AND SOIL MICROORGANISMS IN THE
DECOMPOSITION OF ORGANIC MATTER

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ABSTRACT

THE INTERRELATIONSHIPS OF SOIL ANIMALS
AND SOIL MICROORGANISMS IN THE
DECOMPOSITION OF ORGANIC MATTER

By

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The interrelationships of soil animals and soil microorganisms in degrading uniformly labeled ^{14}C-yeast was studied using a radioisotopic method. The gut of the animal used, *Tracheoniscus rathkei* (Brandt), was examined for its ultrastructure and microbial population. After 28 days, the animal-microbial system (a) respired 56.4 percent of the labeled food while only 33.8 and 50.8 was respired in the animal (b) and microorganism (c) system respectively. The animals were responsible for the high initial degradation rate of the food while the soil microorganisms degraded the feces. Wood fed animals were more efficient utilizers of the yeast than yeast fed animals as indicated by higher assimilation, 44.4% vs. 37.8%, of the original food and lower fecal excretion, 28.2% vs. 37.4%, of the original food. These differences suggest a possible difference in the microbial population associated with the animal gut. The feces from yeast fed animals decomposed in the soil at a faster rate than the feces from wood fed animals. This indicates that the feces from the former were less resistant than the feces from the latter. Pure yeast decomposed rapidly in the soil but the rate declined abruptly after three days. The feces decomposed at a slower

rate but for a longer period. The feces degraded faster in the soil of higher moisture level than at the lower moisture level. Moisture did not affect the decomposition rate of pure ^{14}C-yeast. Bristle-like protrusions extending from the chitinous wall lining of the hindgut was observed. At least six microscopically distinct types of bacteria and two kinds of fungi were isolated from the gut. The estimated microbial population was 3 to 7 x 10^6 bacteria/gut. The enrichment for cellulolytic microorganisms from the gut was successful based on growth and the disappearance of ground filter paper. The radioisotopic method employed for following the fate of decomposing organic material had reproducible results and showed a relatively high recovery of the labels.

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By

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Dedications

To my parents, brothers, and sisters whose memory
gave me the inspiration to finish
this little piece of work

To Dr. Thomas S. C. Wang
whose wisdom enlightened me

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The balance of our dynamic terrestrial ecosystem depends on nutrient recycling and rate of energy flow mediated by its biotic components. Traditionally, soil scientists have assumed that the major agents of degradation of organic materials are microorganisms. Recently, the role of soil animals in decay has been studied intensively and results suggested that they are important in decomposition (Bocock, 1964; Clarke, 1965; Torne, 1967). On the contrary, however, there is evidence showing that soil fauna are insignificant in the metabolism of plant residues in the soil (Bleak, 1970; Curry, 1969; Zachariae, 1963).

This inconsistency is due to the lack of concerted efforts among microbiologists, zoologists, and soil scientists. As van der Drift (1970) and Coupland *et al.* (1969) stressed, workers in different disciplines of soil biology must coordinate their research to understand the complex interactions of the diverse groups of micro- and macroorganisms which drive the biological decomposition processes. In addition, the methods that have been used to study them were as varied and numerous as the number of investigators. Heath *et al.* (1964) recognized this variety and the limitations of the methods used.

It is therefore the purpose of this paper to demonstrate that an integrated approach is possible: that soil microorganisms and soil animals, as they interact, are efficient decomposers. Since currently used biocides reach the soils directly or indirectly and may indiscriminately inhibit beneficial populations (Edwards, 1969), it is evident

that an understanding of their interrelationships is necessary. It is also the purpose of this paper to introduce a radioisotopic method that is sensitive and quantitative for assessing the biological activity in soils.

MATERIALS AND METHODS

Soil animal

Tracheoniscus rathkei (Brandt), commonly known as woodlice, was used in this study. It is a terrestrial Isopod and one of the most active soil arthropods (van der Drift, 1962; Kuhnelt, 1961; Macfadyen, 1962). It thrives mainly on forest litter and wood but can live on almost any kind of organic material as long as the relative humidity is high. The choice was based on its availability, easy maintenance in the laboratory, ready collection of feces, and ability to withstand low oxygen and wide fluctuation of temperatures. They were collected from campus woodlots and reared in the laboratory on a diet of either Baker's yeast or wood according to the method of Butcher *et al.* (1969). In case of mass rearing, 10 x 19.5 cm plastic boxes were used instead of rearing jars.

Preparation of the substrate

Uniformly labeled ^{14}C-yeast was used to represent plant residues at various degrees of decomposition because it was a suitable food for the soil animals and easy to prepare. In addition, previous workers have suggested that the fungal mycelium and bacterial cells associated with decomposing organic residues are the major source of nutrition of grazing animals (Cmelik and Douglas, 1970; Cragg, 1961; Macfadyen, 1962; Minderman and Daniels, 1966).

The yeast was prepared by aerobically growing *Saccharomyces cerevisiae* for 24 hr in 200 ml of medium (pH 5.5) as modified from Olson and Johnson

(1949). It consisted of KH_2PO_4, 0.8 gm; NH_4NO_3, 0.1 gm; $MgSO_4 \cdot 7H_2O$, 40.0 mg; $CaCl_2 \cdot 2H_2O$, 4.0 mg; $FeCl_3 \cdot 6H_2O$, 2.0 ppm; $CuSO_4 \cdot 5H_2O$, 0.10 ppm; H_3BO_3, 1.0 ppm; $MnSO_4 \cdot 4H_2O$, 1.75 ppm; $ZnSO_4 \cdot 7H_2O$, 1.0 ppm; glucose, 2.0%; yeast extract, 0.015% biotin, 0.16 ppm; thiamin, 0.10 ppm; and pyridoxin, 0.10 ppm. This medium was derived to give optimal yield while minimizing the quantity of unlabeled carbon materials added. To supply the needed label, 0.5 mC of uniformly labeled ^{14}C-glucose (Tracerlab) was added (2.78×10^{-3} mM) in the form of 50 ml-aqueous solution (180 mC/mM), rinsing the containing vials with 50 ml of the salt solution used. The medium was then inoculated with 1 ml of the yeast in the logarithmic phase growing in 2% glucose-yeast extract-mineral salt medium. A 500 ml-flask containing the above medium was attached to a rotary shaker operating at 125 rpm. This, together with the removal of CO_2 from the perfusing atmosphere, was necessary to insure aerobic growth for high yield of cells. To aerate the flask, compressed air that had passed through drierite (W. A. Drierite Co.) and ascarite (Arthur H. Thomas Co.) to remove CO_2 was bubbled into the medium (Figure 1). The respired CO_2 was trapped from the exhaust by a series of three gas scrubbing towers containing 1 M NaOH. After incubation, the trap contained 4.35% of the original label.

The cells were harvested in the late log phase at an O.D. of 0.75 by centrifuging the suspension in 250 ml-polypropylene bottles at 5000 rpm for 25 min in a refrigerated centrifuge (Sorvall) set at 4° C. After discarding the supernatant liquid and including the washings from the grower flask, the cells were washed by resuspending them twice in 250 ml of refrigerated distilled water.

The washed yeast cells were carefully pipetted out on a preweighed ovendry filter paper (Whatman No. 42), with two extra filter papers fitted to a watch glass to absorb excess water. The rate of flow was slower than

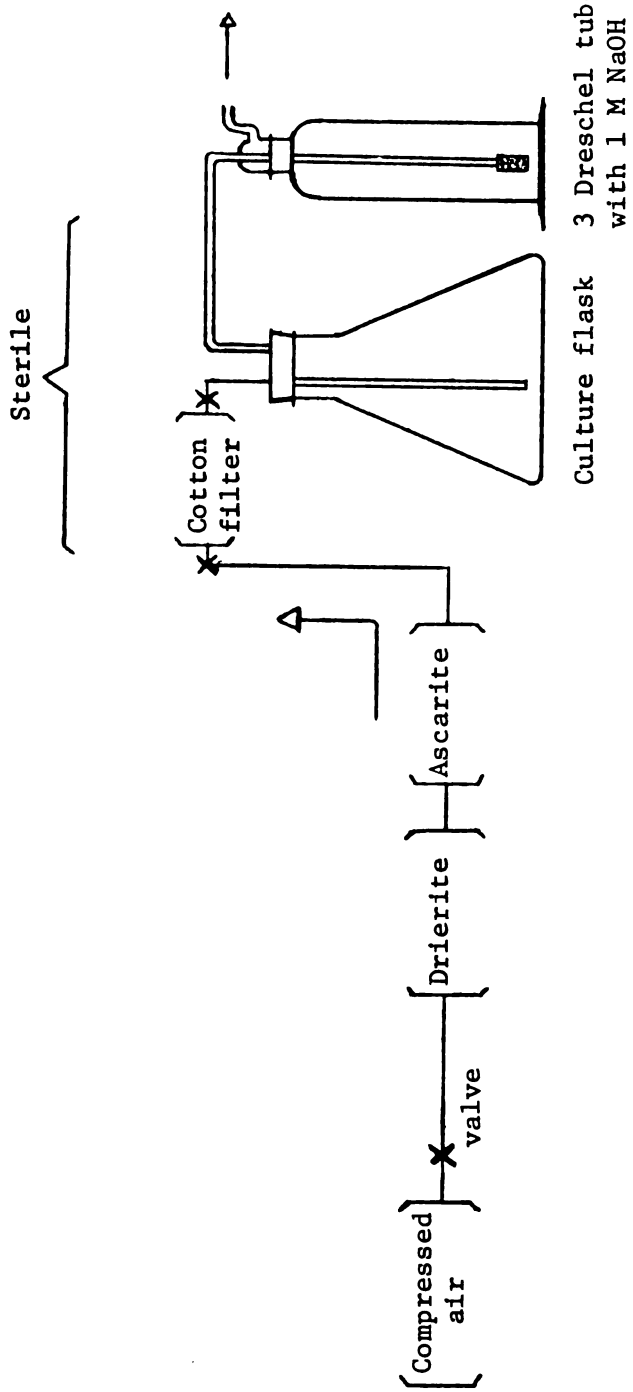

Figure 1. A schematic diagram for growing uniformly labeled ^{14}C-yeast.

the rate of water absorption to retain the cells in a smallest possible area thus facilitating the separation of the yeast cells from the filter paper after oven drying for 12 hr at 60° C and weighing. The caked dry yeast was ground into a fine powder in an agate mortar and placed in a vial, and stored in a colored bottle containing drierite as desiccant overlaid with cotton at 4° C. The total yield of labeled yeast cells was 0.69 g which contained 8.35% of the original label and had a specific activity of 0.06 μC/mg.

Metabolism experiment

All experiments were done in the rearing jar (Figure 2) inside a temperature controlled walk-in incubator (Chicago Electrical and Surgical Co.). The cap liner was repasted with rubber cement to prevent separation after prolonged tight usage. The temperature was maintained at 20 ± 2° C and the relative humidity was kept high by placing a pan of water in the incubator. The incubator was kept dark.

The above rearing jars used to determine the organic matter breakdown contained the following components: (a) three Isopods and soil containing the natural microbial population; (b) three Isopods and charcoal-plaster of Paris; and (c) soil containing the natural microbial population. The soil was freshly obtained from the animal collection site; 25 g per jar was used. The labeled yeast was fed or added at the rate of 2.5 mg (0.15 μC) per jar and placed on a glass cover slip in the treatments containing animals (a and b) or spread on the soil surface where animals were absent (c). This quantity of yeast was previously determined as the amount which three animals would totally consume within 24 hr. Non-labeled Baker's yeast was supplied to the animals one day after the initial feeding and at every other inspection thereafter. Inspections

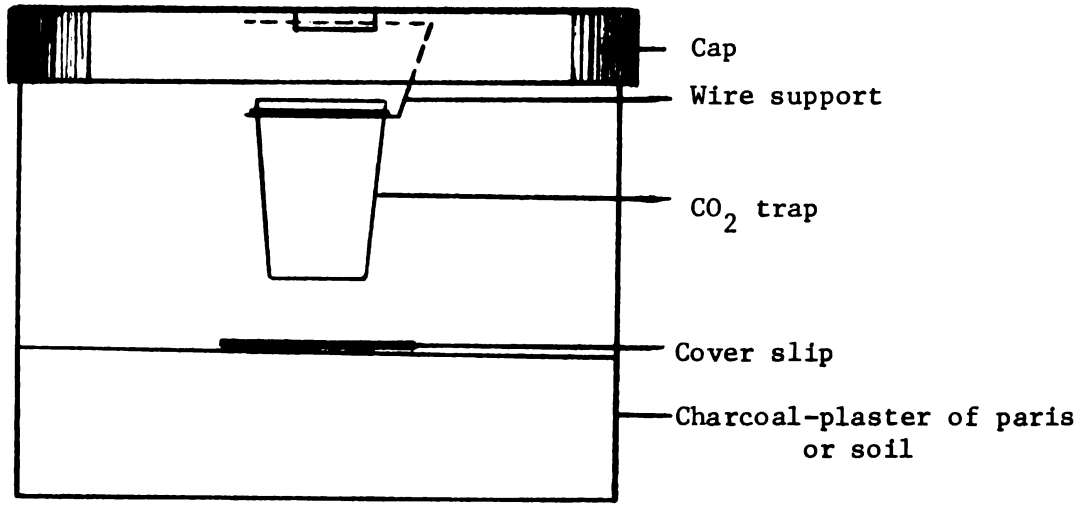

Figure 2. A side view of the screw-cap incubation jar (50 x 53 mm.) showing the various components.

occurred after 1 to 7, 9, 12, 15, 18, 23, and 28 days; during these periods, respired $^{14}CO_2$ was measured while the feces were collected after 3, 7, 23, and 28 days. The feces in the first treatment (a) were not collected but allowed to be metabolized by soil microorganisms. The animals were anesthetized with chloroform in a cotton ball after 28 days and collected. The feces and the animals were picked using disposable needles in a 1 cc syringe (Tomac) and wrapped in glacine weighing paper. Both were immediately dried (60° C) and kept in a freezer to minimize decomposition prior to radioactivity determination. Each treatment was replicated six times.

Yeast-fed and wood-fed animals were compared in an experiment similar to treatment (b) above but lasting only for 48 hr. All of the animals used were reared for two months in the laboratory. Respired $^{14}CO_2$ and feces were collected and measured after 24 and 48 hr, while the animals were collected after 48 hr. One half of the first fecal collection was used for radioactivity assay while the other half was saved for biodegradability determination below. The experiment was replicated eight times.

The biodegradability of the above labeled feces and the labeled yeast was compared in a soil containing the natural microbial population (similar to c). The soil was held at two moisture levels, 16.4% which was near field capacity (17.4% measured at 1/3 atm for 48 hr) and 11.4%. The higher moisture level was that of the freshly collected soil (October) while the other was air dried to the precalculated weight. Respired $^{14}CO_2$ was measured daily for the first 10 days and then at 12, 14, and 16 days. The experiment was replicated eight times.

Radioactivity determination

Respired CO_2 was trapped in 2 ml of 1 M NaOH contained in a 2 ml-disposable polystyrene beaker inside the jar as shown in Figure 2. The beaker and contents were both transferred directly to a glass scintillation vial.

Samples of yeast, feces, and animals were first combusted to CO_2 using a combustion flask (Figure 3) as modified from Dobbs (1963). The sample to be burned was wrapped in a black paper (Arthur H. Thomas Co.) and oven dried before combustion. Ignition was initiated by an Infra Red igniter (Arthur H. Thomas Co.). After combustion, 20 ml of 1 M NaOH was injected into the flask, swirled for 15 sec and allowed to stand for 2 hr. A one milliliter aliquot was pipetted into a glass scintillation vial. A mixture (1:1 v/v) of Bray's scintillator (Bray, 1960) and Cab-O-Sil (New England Nuclear) was added to the sample in the scintillation vial to a total volume of 20 ml. The mixture was vigorously shaken, equilibrated to scintillation counter temperature for 30 min, and counted using a Packard Liquid Scintillation Spectrometer, model 3310. All counts were corrected for quenching by internal standardization and for machine efficiency. The NaOH as CO_2 trap was used throughout because it showed little quenching, was cheap, stable and did not affect the animals. Contrary to previous claims, NaOH did not produce extra background count due to spontaneous breakdown of the scintillators.

Gut examination

The gut was fixed according to normal procedure using buffered 6.25% glutaraldehyde (pH 7.2) at 4° C and postfixed with buffered 1% osmium tetroxide (pH 7.2); and the thin section double stained with uranyl nitrate and lead acetate. A Philips electron microscope model EM 100 was used for examination.

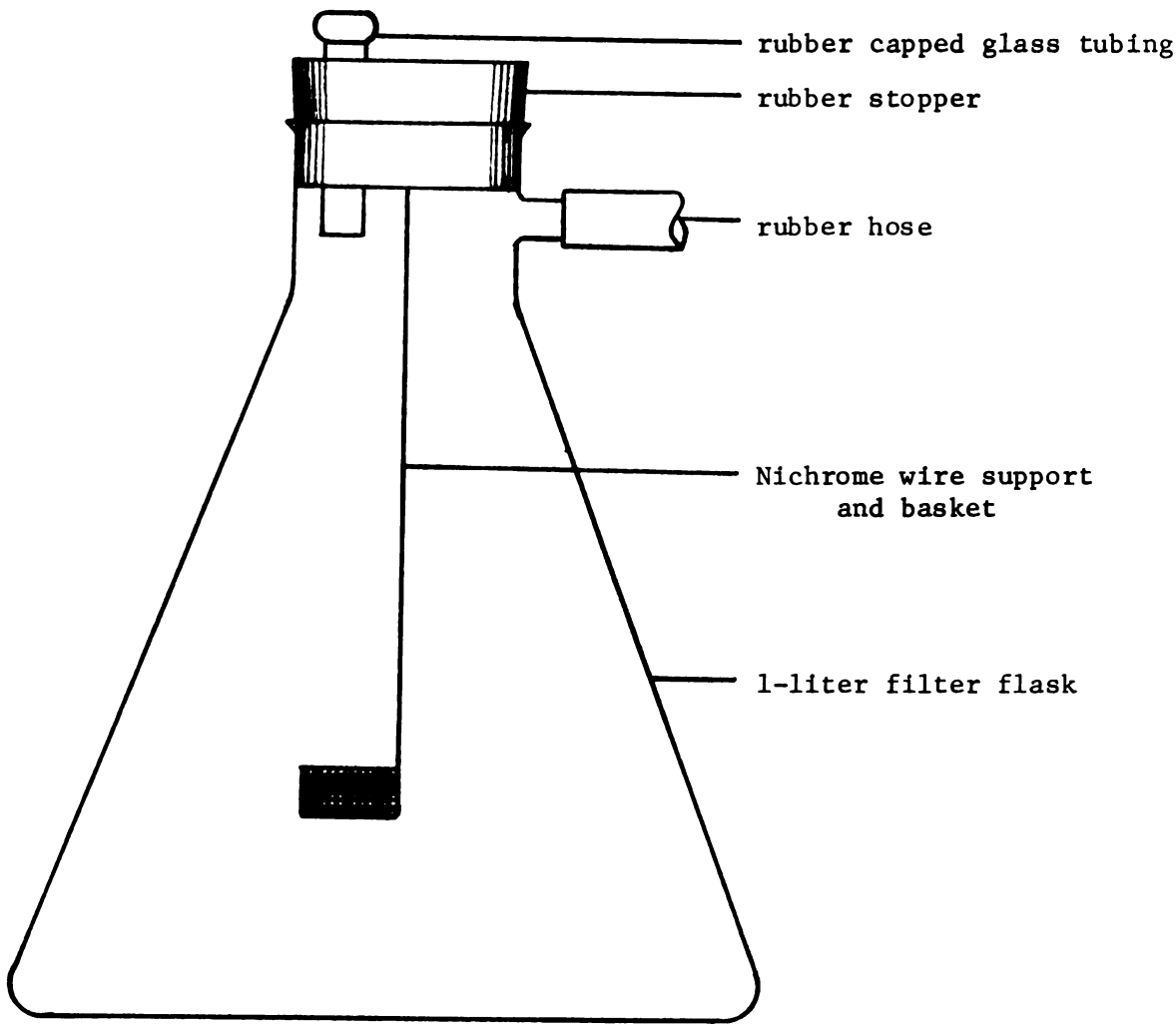

Figure 3. A side view of the modified oxygen combustion flask showing various parts. Rubber hose is connected to an adapter for evacuation and oxygen flushing. The basket holds the wrapped sample during ignition. Rubber capped glass tubing accepts the CO_2 trap after combustion.

Microorganisms in the gut of yeast feeding and wood feeding animals were isolated in YEA (yeast extract agar) and HIA (heart infusion agar). The gut was removed by pulling away from each other the head and the last two to three body segments with sterile fine-tipped forceps. This was done in a sterile disposable petri-dish. Immediately after removal an insect stuffing pin which was held in glass tubing and ethanol-flame sterilized was inserted into the still moist foregut and stabbed four to five times in the agar plates. This was repeated two times, each preceded by sterilization. Each stabbing was alternated between YEA and HIA. The same procedure for the hindgut immediately followed to avoid rapid drying of the gut after removal. As a control after each gut had been sampled, the pin was resterilized as before and stabbed into agar.

To estimate the relative counts of bacteria in the gut, the guts of five previously yeast fed Isopods (12 hr) and five starved Isopods were aseptically crushed with glass beads in 0.9 ml distilled water and made to 1.0 ml with standardized 1.95 μ-polyvinyl-toluene beads (Diagnostic Products). The number was calculated from the ratio of bacteria and beads under the phase microscope and from the known concentration of the initial bead suspension (4.6×10^8 beads/ml). Each treatment was duplicated. Each replicate was sampled and counted twice.

Cellulose decomposers were enriched (Aaronson, 1970) from 0.5 g of feces and five crushed guts of Isopods one week after collection of the animals. Each treatment was duplicated and as a control, the enrichment medium was not inoculated.

RESULTS

Soil arthropod-microorganism vs. arthropod or microorganism system

The combined activity of soil arthropod and microorganisms (system a) was more efficient in degrading uniformly labeled ^{14}C-yeast than either the arthropods (b) or microorganisms (c) as shown in Table 1. After 28 days, the animal-microbial system respired 56.4% of the labeled food while only 33.8% and 50.8% was respired in the animal and microorganism system, respectively.

Table 1. The fate of uniformly labeled ^{14}C-yeast 28 days after feeding to woodlice or acted upon by soil microorganisms

Degradation system	Percent of the original food			
	respiration a/	feces b/	assimilation c/	total
a. soil arthropod-microorganisms	56.4		17.1	73.5
b. animals	33.8	29.8	14.9	78.5
c. microorganisms	50.8			

a/differences significant at 5% level, T-test

b/difference between two computed values of decomposed feces insignificant at 5% level, T-test

c/difference insignificant at 5% level, T-test

The feces accounted for 29.8% of the label. Calculated from the table, two values of fecal decomposition may be ascribed to the soil

microorganisms: directly as the difference in respiration of treatment (a) and treatment (b), 22.6%; and indirectly as the difference of the label in the undecomposed feces (difference between total recovered label in system (a) and system (b), 5.0%) with the label present in the feces, 24.8%. This suggests that at least 75.8% or three-quarters of the fecal material was converted to CO_2 by microorganisms.

The animal retained an average 15.8% of the labeled material originally fed. It is assumed that the bulk of the labeled atoms are in stable body components as shown by the low rate of respiration (Figure 4) on the 28th day. All the systems were respiring almost at the same slow rate on the last sampling as seen in the merging of the curves. Clearly, the presence of the animals boosted the degradation of the yeast on the first day while the microorganisms took 3 days to reach their maximum decomposition rate. When the same data are presented in a cumulative manner (Figure 5), it was evident that decomposition was approaching a constant rate and that no further degradation could result in one system overtaking the other.

Unavoidable losses or errors resulting from inspections, adsorption to substratum, combustion, counting, and the length of time involved are assumed to account for the recovery of only 78.5% of the label originally introduced.

Yeast fed vs. wood fed Isopods

Table 2 shows that the respiration of yeast and wood fed animals after two days accounted for 9.6% and 11.7% of the total initial material, respectively. The difference was not statistically significant at the 5% level, T-test. In the same order as above, the percent of original material present in the feces was 37.4% and 28.2% while assimilation took

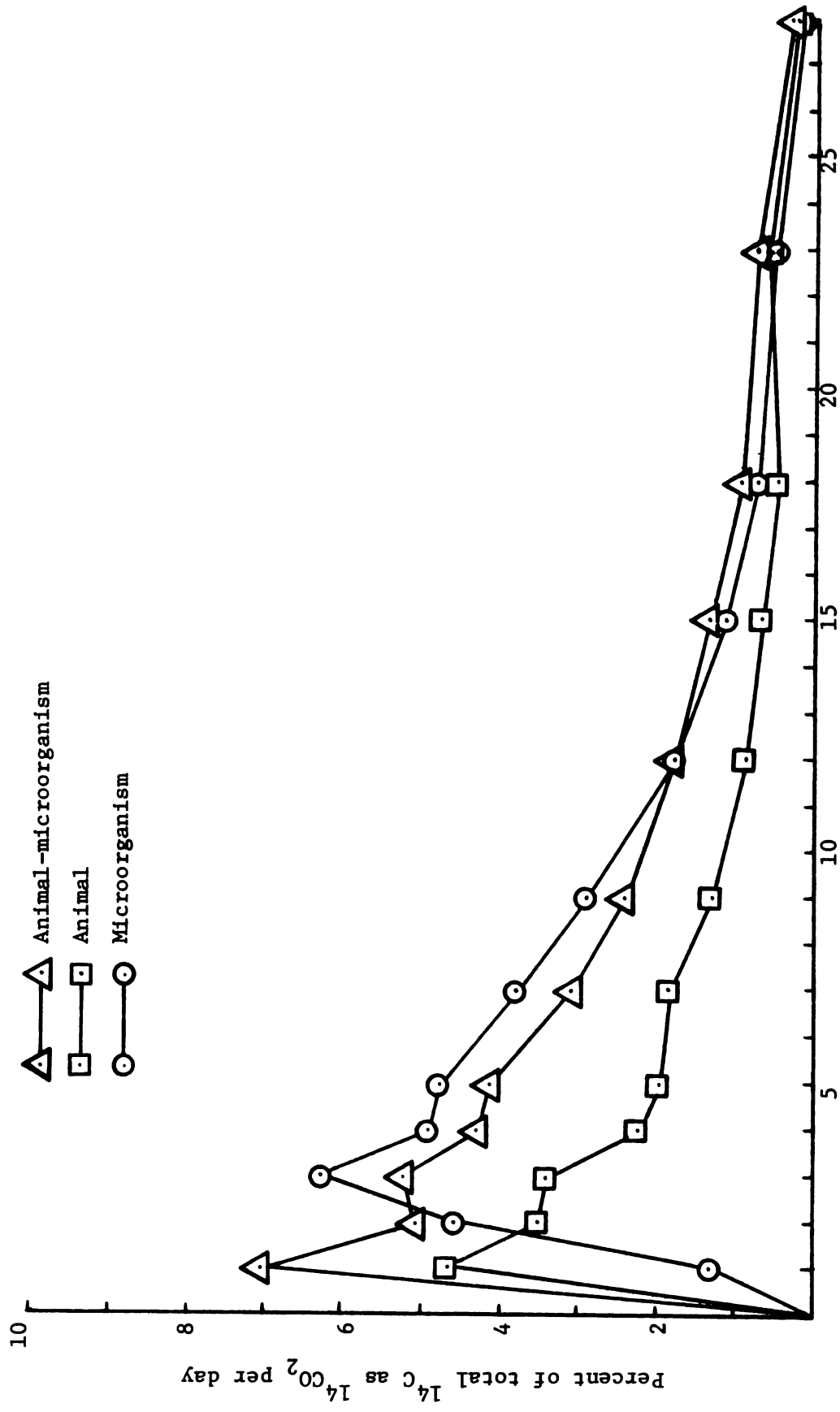

Figure 4. Percent decomposition per day of ^{14}C-yeast in system: (a) animal-microorganism; (b) animal; and (c) microorganism.

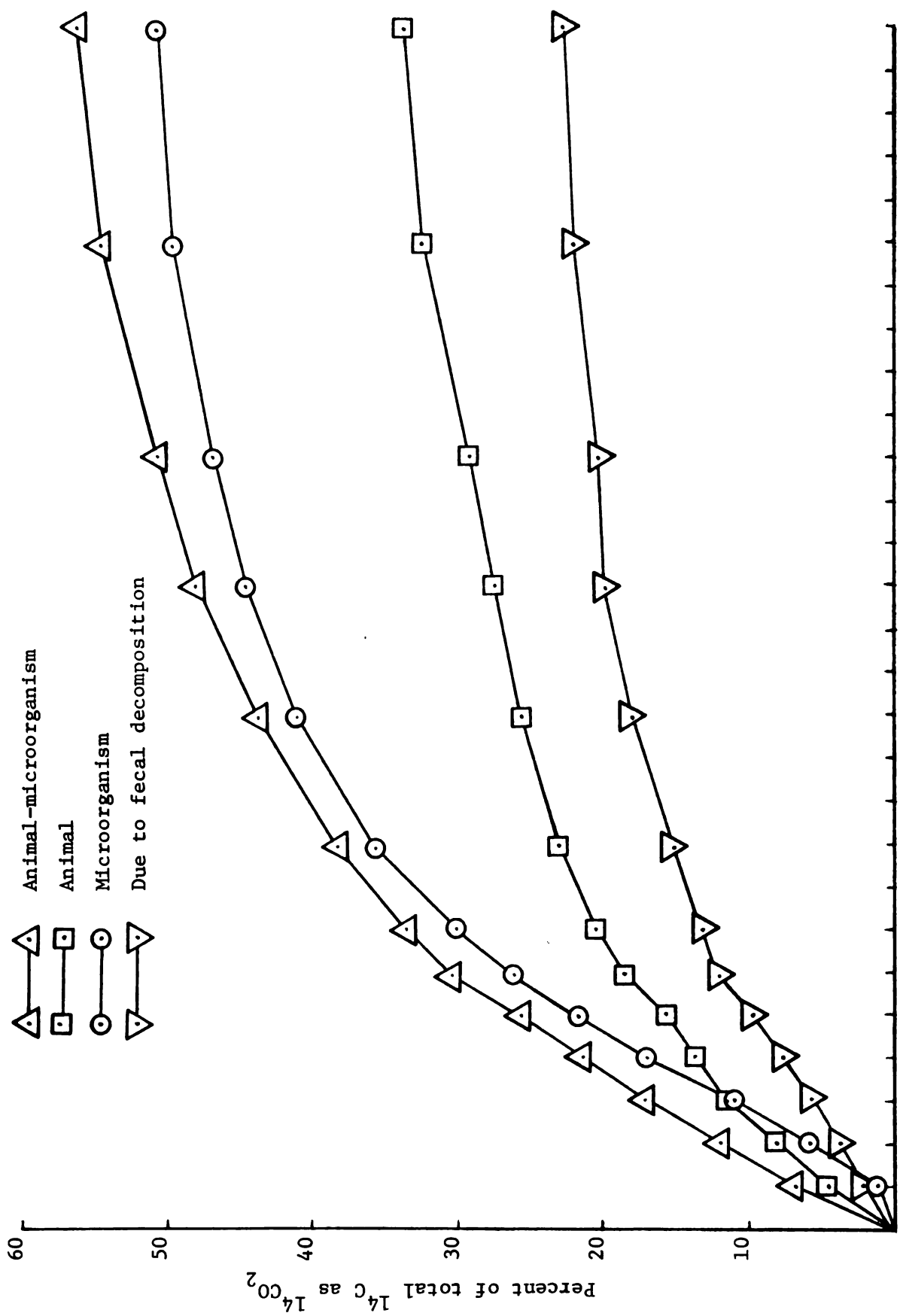

Figure 5. Progressive decomposition of ^{14}C-yeast in system: (a) animal-microorganism; (b) animal; and (c) microorganism.

Table 2. Distribution of ^{14}C label after one and two days following feeding of woodlice with uniformly labeled yeast for one day

Laboratory food	Percent of the original food					
	CO_2		feces		animal	total
	1 da a/	2 da b/	1 da c/	2 da d/	2 da* e/	2 da* f/
Yeast	5.8	3.8	33.9	3.5	37.8	84.8
Wood	6.5	5.2	23.9	4.3	44.4	84.3

*after 2 days

a/, b/, d/, and f/not significant at 5% level, T-test

c/ and e/significant at 5% level, T-test

37.8% and 44.4%, respectively. In both cases the difference between the animals was statistically significant at the 5% level. The difference in feces and assimilation between yeast and wood fed animals is possibly due to a difference in the microbial population associated with the animal gut as conditioned by the previous type of feeding. Wood fed animals were more efficient utilizers of the yeast substrate as indicated by higher assimilation and lower fecal excretion. The lesser efficiency of yeast fed animals is indicated by the opposite of the above. The maximum nine-fold decrease in radioactivity of the feces from the first to second day and the high assimilation compared to what had been respired indicates that the potential ability of these animals to utilize decomposable organic materials is high.

Biodegradability of feces vs. yeast in soil at two moisture levels

The percent decomposition per day of the labeled feces and yeast at two moisture levels (16.4% and 11.4%, respectively) are shown in Figure 6. Yeast, which is more nutritious and containing more digestible materials

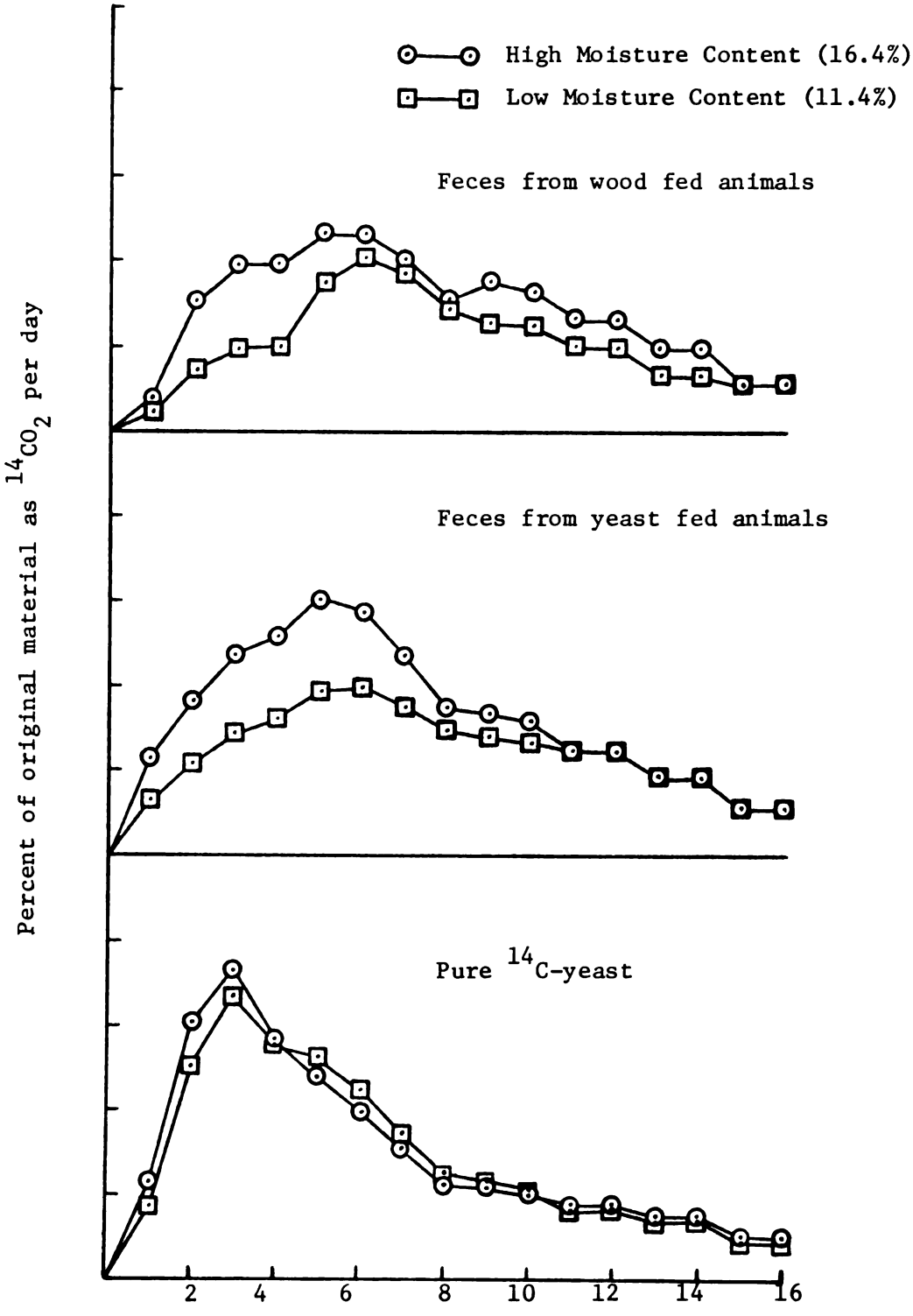

Figure 6. Percent decomposition per day in the soil of pure ^{14}C-yeast and feces from yeast feeding and wood feeding woodlice fed with ^{14}C-yeast.

than feces, decomposed rapidly in soil (maximum rate on 3rd day at 7.39% and 6.65% per day, respectively) though the rate declined abruptly after three days. On the other hand, the feces which had passed through the gut and easily decomposable materials removed, decomposed at a slower rate but for a longer period. The feces from yeast fed animals decomposed at a faster rate (peak on 5th day at 5.93% and 6th day at 3.97% per day, respectively) than the feces from wood fed animals (peak on 5th day at 4.64% and 6th day at 4.01%, respectively). This difference in rate of decomposition might be explained by the high assimilation of the labeled food by the wood fed animal, giving off more resistant feces. Conversely, the yeast conditioned animals which had lower assimilation excreted relatively less resistant feces. The high moisture content favored more rapid decomposition of the feces but not the yeast.

Gut examination

A thin cross section of the hindgut of woodlouse (Figure 7) shows a thin chitinous wall lining (intima) and bristle-like protrusions.

At least six microscopically distinct types of bacteria and two kinds of fungi were isolated from the gut on YEA and HIA. The bacteria ranged from very short rods to long rods. Motile species were observed. No attempt to identify the isolates was made. Counting by comparison with standardized 1.95 μ-polyvinyl-toluene beads revealed a population 3 to 7 x 10^6 bacteria/gut. The enrichment for cellulolytic microorganism based on growth and disappearance of ground filter paper, was successful only from the gut but not from the feces.

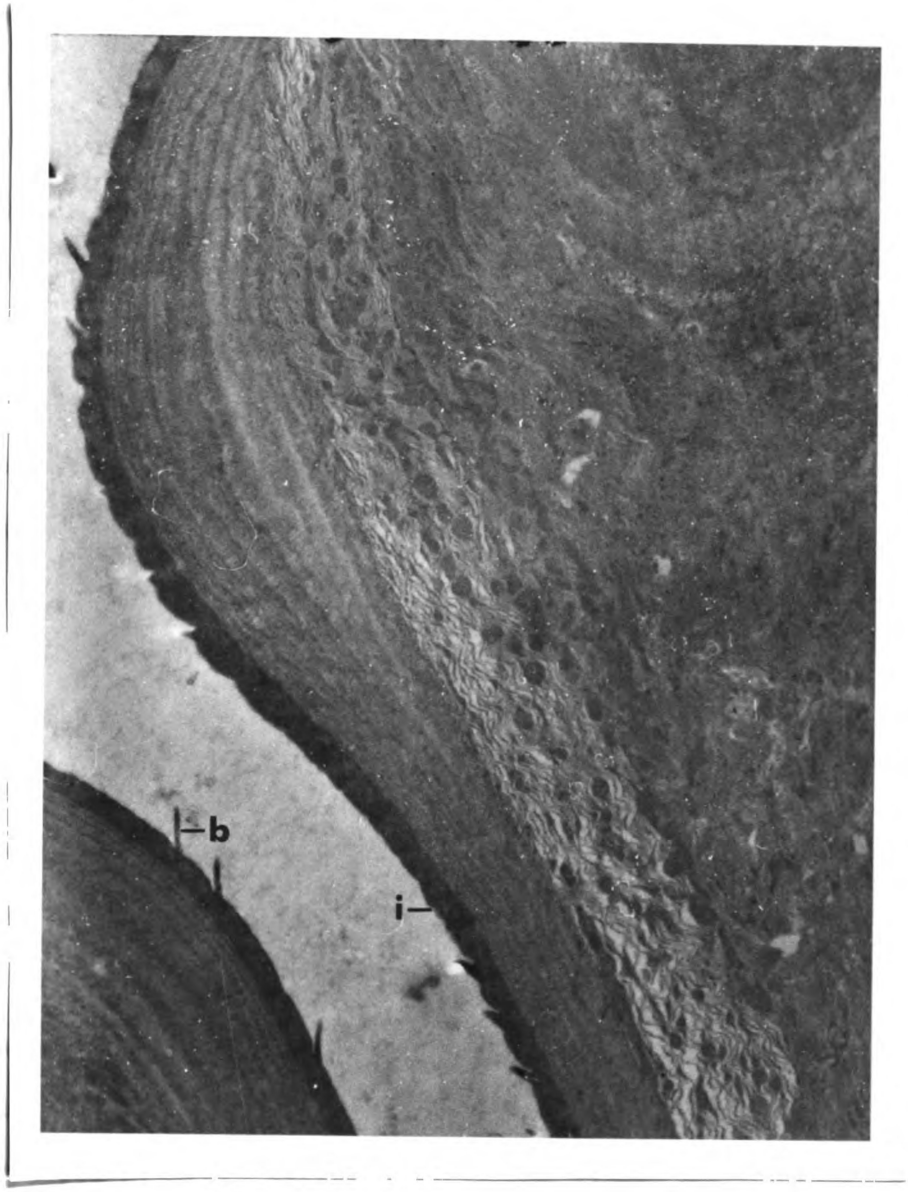

Figure 7. An electron micrograph of a thin cross section of hindgut epithelium showing chitinous intima (i) and bristle (b), 5625 X.

DISCUSSION

The associated activity of soil animals and soil microorganisms appears to result in a more rapid biological disintegration of plant residue. Kurcheva (1960) showed that in the absence of mesofauna, bacteria and fungi decomposed only 10% leaf-fall after 140 days while in the presence of the animals the decomposition was 50%. Odum (1967) estimates that 90% of the net production of the forest is consumed as dead materials. The estimated production of forests are 6.8-9.4 metric tons leaf-fall/ha/yr in the tropics (Cornforth, 1970) and 2.3-4.0 metric tons leaf-fall/ha/yr in temperate deciduous forest (Thomas, 1970), indicating the tremendous amount of organic matter that must be degraded. With the "detritus food chain" as a more important energy pathway in many ecosystems compared to "grazing food chain" (Odum, 1967), the interrelationships of soil biotic communities in degrading biological materials are consequently indispensable.

The high initial rate and total respiration with the woodlice and soil microorganisms combined was largely due to animal respiration. Some soil animals are known to possess digestive enzymes. For example, earthworms contain cellulase and chitinase (Kühnelt, 1961) while the wood-boring isopod *Limnoria* secretes cellulase (Vonk, 1960). The wood feeding insect *Ips rexdentatus* is endowed with a large enzymatic arsenal enabling it to break down many oligosaccharides and polysaccharides (Fay *et al.*, 1970). Soil macrofauna feeding on freshly fallen litter can remove digestible substances like protein, carbohydrates, and fats (Kühnelt, 1961).

The possibility that substrate metabolism was enhanced by the animal due to the presence of gut flora could not be discounted. The microbial numbers and types vary with time, depending on previous food's state, animal's feeding habit, and condition inside the gut (Ghilarov, 1962); and these microorganisms could also give off enzymes such as cellulase (Vonk, 1960; Törne, 1967 and 1968; Honigberg, 1970) and chitinase (Vonk, 1960). Cellulose digesting protozoa are always associated with wood-feeding roach *Cryptocercus* (Cleveland *et al.*, 1934). In insects, micro-symbionts secrete vitamins and amino acids that are eventually utilized by the host (Henry, 1962). Microbes could also synthesize polysaccharides (Hobson, 1970) with subsequent release for absorption or breakdown by other flora.

The chitinous bristles lining the hindgut wall are probably an indirect evidence that gut microorganisms are interacting with soil animals in organic matter decay. Buchner (1965) said that related species containing these appendages use the bristles to hold food in the gut or guarantee permanence of the bacterial strain. Cleveland (1934) showed that when the sphincter of roach contracted, the bristles overlap and close the lumen to the passage of protozoa and wood particles. Buchner (1965) noted that proteases are found in the intestinal secretions of ant larvae though they remain ineffective when pH is alkaline, allowing the ingested microorganisms to pass the midgut unaltered. But, as soon as a sufficient amount of acid is produced by bacterial activity to neutralize the intestinal secretion, the protease is activated and bacteria are digested. In addition, bacteriolytic enzymes are produced by bacteria themselves (Hoogengraad and Hird, 1970) resulting in non-digestive lysis of the gut flora (Bocock, 1962). This may partially explain why the enrichment for cellulolytic microorganism from woodlice feces failed but succeeded in pure gut contents.

A second source of increased respiration in the combined soil animal-microbial system is the decomposition of the feces by the soil microorganisms. It could be expected that increased surface area and moisture due to passage of food through the animal would result in a more favorable condition for decomposition. The enrichment of droppings with nitrogen (Bocock, 1962 and 1964) may stimulate feces degradation even when lignin is high. This has been found to be true especially if soft leaf materials high in nitrogen were fed to soil animals (Heath *et al.*, 1966). Edney (1954) found that the main nitrogenous excretion of woodlice is NH_3, accounting for 70-80 percent of the total non-protein nitrogen excreted. Uric acid was also detected. Dünger (1958), however, claimed that there is no nitrogen enrichment in the gut but conceded that the amount of humic acids increased. Feces could also be enriched with vitamins promoting the growth of other organisms as suggested by Stebaev (1968). Proteins in the form of enzymes associated with the excrements provide favorable nutrients to support vigorous microbial growth and organic matter decay (Rangarwami, 1966).

The difference in the degradation rate of the yeast and the two types of feces shows that the biodegradability of organic residues depends on their physical and chemical nature as well as the conditions of the environment in which they are located. Wallwork (1958) noticed that factors affecting food preferences are particle size, stage in chemical decay of food and moisture content. In another instance van der Berg and Ryke (1967) pointed out that Acari are chemical specific rather than hardness specific.

The method for following the fate of decomposing organic material described here may be said to be reliable. The results are reproducible and the recovery was relatively high. But like the other methods employing radioactive tracers, these results cannot become meaningful once the

consumer-food relationship changes (Shure, 1970). Its potential might be limited in some ecosystems like flowing streams and river. Low oxygen concentration does not seem to affect the animals. In the laboratory, woodlice, Collembola, and mite cultures were able to stand long periods without opening the screw cap.

Other radioisotopic methods have been employed using ^{182}Tantalum (Murphy, 1962) and ^{184}Cesium (Witkamp, 1969). The main disadvantages using these isotopes are their incompatibility with biological systems and the fact that they do not quantitatively relate to energy flow. Uniformly distributed ^{14}C which is compatible with biological systems can quantitatively relate to energy flow. Since respiration measurements yield better values than biomass estimates for appraisal of material and energy flow (Kühnelt, 1961), it is felt that the method developed is advantageous.

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