 µl O$_2$ g$^{-1}$ h$^{-1}$ in casts (Tables 1 and 3). Bacterial and fungal volume were significantly higher in casts than in soil, but the fungal-to-bacterial volume ratio only changed a little (1.9 in soil, 1.7 in casts).

### Table 3

Properties of fresh (1 d old) faeces of $L.\ terrestris$. Treatments: LL = lime forest soil + lime litter; BL = beech forest soil + lime litter; BB = beech forest soil + beech litter; B0 = beech forest soil, no litter

<table>
<thead>
<tr>
<th>Treatments</th>
<th>LL</th>
<th>BL</th>
<th>BB</th>
<th>B0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Litter content (%)</td>
<td>12.9 a</td>
<td>18.3 a</td>
<td>5.6 b</td>
<td>0</td>
</tr>
<tr>
<td>C (%)</td>
<td>6.5 a</td>
<td>12.5 b</td>
<td>9.1 a</td>
<td>7.4 a</td>
</tr>
<tr>
<td>N (mg g$^{-1}$)</td>
<td>4.2 a</td>
<td>8.7 b</td>
<td>7.3 c</td>
<td>6.8 c</td>
</tr>
<tr>
<td>C/N (ratio)</td>
<td>15.8 a</td>
<td>15.2 a</td>
<td>12.7 b</td>
<td>11.0 c</td>
</tr>
<tr>
<td>pH (in CaCl$_2$)</td>
<td>5.9 a</td>
<td>7.0 b</td>
<td>7.2 b</td>
<td>7.1 b</td>
</tr>
<tr>
<td>Basal respiration (µl O$_2$ g$^{-1}$ dry wt h$^{-1}$)</td>
<td>28.4 ab</td>
<td>37.8 b</td>
<td>15.1 a</td>
<td>7.3 c</td>
</tr>
<tr>
<td>Microbial biomass (mg C$_{mic}$ g$^{-1}$ dry wt)</td>
<td>2.26 ab</td>
<td>3.52 b</td>
<td>1.48 ac</td>
<td>1.00 c</td>
</tr>
<tr>
<td>$q_{O_2}$ (µl O$<em>2$ mg$^{-1}$ C$</em>{mic}$ h$^{-1}$)</td>
<td>12.0 a</td>
<td>10.7 a</td>
<td>10.1 ab</td>
<td>7.5 b</td>
</tr>
<tr>
<td>Fungal volume (mm$^3$ g$^{-1}$ dry wt)</td>
<td>5.00 a</td>
<td>3.82 b</td>
<td>1.68 c</td>
<td>2.16 c</td>
</tr>
<tr>
<td>Bacterial volume (mm$^3$ g$^{-1}$ dry wt)</td>
<td>1.54 a</td>
<td>1.81 a</td>
<td>1.48 a</td>
<td>1.27 a</td>
</tr>
<tr>
<td>Total microbial volume (mm$^3$ g$^{-1}$ dry wt)</td>
<td>6.53 a</td>
<td>5.63 a</td>
<td>3.17 b</td>
<td>3.43 b</td>
</tr>
<tr>
<td>Fungal-to-bacterial volume ratio</td>
<td>4.0 a</td>
<td>2.2 ab</td>
<td>1.2 b</td>
<td>1.7 b</td>
</tr>
</tbody>
</table>

Different letters in a row indicate significant difference between treatments (Tukey’s HSD test based on log-transformed data, $n = 5$, $P < 0.05$).

### Table 4

Correlations ($r$ values) between carbon content, litter content, and microbial characteristics in fresh (1 d old) faeces of $L.\ terrestris$

<table>
<thead>
<tr>
<th>Carbon content</th>
<th>Litter content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal respiration</td>
<td>0.717**</td>
</tr>
<tr>
<td>Microbial biomass</td>
<td>0.807**</td>
</tr>
<tr>
<td>Fungal volume</td>
<td>-0.074 ns</td>
</tr>
<tr>
<td>Bacterial volume</td>
<td>0.437 ns</td>
</tr>
<tr>
<td>Total microbial volume</td>
<td>0.066 ns</td>
</tr>
</tbody>
</table>
of microbial activity (except bacterial volume) were significantly lower in the latter. In fresh casts the linear correlation of both microbial biomass and respiration with litter content was much stronger than with total C content (Table 4). Fungal, bacterial and total microbial volume were correlated only with litter content, but not with the C content.

### 3.4. Ageing of casts

Total carbon and nitrogen contents varied only slightly between cast samples of different age. The pH values were 0.2–0.7 units higher in 100 d old casts than in fresh casts. Conversely, as indicated by significant age effects, microbial characteristics of *L. terrestris* faecal material were strongly affected by cast age (Table 2). In treatments with lime litter (LL and BL) microbial biomass, basal respiration and fungal volume showed similar patterns (Fig. 1). During 100 d of incubation all three properties significantly decreased in LL and BL treatments. Bacterial volume declined strongly between d 10 and 100. Changes in

![Graph](image.png)

**Fig. 1.** Changes in basal respiration (a), microbial biomass (b), bacterial (c) and fungal (d) volumes in *L. terrestris* cast material during 100 days of incubation. Treatments: LL=lime forest soil+lime litter; BL=beech forest soil+lime litter; BB=beech forest soil+beech litter; B0=beech forest soil, no litter. HSD=Tukey’s honestly significant difference, n=5, P<0.05.

| Table 5 | Two factorial ANOVA table on the effects of Treatment (lime forest soil+lime litter; beech forest soil+lime litter; beech forest soil+beech litter) and Age on the differences between actual and ‘expected’ values of microbial activities in *L. terrestris* casts (for details on the calculation of ‘expected’ values see text). F values; "P < 0.01; **P < 0.001; ns = not significant |
|------------------|------------------|------------------|------------------|
| Treatment (d.f. = 2) | Age (d.f. = 3) | Treatment × Age (d.f. = 6) | % SS explained |
| Basal respiration | 24.2** | 46.3** | 5.6** | 82.1 |
| Microbial biomass | 3.1 ns | 18.3** | 5.1** | 65.6 |
| qO₂ | 27.3** | 38.5** | 8.4** | 82.1 |
| Fungal volume | 0.9 ns | 38.5** | 7.8** | 77.4 |
| Bacterial volume | 7.8* | 9.1** | 3.4* | 56.9 |
| Total microbial volume | 3.0 ns | 51.9** | 7.4** | 81.1 |
| Fungal-to-bacterial volume ratio | 8.5** | 0.1 ns | 4.7** | 48.4 |
Fig. 2. Differences (%) between actual and ‘expected’ values of basal respiration (a), microbial biomass (b), specific respiration (c) and microbial volume (d) in L. terrestris cast material of different age (see text for details on the calculation of ‘expected’ values). Treatments: LL = lime forest soil + lime litter; BL = beech forest soil + lime litter; BB = beech forest soil + beech litter. Bars sharing the same letter are not significantly different, Tukey’s HSD test. Asterisks indicate significant differences between actual and respective ‘expected’ values, Student’s t-test. For both tests $n = 5$, $P < 0.05$. 

microbial activities during cast incubation were less conspicuous in BB and B0 treatments. In both treatments $C_{\text{mic}}$ remained almost constant throughout the incubation. Basal respiration sharply declined from day 5 to d 10; fungal volume increased during the first 5–10 d (Fig. 1).

3.5. Comparison with ‘expected’ values

Generally, differences of the properties studied from ‘expected’ values were not very pronounced, yet were often significant (Fig. 2). Predictions of microbial biomass in fresh cast material were remarkably close to the actual values; in all three treatments the difference was less than 10% and not significant.

As revealed by 2-way ANOVA, differences between ‘expected’ and actual values of microbial biomass, fungal and total microbial volume in L. terrestris faeces were not significantly affected by treatments (Table 5, cf. Table 2). This indicates that similar alterations of microbial communities occurred during passage through the gut of L. terrestris in all treatments with litter materials. Conversely, cast incubation strongly affected all properties studied except the fungal-to-bacterial volume ratio. Significant treatment x age interactions indicate that the microbial succession differed in cast material from different treatments (Table 5). However, temporal changes of $C_{\text{mic}}$ and basal respiration were generally similar in all treatments. Basal respiration which exceeded ‘expected’ values by 30–120% in fresh casts, decreased steadily and was significantly below ‘expected’ values by d 10 of the incubation (Fig. 2). Microbial biomass declined sharply during the first 5 d, especially in LL and BL treatments.

Differences between ‘expected’ and actual values in microbial volume changed in a similar way in LL and BL treatments, with a maximum in fresh (+58 and +57%) and a minimum in 100 d old faeces (−18 and −26%; Fig. 2). In BB treatment microbial volume was at a maximum at day 5 (69% above ‘expected’ values), but then declined below the ‘expected’ values (−6%).

The only factor not affected significantly by cast age was the ratio between fungal and bacterial volume. This value changed little and irregularly (model explained less than 50% of total variation; Table 5). Differences from ‘expected’ values in the ratio between fungal and bacterial volume in fresh casts were only significant in the BB treatment (−33%; data not shown). During cast incubation the fungal-to-bacterial volume ratio tended to decrease in treatments with lime litter (from 4.0 to 3.8 and from 2.1 to 1.7 in LL and BL, respectively) but increased in BB and B0 treatments (from 1.2 to 1.6 and from 1.7 to 2.5, respectively). In 10 days old casts actual values were not significantly different from ‘expected’ values in any treatment.

4. Discussion

A significant decline in L. terrestris body mass in BB and B0 treatments and a low content of beech litter in faeces demonstrate that beech litter and beech forest soil were not adequate food for L. terrestris. This suggests that other kinds of organic debris (presumably ground flora residues) comprise a main part of L. terrestris diet in the Göttinger Wald (Judas, 1992). Therefore, data obtained in BB and B0 treatments should be considered with caution. Conversely, a high litter consumption rate and the earthworms’ weight increase in LL and BL treatments indicate that lime litter was a suitable food substrate, which is in accordance with results of food choice experiments (Satchell and Lowe, 1967; Hendriksen, 1990). Thus, data from these treatments may more adequately reflect the processes occurring under field conditions.

L. terrestris faeces consist of a mixture of ingested litter and mineral soil, somewhat affected by the gut passage. Most chemical and microbial characteristics (except specific respiration) in fresh faeces were less than the corresponding values in the litter, but much higher than those in the soil (Tables 1 and 3). In order to minimise the effects of variations in the litter content in cast material and to compare faeces from different treatments, we estimated the effects of L. terrestris gut passage and subsequent ageing of faecal material on the microbial community by comparing actual and ‘expected’ values. In calculating ‘expected’ values we did not take into account a rate of litter (or soil) carbon assimilation during the gut passage. Daniel (1991) found very high (43–55%) assimilation of ingested dandelion (Taraxacum officinale) leaves by juvenile L. terrestris. Assimilation efficiency of weathered ryegrass litter is much lower as indicated by recalculating data presented by Cortez and Hameed (1988). Despite the fact that assimilation of C from litter may have affected the ‘expected’ values of microbial activity, we suggest that our approach allowed valid comparison of cast material with both soil and litter materials ingested by L. terrestris. This is supported by the close similarity of ‘expected’ and actual values of all the parameters studied. Even the highest differences between ‘expected’ and actual values (basal respiration and $qO_2$ in BB treatment) did not exceed 150% (Fig. 2), whereas $C_{\text{mic}}$ and basal respiration in litter and soil differed by orders of magnitude (Table 1).

Most of the microbial community characteristics (except bacterial volume) were very different in casts from different treatments (Tables 2 and 3, Fig. 1). However, the comparison with ‘expected’ values...
revealed a strong similarity of microbial community alteration during the gut passage (Table 5, Fig. 2). Alternatively, this similarity may be interpreted as an indication of a strong dependence of the faecal microbial community on properties of soil and litter consumed by *L. terrestris*.

Close similarity of actual and ‘expected’ values of Cmic in 1 d old casts supports the assumption that no substantial alteration of microbial biomass occurs during passage of soil and litter through the gut of *L. terrestris*, previously shown for epigeic (Daniel and Anderson, 1992) and endogeic earthworm species (Scheu, 1987). Microscopic measurements of microbial volume generally support this conclusion. However, unlike Cmic, microbial volume in 1 d old *L. terrestris* faeces was 20–60% greater than ‘expected’ values. This discrepancy may partly be explained by a low efficiency of microscopic measurements in undisturbed soil and especially in the litter (Ineson and Anderson, 1982; Newell, 1992). Indeed, microbial volume to biomass conversion (using factors of Bakken and Olsen, 1983) showed that data obtained by microscopy were 25–55% of SIR-determined Cmic in soil and casts, whereas for both lime and beech litter samples it was only about 11%. The underestimation of microbial volume in litter was not corrected for in calculations, so could significantly reduce estimated ‘expected’ microbial volume in *L. terrestris* casts.

In spite of the difference described above, highly significant (*P* < 0.001) linear correlations were obtained between both the physiological (basal respiration and Cmic) and the microscopic approximations of microbial biomass and activity in the materials studied. Correlation coefficients (r) were 0.978 between basal respiration and Cmic, 0.873 between basal respiration and microbial volume and 0.862 between Cmic and microbial volume. Very similar correlation coefficients between SIR-determined Cmic and microbial volume were reported by West et al. (1986).

For the valid extrapolation of data obtained in laboratory microcosms to field conditions, knowledge on processes occurring during ageing of cast material is necessary. Although upper soil layers in the majority of temperate ecosystems contain huge amounts of invertebrate faeces, only a tiny proportion of these casts is ‘fresh’ at any given moment. Our data clearly show that even a comparatively short incubation leads to considerable changes in the microbial community of casts. Both basal respiration and microbial biomass sharply decrease from the very beginning of incubation. A similar trend occurred for the microbial volume in LL and BL treatments (Fig. 2).

Similar patterns of Cmic decline were found in ageing faeces of the endogeic earthworm *Aporrectodea caliginosa* by Scheu (1987). However, in his 30 d experiment the basal respiration in *A. caliginosa* casts was never less than that in the control soil. The same occurred in the B0 treatment in our study.

Parle (1963) also showed a gradual decline in the respiratory activity of cast material of the anecic species *Aporrectodea longa* during 40 d of storage on the soil surface. Nevertheless, a significant increase in the length of fungal hyphae had been recorded during the first 15 d after cast deposition. This observation has been extensively cited and strongly influenced current opinions on earthworm–microflora interactions. However, another observation of Parle (1963) has been widely overlooked. After applying glucose to cast material and measuring O2 consumption over a 1 h period in a Warburg apparatus, he suggested “a decline in the number of actively respiring organisms with age of the casts”. In SIR terminology this is equivalent to a decrease in the initial respiratory response (Anderson and Domsch, 1978) and thus in microbial biomass in ageing casts. A very similar inconsistency between physiological and microscopic methods occurred in the BB treatment of our experiment. The increase in microbial volume during the first 5–10 d of cast incubation was not reflected in SIR-determined microbial biomass, nor in the basal respiration. The latter two values decreased during this period (Fig. 2). Stains used in our study presumably stain active, inactive and also dead microorganisms. Therefore, an increase in microbial volume during the first days of cast incubation may at least partly be ascribed to the accumulation of dead microbial cells.

The reason for the sharp decline of Cmic during the initial phase of cast incubation, which was detected in all litter-containing treatments, is not clear. In his experiments with *A. caliginosa*, Scheu (1987) had shown that microbial growth in fresh casts was primarily limited by available carbon. To check this assumption, we monitored the respiratory response of fresh casts during 40 h after glucose application. Significant microbial growth occurred in cast material from B0 and BB treatments. However, no increase in respiration was recorded in casts from BL and LL treatments, indicating that microbial growth was not limited by available carbon (unpublished data).

The decline in microbial biomass might have been caused by enhanced grazing of protozoa, nematodes or other microbivores, which have been shown to be abundant in earthworm casts and earthworm-worked soil (e.g. Brown, 1995; Winding et al., 1997). However, a continuous decline in the specific respiration of ageing casts contradicts the hypothesis of high grazing pressure. Others mechanisms, presumably antagonistic interactions between members of the faecal microbial community might explain the observed decline in microbial biomass. Several workers have shown that different bacterial and fungal species are differentially affected by earthworm gut passage (e.g. Striganova et
al., 1988; Pedersen and Hendriksen, 1993; Byzov et al., 1996; Moody et al., 1996). Parkinson et al. (1979) have shown that even relatively low rates of selective grazing by litter invertebrates may strongly modify the competitive colonising ability of different fungal species. Since the decrease in Cmic and microbial volume only occurred in litter-containing treatments, we suggest that the observed decline was caused by an interaction between ‘soil’ and ‘litter’ microbial communities in *L. terrestris* casts. However, this question is not within the scope of our study, so cannot be answered with certainty.

A decrease in microbial biomass in aged *L. terrestris* faeces agrees with data presented by Devliegher and Verstraete (1995). They compared the microbial biomass and activity in *L. terrestris*-worked soil of laboratory microcosms with a ‘mixed control’ where food substrate (lettuce) was manually mixed into the soil, and an ‘unmixed control’ where lettuce was left on the soil surface. Devliegher and Verstraete (1995) concluded that processes associated with the passage of soil and organic matter through the gut of *L. terrestris* (GAP) significantly decreased microbial biomass (by ca. 20%) and respiration (by ca. 40%). Soils were studied after 5 weeks of incubation, i.e. the average age of *L. terrestris* casts presumably was about 17 d. The reported decrease in microbial biomass and respiration is remarkably similar to that in 10 d old cast material from litter-containing treatments in our experiment (10–30 and 10–20%, respectively). A decrease in microbial biomass in earthworm-worked soil after several weeks of incubation has also been reported in experiments with endogeic and epigeic earthworm species (e.g. Wolters and Joergensen, 1992; Zhang and Hendrix, 1995).

Wolters and Joergensen (1992) suggested that the depression of microbial biomass and the increase in specific respiration in fresh casts might be attributed to an increase in the relative importance of procaryotes, which quickly mineralise labile organic substrates in fresh casts. As casts age, labile substrates are exhausted and fungi become more important, resulting in a lower specific metabolic activity. The results of our experiment contradict this suggestion. A decline in qO2 during cast incubation was not paralleled by any significant alteration of the fungal-to-bacterial volume ratio (r = 0.06). Similarly, using plate-counts Devliegher and Verstraete (1995) did not find an alteration in the fungi-to-bacteria ratio in *L. terrestris*-worked soil.

Specific respiration was very high in fresh *L. terrestris* casts. Indeed, during the first 5 d of cast incubation qO2 values were not only significantly higher than ‘expected’ values, but higher than those in the control litter samples (Fig. 2; Tables 1 and 3). High specific respiration indicates the presence of easily-available organic material in the soil (e.g. Parkinson and Coleman, 1991; Cheng et al., 1996). Presumably, the availability of easily-degradable carbon sources increases during the gut passage due to the fragmentation of litter, digestion and addition of water and intestinal mucus.

An increase in microbial respiration in fresh casts and the subsequent decrease in the respiratory activity during cast incubation may reflect both a change in the structure of the microbial community and in the ratio between dormant and active stages of microorganisms. Alternatively, a high specific respiration has been interpreted as an indicator of early stages of soil succession (Insam and Domisch, 1988; Insam and Haselwandter, 1989; Anderson and Domisch, 1990). Furthermore, both microbial biomass and volume underwent sharp changes during the first 5–10 d of cast incubation. Thus, energetic characteristics of the microbial community in freshly deposited *L. terrestris* casts meet three criteria proposed by Odum (1985) to characterise stressed communities, i.e. high respiration rate, high respiration-to-biomass ratio and unbalanced production-to-respiration ratio. Therefore, the microflora of fresh *L. terrestris* faeces may be characterised as being in a stressed, unstable, or transitional phase.

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References


