Changes in microbial biomass C, N, and P and enzyme activities in soil incubated with the earthworms *Metaphire guillelmi* or *Eisenia fetida*

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Accepted 6 June 2000

**Abstract**

Earthworms are ubiquitous in soil and ingest large amounts of soil, organic matter and leaf litter. To assess changes in organic matter fractions after passage through the earthworm gut, we measured microbial biomass C, N and P and the fungal-to-bacterial ratios in worm-worked soil (WWS), obtained by incubating soil for 24 h with large numbers of the anecic earthworm *Metaphire guillelmi* (1:5 ratio of fresh weight worms: dry weight soil). Microbial biomass C, N and P were estimated by the fumigation–extraction methods, and fungal-to-bacterial ratios by selective inhibition using substrate induced respiration. Enzyme activities in the gut of *M. guillelmi* were also compared with an epigeic earthworm species *Eisenia fetida*. Activities of cellulase, protease, chitinase, acid and alkaline phosphatases, in gut, casts and uningested soil were measured.

In WWS, microbial biomass decreased (130 μg C g⁻¹ soil), and there was a concomitant increase of available nutrients (27 and 10 μg g⁻¹ soil for ninhydrin-reactive N and inorganic P, respectively). There was no difference between the glucose-sensitive microbial biomass (MB) and the control but the respiratory quotient was greater (2.85 ± 0.17 and 2.95 ± 0.07 μg CO₂-C g⁻¹ soil h⁻¹ for WWS and control, respectively). The fungal-to-bacterial ratio was slightly higher in WWS than in uningested soil (1.61 vs. 1.35).

Cellulase activity was greater in the gut of the epigeic earthworm than in that of the anecic one (152.8 ± 18.7 vs. 18.9 ± 1.3 μg glucose g⁻¹ worm fw h⁻¹); conversely, protease and phosphatase activities were significantly higher in gut of the anecic specie as opposed to the epigeic species. The activity of cellulolytic enzymes was slightly higher in casts than in soil; while activities of protease, acid (pH 6.5) and alkaline (pH 9.0) phosphatases were lower in earthworm casts than in the uningested soils (protease, acid and alkaline phosphatase activity were 29.5 ± 1.7 mg tyrosine g⁻¹ worm fw h⁻¹, 570 ± 2.9, 748 ± 7.3 μg p-nitrophenol g⁻¹ soil h⁻¹ in soil and 17.8 ± 2.0 mg tyrosine g⁻¹ worm fw h⁻¹, 327 ± 26.7, 549 ± 19.7 μg p-nitrophenol g⁻¹ soil h⁻¹ in casts, respectively). We conclude that micro-organisms are used by earthworms as a secondary food resource, and that passage through earthworm gut decreases the total soil MB and increase the active components of MB. © 2000 Elsevier Science Ltd. All rights reserved.

**Keywords:** Earthworm; Microbial biomass; Protease; Phosphatase; Cellulolytic enzyme; Substrate-induced respiration

1. Introduction

Earthworms are ubiquitous soil animals and dominate the invertebrate biomass in temperate soils. Worms ingest a large amount of soil, organic matter and surface litter. In soils where they are abundant, the surface layers may be considered as earthworm casts of different age (Lavelle, 1978). Thus earthworms are thought to have a profound influence on soil organic matter decomposition and nutrient transformations. Earthworms prefer a diet of decomposed and decomposing organic matter, which suggests that micro-organisms are also ingested as food (Edwards and Fletcher, 1988). As micro-organisms are a key factor in soil nutrient transformations and the soil microbial biomass (MB) serves both as a pool and as a source of plant nutrients, ingestion of MB by worms could affect these processes and nutrient supply. Previous work in this area has given contradictory findings. Plate counts of proteolytic bacteria, total bacteria and actinomycetes were reported to be increased by passage through earthworm intestinal tracts (Parle, 1963; Daniel and Anderson, 1992; Devliegher and Verstraete 1995); while Pedersen and Hendriksen (1993) reported that numbers of some bacteria increased and others decreased. MB was reported to decrease (Bohlen and Edwards, 1995; Devliegher and Verstraete 1995), increase (Scheu, 1992) or remain unchanged (Daniel and Anderson, 1992) after passage through the earthworm gut.

Little information is available concerning the capacity of earthworms to degrade organic matter. Most studies on earthworm digestive enzymes have concentrated on Lumbricidae (Zhang et al., 1993), with cellulases being...
the most commonly studied enzymes (Parle, 1963; Loquet and Vinceslas, 1987; Urbášek, 1990). Proteases, phosphatases and chitinases, important in soil N and P transformations, have been less well studied in earthworms.

Our objectives were, firstly, to determine the effects of passage through earthworm guts on the soil microbial community, especially microbial biomass C, N, P and respiration, and secondly, to determine the activity of the digestive enzymes of earthworms and their casts.

2. Materials and methods

2.1. Soil and earthworms

Surface soil (0–10 cm) was taken from the experimental station in the campus of China Agricultural University, Beijing. The soil had the following physical and chemical properties: pH (in H₂O) 7.4, clay 16%, silt 41%, sand 43%, soil organic matter 1.8%, total N 0.1%. The soil was air-dried and sieved (<2 mm) and well mixed. Four parts (v/v) of soil were mixed with one part of sieved (<2 mm) wheat straw compost; the mixture was adjusted to 50% water holding capacity and were conditioned at 25°C.

Specimens of the anecic earthworms *Metaphire guillelmi* (Sims and Easton, 1972) were collected by digging from a green food production base in a Western suburb of Beijing and cultivated in the laboratory for 1 month. Adult earthworms, with fresh weight varies between 2.3 and 2.5 g, were washed free from soil and blotted with filter paper before fresh weight determinations were made. The earthworms were placed in the incubated mixture for 1 d prior to the start of the experiment, in order to replace their gut contents by the mixture. Adult epigeic earthworms *Eisenia fetida* (fw varied from 0.4 to 0.5 g) were purchased from the Chinese Agricultural Academy’s laboratory stock.

2.2. Microbial biomass determination

To ensure the soil contained a large proportion of earthworm casts and that the casts in the worm-worked soil (WWS) could be relatively homogeneous in age, a large number of earthworms were added to a comparatively small volume of soil and a short treatment time was chosen. The gut transit time varies from 2 to 20 h for most species (Parle, 1963; Lavelle, 1978; Lee, 1985), so a residence time of 24 h was selected for the experiment. A preliminary experiment showed that by adding 1 g fresh weight worms to every 5 g dry weight soil, more than 50% of soil passes through earthworm intestine during 24 h of treatment. Although the earthworms were a little crowded, there was no weight loss during the experiments.

To a sample of 200 g (dw equivalent) of the conditioned soil, earthworms were added at a ratio of 1 g fresh weight worms to every 5 g (dw equivalent) soil. After 24 h at 25°C, the earthworms were withdrawn. The worm-worked soil was further incubated for 3 d (3 d WWS) at 25°C with the soil moisture adjusted to 50% water holding capacity. The worms removed from the pots were washed free from soil, weighed and reintroduced into another sample of the conditioned soil. After 24 h, the earthworms were removed, the WWS were further incubated for 2 d (2 d WWS). The earthworms were washed once more, weighed and re-introduced into a new sample in order to obtain 1 d WWS. Then, each WWS sample was homogenised and subsampled in triplicate to determine soil moisture content and measure microbial biomass C, N and P. Worm-free soils were used as the controls and were treated in the same way as the WWS samples.

For biomass C and N estimation, triplicate samples of 15 g (dw equivalent) were extracted with 80 ml of 0.5 M K₂SO₄ immediately after exposure to worms or after a subsequent 24 h fumigation with CHCl₃. Organic C in the extracts was measured in 5 ml aliquots by the dichromate oxidation method (Vance et al., 1987). Ninhydrin-reactive N (N₉₅) in the extracts was analysed by reacting aliquots (2 ml) with a ninhydrin reagent (Moore and Stein, 1954) and reading absorbance at 570 nm. Microbial biomass C and N were calculated, respectively, by the equations $E_{C} = E_{C} \times 2.64$ (Vance et al., 1987) and $E_{N} = E_{N} \times 5.0$ (Joergensen and Brookes, 1990), where $E_{C}$ and $E_{N}$ are the difference between organic C or ninhydrin-reactive N extracted by 0.5 M K₂SO₄ from fumigated and non-fumigated soil, respectively.

For microbial biomass P determination, triplicate samples of 15 g were extracted with 80 ml of 0.5 M NaHCO₃ (pH 8.5) immediately at the end of incubation or after a subsequent 24 h fumigation with CHCl₃. Inorganic P (Pᵢ) was analysed in aliquots (1 ml) of the extracts by the ammonium molybdate–ascorbic acid method described by Murphy and Riley (1962). A spike of KH₂PO₄ equivalent to 25 μg P g⁻¹ soil was used to correct for P fixation during the NaHCO₃ extraction. Biomass P was calculated by $B_{P} = E_{P} / 0.38$, where $E_{P}$ is the difference between NaHCO₃-P, extracted from fumigated and non-fumigated soil (Brookes et al., 1982). NH₄⁺-N and NO₃⁻ were measured in the same extracts as microbial biomass C and N. NH₄⁺-N was analysed by indophenol blue method (Keeney and Nelson, 1982), NO₃⁻-N was analysed by a colorimetric method of nitration of salicylic acid (Cataldo et al., 1975).

2.3. Substrate-induced respiration (SIR) rate and bacterial-to-fungal ratio

Triplicate WWS samples were obtained by exposing earthworms to conditioned soil for 24 h, at a 1:5 ratio of worms to soil (fw/dw). Then, SIR was determined in triplicate samples (ca. 10 g dry weight) of WWS or uningested soil with a continuous air-flow system modified and adapted from Cheng and Coleman (1989). Glucose was added to soil with talcum as a carrier. Preliminary experiments showed that 10 mg glucose g⁻¹ soil was optimal for a maximal
2.4. Enzymatic activity in gut of the earthworms

Adult earthworms were washed free from soil and incubated in soil or soil/decomposed straw mixture (worm to soil or mixture at 1:5 ratio, fw/dw) for 24 h. Sub-samples of fresh casts were collected, the earthworms were washed and weighed. Four individuals of *M. guillelmi* were sampled and dissected. After dissection, the gut wall and its contents of one *M. guillelmi* worm were homogenised in a known volume of 50 mM pH 4.8 citrate buffer at 4°C to determine cellulase activity, or in pH 8.1 Tris–HCl buffer to determine protease activity. The homogenates were centrifuged for 10 min at 6000 rpm, the supernatant was stored at −18°C until the enzymes were assayed. Because of their small size, *E. fetida* (2 individuals for each replication, 6 replications) were homogenised without dissection. The enzymatic activities of both species were expressed per unit fresh weight of worm.

Cellulase activity was determined using a filter paper assay (Mandels et al., 1976). Briefly, 1 ml of 50 mM pH 4.8 citrate buffer, 1 ml centrifuged homogenate, 0.5 ml toluene and a filter paper strip were incubated at 50°C for 24 h. The reducing-sugar concentration was estimated by the dinitrosalicylic acid method (Miller, 1969). Glucose was used as the standard for reducing-sugars.

Protease activity was measured as described by Ladd and Butler (1972). Centrifuged homogenate (1 ml) was incubated with 2.5 ml of 1% sodium caseinate in 100 mM Tris buffer pH 8.1 at 50°C. After 60 min the reaction was stopped by precipitating the enzyme and the remaining caseinate by adding 1 ml of 17.5% trichloroacetic acid. After centrifugation, 2 ml of the supernatant was mixed with 3 ml of 1.4 M Na₂CO₃. Then 30 min later, 1 ml of 2-fold diluted Folin-phenol reagent (Lowry et al., 1951) was added and the mixture was homogenised immediately. Absorbance at 700 nm was determined 20 min later and related to those of tyrosine standards (0–1000 μM) treated in a similar way.

Acid (pH 6.5) phosphatase activity was determined according to the method adapted from Tabatabai (1982). Centrifuged homogenate (500 μl) solution was incubated with 4 ml of modified universal buffer (MUB) at pH 6.5, and 1 ml of 25 mM sodium p-nitrophenyl phosphate in pH 6.5 MUB at 37°C. After 30 min, the reaction was stopped by adding 4 ml of 0.5 M NaOH solution. After a further 20 min at room temperature, the concentration of p-nitrophenol was determined by reading the absorbance at 420 nm and related to those of 1% p-nitrophenol standards.

Chitinase activity was determined by incubating 1 ml of centrifuged homogenate with 1 ml of citrate-phosphate buffer pH 5.2 and 1 ml of 0.2% chitin suspension for 24 h (Skujins et al., 1965). After stopping the reaction in boiling water and centrifugation, liberated β-N-acetyl-β-d-glucosamine in the supernatant was measured as described by Reissig et al. (1955).

2.5. Enzymatic activity in casts and soil

Protease activity of earthworm casts and soil was determined using the same method as for earthworm extracts. Preliminary experiments showed that no glucose liberated from degradation of cellulose or filter paper under conditions of our test was detectable by the DNS method (Miller, 1969) or by reducing sugars methods (Smoggyi, 1945). Thus the CO₂ evolution rate from casts and soil incubated with micro-crystalline cellulose (MCC) was used as an indicator of cellulolytic enzyme activity. Earthworm casts or soil (20 g) were mixed thoroughly with 200 mg MCC, and incubated at 25°C for 7 d. CO₂ evolved was absorbed by 5 ml of 1.0 M NaOH which was replaced daily. Residual alkali was titrated against 300 mM HCl after precipitating carbonate by excess of BaCl₂, with phenolphthalein as indicator.

Acid (pH 6.5) and alkaline (pH 9.0) phosphatase activities of casts and soil were determined by the method described by Tabatabai (1982).

Enzymatic activities of earthworms are presented on a live weight basis, and other results are presented on an oven-dry soil weight basis. All results are the means of independent assays (number of mean are indicated in corresponding tables and figures). Student’s *t*-test was used to compare significant difference between the means.

3. Results

3.1. Effect of earthworm on total soil microbial biomass

Soil microbial biomass (MB), C, N and P had decreased after 24 h of earthworm treatment, while soil extractable
organic C, ninhydrin-reactive N (Nnin) and NaHCO₃-Pi had increased (Table 1).

In the WWS, Nmin consisted mainly of NH₄⁺-N, which was nitrified during the subsequent incubation as indicated by an increase of soil NO₃⁻-N (Fig. 1). Inorganic P was higher in WWS than in the control soil and the amounts increased during the subsequent incubation (Fig. 2).

### 3.2. Effect of earthworms on the soil microbial community

Although the total MB decreased during the earthworm treatment, the glucose-sensitive component of the MB was not considerably affected, as there was no significant difference in glucose-induced respiration between the earthworm treatment and the control (2.85 ± 0.17 and 2.95 ± 0.07 μg CO₂-C g⁻¹ soil h⁻¹ for WWS and control, respectively). The Metabolic quotient (qCO₂), was significantly higher in WWS than in the control soil (10.66 ± 1.9 and 5.09 ± 0.48 μg CO₂-C mg⁻¹ microbial biomass-C h⁻¹ for WWS and control, respectively). This resulted from a combination of an increase of basal respiration and a decline of MB in the earthworm treatment. The fungal-to-bacterial ratio of the glucose-sensitive MB was slightly higher in WWS than in the uningested soil (1.61 vs. 1.35). The total combined inhibition reached 41% for the earthworm treatment and 48% for the control. Inhibitor selectivity was satisfactory, as the inhibitor additivity ratio was close to 1.0 (1.12 and 1.04 for control and WWS, respectively).

### 3.3. Cellulase, protease and phosphatase activities in earthworm gut

Cellulase activity was 7-fold greater in the guts of E. fetida than that in the guts of M. guillelmi (Table 2). In contrast, protease and acid phosphatase activity of E. fetida were significantly lower than in M. guillelmi (135.5 ± 1.2 mg tyrosine g⁻¹ fw h⁻¹, 474 ± 0.11 μg p-nitrophenol g⁻¹ fw h⁻¹) (Table 2).

Chitinase activity was not detected in the gut of either of the two earthworm species.

### 3.4. Digestive enzymes in soil and casts of Metaphire guillelmi

Activities of protease, acid and alkaline phosphatases in the casts of the earthworm M. guillelmi were 17.8 ± 2.0 mg tyrosine g⁻¹ soil h⁻¹, 327 ± 26.7 and 549 ± 19.7 mg p-nitrophenol g⁻¹ soil h⁻¹, respectively, while those in the

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**Table 1**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Microbial biomass a (μg g⁻¹ soil)</th>
<th>Soil extractable C, N and P (μg g⁻¹ soil)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>N</td>
</tr>
<tr>
<td>Control</td>
<td>920.6 (67.5)</td>
<td>205.25 (3.4)</td>
</tr>
<tr>
<td>Earthworm treatment</td>
<td>791.1 (11.1)</td>
<td>146.35 (10.9)</td>
</tr>
</tbody>
</table>

a Kc for biomass C, N, P were used as 0.41, 0.2 and 0.37, respectively.

b *, ** and *** represent, respectively, P < 0.05, P < 0.01 and P < 0.001 probability of significant difference between earthworm treatment and control (t-test).
570

further increase CO\textsubscript{2} evolution rate either in earthworm
3-fold greater than that in soil. Cellulose amendment did not
higher CO\textsubscript{2} evolution rate in earthworm casts compared to
no difference of CO\textsubscript{2} evolution rate between casts and unin-
wheat straw addition), in the absence of cellulose, there was
CO\textsubscript{2} evolution rate between worm casts and soil was maxi-
imum at day 5, with the CO\textsubscript{2} evolution rate of the casts being
3-fold greater than that in soil. Cellulose amendment did not
further increase CO\textsubscript{2} evolution rate either in earthworm
casts or in the control soils.

When the earthworm culture medium was soil (without
wheat straw addition), in the absence of cellulose, there was
do no difference of CO\textsubscript{2} evolution rate between casts and uningested
soils, except on day 2, where there was a slight increase in casts; however, cellulose amendment caused a
higher CO\textsubscript{2} evolution rate in earthworm casts compared to the
uningested soils (Fig. 4).

4. Discussion

4.1. Effects of earthworm on micro-organisms

Our data and published information shows that various
components of soil MB respond differently to passage
through earthworm gut. This partly explains why different
results were obtained depending upon which components of
the biomass were measured. Total soil MB is assessed by

control soil were \(29.5 \pm 1.7 \text{ mg tyrosine g}^{-1} \text{ soil h}^{-1}, \)
\(570 \pm 3\) and \(748 \pm 7.3 \text{ mg } p\text{-nitrophenol g}^{-1} \text{ soil h}^{-1}, \)
respectively (Table 3).

The CO\textsubscript{2} evolution rates were higher in casts than in the
control soil when a mixture of soil and decomposed wheat
straw was used as the medium for earthworm culture, which
suggested that the microbial activity was greater in casts
compared to the control soil (Fig. 3). The difference of the
CO\textsubscript{2} evolution rate between worm casts and soil was maxi-
mum at day 5, with the CO\textsubscript{2} evolution rate of the casts being
3-fold greater than that in soil. Cellulose amendment did not
further increase CO\textsubscript{2} evolution rate either in earthworm
casts or in the control soils.

When the earthworm culture medium was soil (without
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no difference of CO\textsubscript{2} evolution rate between casts and uningested
soils, except on day 2, where there was a slight increase in casts; however, cellulose amendment caused a
higher CO\textsubscript{2} evolution rate in earthworm casts compared to the
uningested soils (Fig. 4).

In summary, soil total MB was negatively affected by
chloroform-fumigation methods, SIR method measures the
active MB or more exactly the substrate sensitive compo-
nent of MB, while plate counting methods provide a
measure of the number of cultivable (or viable) micro-
organisms. In our study, passage through the earthworm
gut reduced total soil MB (Table 1), which is in accordance
with results reported by Devliegher and Verstraete (1995)
and Bohlen and Edwards (1995). These authors also used
fumigation technique to determine MB. However, Daniel
and Anderson (1992) found no change of soil MB after
gut passage although they used the same fumigation-extrac-
tion method. In their experiment, the comparison of MB was
made between earthworm casts and uningested soil; but that
may be an effect of the selective behaviour of earthworms
on soil MB. Earthworms selectively consume soil fractions
with high concentration of micro-organisms (Hendriksen,
1990), and not all the ingested micro-organisms are killed
during passage through earthworm intestine (Edwards and
Fletcher, 1988; Hendriksen, 1990). In that study, the detri-
mental effect of the earthworm gut on soil MB would have
been counteracted by enriching casts with micro-organisms
through selective feeding behaviour (Hendriksen, 1990).
In our study, soil MB was compared between WWS and the
uningested soils, thus the indirect effect of earthworm selec-
tive feeding was eliminated.

In WWS, a reduction in soil MB with a concomitant
increase of extractable organic C, mineral N and
NaHCO\textsubscript{3}-extractable P, indicated that release of microbially
immobilised nutrients was enhanced by earthworms (Table
1). The increase in metabolic quotient \(q\text{CO}_2\) of soil MB in the
WWS suggested a rejuvenation of the microbial community,
as the \(q\text{CO}_2\) quotients of “young” micro-organisms are
frequently greater than that of “aged” ones (Anderson and
Domsch, 1978). The release of immobilised nutrients may
have a stimulating effect on microbial activity and activate
dormant microbes. Fischer et al. (1997) demonstrated that
passage of microbes through earthworm guts resulted in a
significant increase in the number of vegetative cells and a
decrease in the number of spores of \textit{Bacillus megaterium}.
Their results suggested that germination of bacterial spores
were enhanced by the gut passage.

Table 2
Activity of digestive enzymes in gut of two earthworm species, means and standard errors and numbers of the mean (in parenthesis)

<table>
<thead>
<tr>
<th>Earthworm</th>
<th>Enzymatic activity\textsuperscript{a}</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cellulase</td>
<td>Protease</td>
<td>Phosphatase</td>
<td></td>
</tr>
<tr>
<td>\textit{E. fetida}</td>
<td>152.8 (18.7, (n = 6))</td>
<td>24.6 (0.3, (n = 4))</td>
<td>127.0 (0.11, (n = 3))</td>
<td></td>
</tr>
<tr>
<td>\textit{M. guillelmi}</td>
<td>18.9 (1.3, (n = 4))</td>
<td>135.5 (1.2, (n = 4))</td>
<td>473.9 (4.9, (n = 3))</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a} Expressed respectively as \(\mu\text{g glucose}, \mu\text{g tyrosine, and } \mu\text{g } p\text{-nitrophenol produced } \text{g}^{-1} \text{ earthworm live weight } \text{h}^{-1}.

\textsuperscript{b, **, ***}: significantly different between \textit{E. fetida} and \textit{M. guillelmi} at \(P < 0.001, P < 0.0001\) and \(P < 0.00001\), respectively (t-test).

| Treatments | Phosphatase activities (\(\mu\text{g } p\text{-nitrophenol g}^{-1} \text{ soil h}^{-1}\)) | Protease activity (mg tyrosine g\(^{-1}\) soil h\(^{-1}\)) |
|-----------|-------------------------------------|---|---|
|           | Acid                                | Alkaline | |
| Soil      | 570 (2.9)*** | 748 (7.3)*** | 29.5 (1.7)** |
| Casts     | 327 (27) | 549 (19.7) | 17.8 (2.0) |

\textsuperscript{a, **, ***}: significantly different between soil and casts at \(P < 0.01\) and \(P < 0.001\), respectively (t-test).
nutrients, rejuvenation of the microbial community and possibly activation of dormant microbes may have enhanced the microbial activity in the earthworm casts.

Reduction of soil MB by the earthworm *M. guillelmi* indicates that this earthworm may consume soil microorganisms. Compared to the epigeic earthworm *E. fetida* which feeds on plant litter, the gut of *M. guillelmi* has much greater protease and phosphatase activities and much lower cellulyolytic enzyme activities (Table 3). This suggests that *M. guillelmi* may assimilate proteins and organic P compounds in the cells of soil micro-organisms rather than the cellulose of plant litter. The preference for partially decomposed organic materials rich in micro-organisms rather than for fresh organic residues by earthworms (Cortez et al., 1989; Moody et al., 1995) is also an indication that earthworms may consume micro-organisms. In the course of organic residue decomposition, exhaustion of more easily decomposable constituents such as sugar and proteins precedes that of cellulose and hemicellulose. Substances remaining in partially decomposed plant residues are mainly cellulose, hemicellulose, lignin, substances newly synthesised by micro-organisms and microbial cells. Lignins are very resistant to microbial attack, and microbial metabolites are thought to be more recalcitrant than the initial substances. Since cellulase activities were very weak in the guts of *M. guillelmi*, microbial biomass must be the only substrate for the earthworms. Thus it is easy to speculate that plant residues are used as primary resources by micro-organisms which in turn, are used by *M. guillelmi* as a secondary resource. As for *E. fetida*, the relatively higher cellulase activity in the gut may allow them to utilise plant materials directly as a food source. The results of the enzymatic activities in the gut and casts of *M. guillelmi* suggested that proteins and phosphorus-containing organic compounds are digested in the gut rather than in the casts, whilst cellulose seems to mainly be degraded in casts.

Consumption of micro-organisms by earthworms may have various consequences on organic matter decomposition. Release of microbial immobilised nutrients and rejuvenation of soil MB may enhance the decomposition of soil organic matter as metabolic activities of micro-organisms remain intense. When earthworm feeding intensity is high and soil nutrients are limited, earthworm feeding may have detrimental effects on microbial population and activity, consequently the decomposition rate of organic residues may be reduced. Although the soil fungal-to-bacterial ratio was not significantly modified by earthworms in our experiment, there is other evidence (Pedersen and Hendriksen, 1993; Toyata and Kimura, 1994) that the composition and succession of the microbial community may be changed by earthworms. Earthworm preferentially feed on fungi degrading water-soluble sugars and cellulose, while the lignin-degrading fungi characteristic of the later stage of plant litter decomposition were less selected (Moody et al., 1995). It is well known that the capacity to decompose complex organic matter varies with microbial community (Campbell, 1983).

### 4.2. Digestive enzymes in gut and casts of the earthworms

The difference in the enzymatic activities of the two earthworms studied may be associated with their ecological categories and feeding habitats. The earthworm *E. fetida* is an epigeic species which feeds mainly on plant litter. Their
high cellulase activity allows them to efficiently assimilate plant materials, which are composed mainly of cellulose; while *M. guillelmi* is an anecic species which feeds mainly on leaf litter mixed with the soil of upper horizons. They appear to prefer micro-organism-contaminated organic matter and assimilated micro-organisms (Edwards and Fletcher, 1988, Zhang et al., in preparation). Our results are similar to those obtained by Urbásek (1990), in the study of cellulase activity in the earthworm gut, who found that epigeic species had higher gut cellulase activity than endogeic earthworms that feed on soil less enriched in organic matter.

In this study, we did not detect chitinase activity in the gut of earthworms. In contrast, in the tropical earthworms *Pontoscolex corethrurus* and *Polypheretima elongata* (Zhang et al., 1993; Lattaud et al., 1997) we found that β-N-acetyl-d-glucosaminidase was rather active, chitin is a component of fungal cell walls and β-N-acetyl-d-glucosamine is the monomer of chitin. Barois and Lavelle (1986) reported that earthworms produce a huge amount of intestinal mucus being a mixture of glyco-proteins, and small glucidic and proteic molecules. They suggested that the mucus was rapidly incorporated into microbial biomass in the gut, since no mucus was recovered in the casts. The weak protease and phosphatase activity in earthworm casts may have resulted from degradation of the enzymes in the posterior part of the gut. Apart from degradation of enzymes, low phosphatase activities may have resulted from inhibition of expression of enzymatic activities by the inorganic phosphate (McGill and Cole, 1981). However, in our experiment, this was not the case: the higher content of NaHCO$_3$-extractable inorganic phosphorus in casts cannot account for the decrease of phosphatase activity (data not shown). Our results contradict the findings of Sharpley and Syers (1976) who found that phosphatase activity was higher in fresh casts of earthworms. In contrast, in the tropical earthworms *L. rubellus* and *A. caliginosa* than in uningested soils; this difference may be partly explained by the fact that different species were used in each experiment.

**Acknowledgements**

The research was funded by the Natural Science Foundation of China. We thank M. Li Nai-Guang and Li Guo-Xue for aid in earthworm collection, Dr Patrick Lavelle professor of the Université Pierre et Marie Curie and Dr Graham Sparling, Landcare Research, New Zealand for constructive critiques and suggestions.

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