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Studies on the decomposition of the faecal pellets of Glomeris Marginata Villers

P.B. Nicholson

STUDIES ON THE DECOMPOSITION OF THE FAECAL

PELLETS OF GLOMERIS MARGINATA VILLERS

P. B. NICHOLSON

The Nature Conservancy;   formerly at

Merlewood Research Station, Grange-over-Sands,

Lancashire;   at present East Suffolk Warden Naturalist,

Carmel Cottage, Cookley, Halesworth, Suffolk

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I. INTRODUCTION

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Dudich Balogh and Loksa (1952) and Nef (1957) calculated that all
the annual litter fall in woodlands is eaten eventually by the soil
fauna.   More than 60% and usually about 90% of this food litter is
returned to the soil as faeces (Bocock 1963, Van der Drift 1951,
Gere 1956, 1962).   Although the faeces are such a large potential source
of plant nutrients their decomposition has been little studied.

Van der Drift and Witcamp (1959) found that respiratory activity
in the decomposing faecal pellets of a trichopteran larva reached a
peak after 5 days and then declined.   This high activity was corroborated
by the evidence from bacterial and fungal dilution counts which were far
higher after 12 days decomposition than they had been in fresh material.
During the first 14 days Mucoraceous fungi were dominant on the pellets
after which they were replaced by Fungi Imperfecti.   After 6 weeks,
substituted Glomeris pellets had lost considerable amounts of cellulose
and there had been small losses of nitrogen and soluble carbohydrates.
Parle (1963) investigated the decomposition of ageing earthworm casts.
He found that respiratory activity declined considerably as the casts aged.
Because bacterial counts made over the same period fluctuated irregularly

he suggested that the bacteria formed resting stages during this period. Direct fungal counts (Jones and Mollison, 1948) increased and reached peak values at 15 days. There was no clear sequence of fungal succession and it was thought that the fungi that grew were probably those dominant in the soil the worm had eaten. Stebaev (1964) found that bacterial counts on crane fly excrements were considerably higher than those on soil samples, whilst fungal counts were lower. However, the variety of fungal and bacterial species recorded from excrements was greater than that for soil. Aspergillus, Penicillium and Mucor spp. were the most frequently recorded fungi from both excrements and soil.

Other work has been concerned with a comparison of the bacterial counts of earthworm faeces and parent soil. Bässalik (1913), Stockli (1928), Ruschmann (1953), Schutz and Felber (1956), Swaby (1949), recorded higher bacterial counts in fresh faeces than soil whilst Day (1950) found that these counts did not differ consistently. Went (1963) suggested that fresh earthworm faeces appeared to have high bacterial counts because the soil food was mixed with plant and soil debris and these counts could not therefore be compared directly with pure soil.

Relevant research on vertebrate faeces has been confined to studies on fungal successions. The well-known succession on ageing rabbit dung has recently been described in detail by Harper and Webster (1964).

There is an initial phase of Phycomycetes followed by Discomycetes and later, Pyrenomycetes. Finally, Basidiomycetes (esp. Coprinus spp.) sometimes appear. Watling (1963) investigated the succession appearing on hawk pellets and his results were similar to those for rabbit dung. There was an early Phycomycete stage but this gave way to Fungi Imperfecti. The succession concluded with an Ascomycete stage. These fungi eventually died out, leaving the substrate depleted of available nutrient.

One point on which most of these workers agree is that microbial activity in fresh faeces is higher than in the parent food. This finding indicates that the contribution of soil animals to the breakdown of dead plant material is considerably greater than assimilation data indicate because the stimulation of microbial activity in the faeces must be taken into account. The degree of stimulation which occurs and its effect on the overall rate of decomposition, are the two problems that originally stimulated this research.

The aim of the present studies therefore was to obtain some fundamental information on the little studied topic of faeces decomposition. The faecal pellets of the millipede Glomeris marginata (Villers) were used for the experiments. This animal was chosen because it is a plant

litter feeder commonly found in base-rich woodlands (Blower 1959), and its ecology and biology have been well studied (Bocock 1963, Van der Drift 1951). Providing the humidity is kept high, this animal feeds readily in laboratory culture. Its principal foods are the dead leaves of ash, hazel, and birch, as well as a little soft rotten wood, moss and mineral soil. However, to obtain a degree of uniformity in these experiments only hazel leaf litter was used as food.

Fresh pellets were collected from Glomeris cultures and allowed to decompose under fixed conditions. Losses in dry weight and qualitative and quantitative changes in the microbial population were estimated. Respiration and changes in chemical composition were taken as indications of the microbial activity. Two field experiments were carried out, the first to compare the pattern of pellet decomposition in the field and in the laboratory, the second to obtain more information about the first 2 to 3 weeks of decomposition when changes in microbial populations were most noticeable.

II. METHODS

II. METHODS

(1) Culture Techniques

The experiments required a large sample of undamaged *Glomeris* pellets of approximately the same age. An apparatus was used that delivered such pellets (Plate 1 and 2). Fundamentally it was a Tullgren funnel apparatus with a *Glomeris* culture in the sample cup. The pellets from this culture were funnelled into a sterile damp chamber below. The funnel was constructed of aluminium and was 25.5 cm high having a diameter of 11.6 cm and 1.4 cm top and bottom respectively. The aluminium culture vessel was 5 cm high and 8.5 cm diameter. It had a perforated metal floor (aperture size 2 mm) and was supported inside the funnel by three metal hooks. Ten "Tullgren" cultures were set up and kept in the laboratory.

When pellets were required, about 30 adult *Glomeris* were placed in the culture chambers with food material. The chambers were then closed with a plastic petri dish top containing a dampened filter paper. The upper half of the apparatus was sealed against moisture loss with a polythene sheet fastened with an elastic band (Plate 2). During collection of the pellets, the funnels were shaken occasionally to dislodge any pellets trapped amongst the food. After 2-3 hours pellets began to drop into the collection vessel. This was a sterile

Plate 1.   Inside of Tullgren sample cup showing <u>Glomeris</u>
culture and moist hazel litter food

Plate 2.   Tullgren funnels modified for <u>Glomeris</u>
            culture.   Each is sealed with polythene
            sheeting at top and bottom

sample jar, 7 cm high, 5 cm diameter, containing a number 1 Whatman filter paper, previously moistened with 1 ml of sterile distilled water.   To prevent moisture loss the lower half of the apparatus was sealed in a polythene bag (Plate 2).   All parts of the apparatus were thoroughly cleaned before each experiment.

(2) Glomeris Food and Feeding

To reduce experimental variability cultures were fed only on hazel litter (Corylus avellana L.).   Large samples of hazel litter were collected in May 1964 (laboratory experiments) and October 1964 (field experiments).   The litter was air dried ($30^{\circ}C$) and stored in polythene bags.   Before they were fed, the Glomeris cultures were starved for 24 hours to empty the gut of food eaten prior to the experiment.   A sample of food litter (approximately 100 g. dry weight) was soaked in sterile distilled water for one hour.   The excess water was squeezed out by hand, and the food was divided between the cultures.   To minimize the effect of changes in the microbial population in the food during feeding the food litter was changed after 12 hours.

When "time 0" (fresh) pellets were required, collections were made from the funnels every three hours.   When air-dried pellets were required the collecting jars were kept dry and open so that the

pellets dried as they were collected. A culture of about 30 mature Glomeris would deliver approximately 1.5 g. of pellets in 12 hours.

(3) Laboratory Experiments

The Tullgren cultures were set up and pellets were collected for 12 hours. The collecting jars were sealed with polythene to prevent moisture loss and stored at temperatures fluctuating between $10-13^{o}C$. In this way the jars served both as collecting vessels and decomposition damp chambers. A large number of these collections were made, thus providing a store of decomposing pellet material of various ages. When a sampling was made, one batch of ten replicate jars was opened and one sub-sample was taken from each jar. For the fungal colonisation experiment, the same jars were used throughout, and no pellet material was removed.

The jars were sampled as follows:

(a) Dry weight changes

One batch of ten replicate chambers was set up, and the pellet content dried and weighed; 6 ml of sterile distilled water was added, and the jars were sealed with polythene sheeting and stored. After eight weeks, they were opened, dried and re-weighed.

(b) Mycelial lengths and bacterial counts

Nine batches, each of ten replicate damp chambers, were set up and stored. Sampling was made on pellets after 0, 3, 7, 14, 21, 28,

42, 56, and 70 days.   At each sampling, one batch was first dried

and ten sub-samples, 0.125 g. each, were taken.   To see what

effect passage through the animal gut had on the fungal count,

counts were also made on the hazel litter food.   Because <u>Glomeris</u>

rejects the leaf main vein and larger lateral veins, these were

removed before testing.

(c) Respiration measurements

Measurements of oxygen uptake and respiratory quotient were

made on pellets which had decomposed for 0, 3, 7, 14, 21, 28, 63 and

77 days.   One batch of ten replicate damp chambers was opened at

each sampling.   Each sampling was made three times, giving

30 replicates/period in all.

(d) Fungal colonization

Thirty replicate damp chambers were set up and stored.   Each

was inspected for growth of superficial fungi after 0, 3, 7, 14, 21,

28, 35, 42, 56, 70 and 84 days.   The damp chambers were brought into

the laboratory, opened, and the pellets viewed under a low-power

microscope (x 20 x 75).   ⁀Superficial fungi were cultured and

identified.

(e) Chemical analyses

Ten replicate damp chambers were set up.   Five of these were

bulked and stored in a refrigerator at $-10^{o}C$.   The other five were

stored in sealed jars as described above, and bulked after 56 days.

Analyses were made for total ash, carbon, nitrogen, soluble carbo-

hydrates and holo-cellulose on each bulked sample.   A supplementary

analysis was made for soluble carbohydrates to find how they were

being used up in the early stages of decomposition.   One batch of

pellets was analysed for soluble carbohydrates after 0, 7 and 28 days.

(4) Field experiments

    (a) The experimental site

The site selected for study (Plate 3) was an area of deciduous

woodland in the grounds of Merlewood Research Station (Nat. Grid Ref.

SD(34)409796).   Bocock (pers. comm.) had recorded densities of

Glomeris as high as 100 animals/sq. m. in this woodland.

The wood is at an altitude of approximately 250 ft. (61 m), and

is on a steep slope with a south easterly aspect.   The average

annual rainfall is 48.3 in. (122.8 cm).

The trees are a mature stand 60-70 feet (20-21 m) high, with

ash (Fraxinus excelsior L.), sycamore (Acer pseudo-platanus L.) and

elm (Ulmus glabra Hudson) dominant.   Hazel (Corylus avellana L.) forms

a sparse coppice understorey.   Dog's mercury (Mercurialis perennis L.),

with occasional patches of ferns, (Dryopteris felix-mas (L. Scholt);

Phyllitis scolopendrium (L. Newm) dominates the ground flora.

The soil is a mull-like rendzina with a moder humus form (Kubiena 1953). It consists of a layer of limestone rubble (stones 1-15 cm diam.) which varies in depth from 25-100 cm. Beneath the uppermost stones there is an organic matter rich layer, dark brown in colour, composed almost entirely of animal faeces. Leaf veins, mollusc shells and fragments of insect cuticle are conspicuous. Deep burrowing earthworms and soil arthropods, including _Glomeris_ are common on the site. (See Appendix Note 1). The lower soil is much intermixed with silt from siliceous glacial drift. Most of the plant litter disappears from the soil surface in the first nine months after leaf fall, but occasionally an F layer is present in small stony hollows and around coppice stools.

Two relatively uniform areas each 15 x 15 m. were chosen for the experiment.

(b) Field techniques

Preliminary searches in the field showed that natural accumulations of _Glomeris_ pellets occured most frequently on the soil surface amongst the organic matter rich layer. Therefore, the pattern of pellet decomposition in the field was followed by placing weighed pellet samples on to the soil surface and testing these samples at intervals.

Because it was difficult to distinguish the sample pellets from natural pellets and other organic material in the soil, they were sealed into terylene net bags, made in the laboratory from 0.7 mm mesh terylene netting. This material was cut to approx. 8 x 4 cm, folded, and the edges fused together over a spirit lamp. The resulting bags (Plate 4) were approximately 4 x 4 cm, and weighed 0.3-0.5 g.

Each bag was first weighed, and 0.5-0.8 g. of air dried Glomeris pellets was added. The bag was thoroughly shaken to allow fine particulate matter to drop through the mesh and be re-weighed. The mouth of the bag was sealed as described above, and the bag was weighed again. Thus each contained a known quantity (i.e. one sample) and any loss in weight caused by the final sealing could be accounted for. Each bag was placed on to the soil and covered with a few small pieces of limestone.

In the first field experiment (started 19th October, 1964), approximately 140 samples were placed in the field. The position of each was marked with a short cane (Plates 3 and 4). Samplings were made after 0, 3, 7, 14, 28, 42, 56, 84, 140 and 365 days.

At each sampling, ten randomly selected samples were brought into the laboratory. Moisture loss in transit was prevented by placing

Plate 3.   One of the 15 x 15 m. plots used for field
            experiments.   In the foreground are canes
            each marking the position of a <u>Glomeris</u>
            pellet sample.

Plate 4.   Terylene netting bag, which contains a <u>Glomeris</u>
            pellet sample, with marker tag and cane.   The
            small covering pieces of limestone have been
            removed for the photograph.

the bags in petri dishes lined with moistened filter paper.   A low-power microscope (x 20 x 75) was used to observe the physical condition of the pellets and fungal growths.   Small animals and contaminating soil were picked off.   Each sample was placed into a Warburg flask and respirometry readings were made for six hours. Subsequently, to allow fungi to spore, the samples were stored in petri dishes (as above) for three days at laboratory temperatures (20-22°C), after which the bags were slit open and inspected with the low power microscope for superficial fungi.   The samples were then dried and weighed.   The pellets were bulked and thoroughly mixed. Two 0.125 g. sub-samples were taken for mycelial length estimations. Ten extra samples were taken at the 84 day sampling, cleaned and dried as above, and bulked for chemical analyses.   To obtain, an accurate determination of the dry weight loss after 365 days, 37 samples were taken.

For the second field experiment, approximately 300 pellet samples were placed in the field as above.   Sampling was made daily for the first 17 days of decomposition and also after 42 and 56 days. At each sampling 15 samples were brought into the laboratory and examined and cleaned as before.   Respirometry readings were taken for six hours from 12 samples, whilst simultaneously, the mycelial

length estimations and bacterial counts of the remaining three samples, were determined. Estimates of moisture content were obtained by drying small sub-samples.

Daily weather readings were taken throughout the field experiments. Rainfall was measured using a standard 5" gauge and temperature fluctuation with a maximum and minimum thermometer placed on the soil surface and covered with loose rubble. Daily pellet moisture content for the first field experiment was measured by taking a sub-sample from a large "time 0" pellet sample, placed on the soil surface on the field site. Detailed rainfall results are not given because they correspond with changes in pellet moisture content.

(5) General Techniques

(a) Dry weight determinations

Standard drying conditions were used throughout the experiments. Samples were subjected to 30°C for 72 hours in an oven with forced air-circulation.

(b) Chemical analyses

Carbon was analysed by dry combustion (Ingram 1956) and nitrogen by a modification of the Kjeldahl method. Ash content was determined as loss on ignition at 550°C. Soluble carbohydrates were measured by the anthrone procedure of Deriaz (1961), holocellulose by the method of Wise et al (1946).

(c) <u>Mycelial length and bacterial counts</u>

The method of Jones and Mollison (1948) was modified as follows for mycelial length estimations and bacterial counts. Each sub-sample was homogenized in a laboratory blender for 15 minutes at 2,000 r.p.m., in 10 ml of water. The resulting suspension was transferred to a 50 ml volumetric flask and made up to the mark with 2.5% water agar. Eight agar films were made from each suspension using a haemocytometer of 100μ depth. These were allowed to dry in air and stained in 2% phenolic-aniline blue for 12 hours, washed in methylated spirits and mounted in Euparal.

A projection system (Plate 5) was used to reduce the strain of counting the large number of agar films. An optical bench was fitted with a monocular microscope mounted horizontally at one end, and a 250 W mercury vapour lamp (200-250 V 250 W AC/DCME/D Box type) at the other. The beam of the lamp was focused on to the condenser of the microscope by a condenser lens (Nelson type 2 lens aplanatic 2.5" f.l.). A satisfactory image of the microscope field was then reflected by a mirror prism on to a sheet of dull white paper. The size of this image using a x 40 objective and a x 6 eye piece was approximately 15 cm diameter. The microscope field diameter was approximately 250μ. Total length of mycelium was measured with a low-geared map-measurer
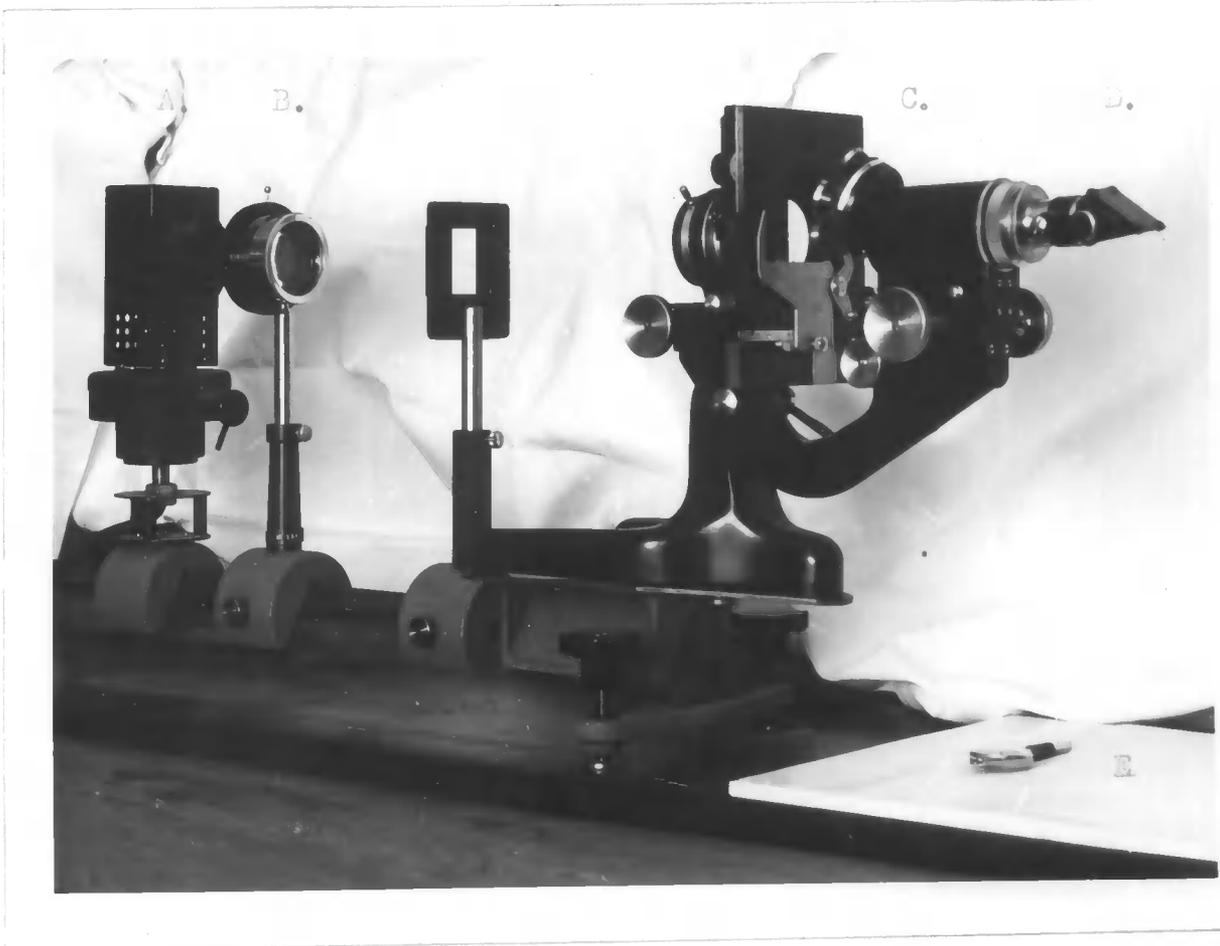
Plate 5.   Projection apparatus used for estimating fungal
mycelium lengths in agar films.   A. Mercury
vapour lamp.   B. Condenser lens.   C. Horizontally
mounted microscope.   D. Mirror prism.   E. White
paper sheet screen for image.

(Number 620 courrimetre H.B. Paris).   The projection system was unsatisfactory for counting bacteria and these counts had to be made by direct microscope viewing using a x 90 oil immersion objective and a x 10 eyepiece.   Ten fields/film were examined for bacteria and fungi.

(d) Respiratory measurements

Respiratory measurements were made in a Warburg apparatus. Carbon dioxide was absorbed continuously in 10% sodium hydroxide and Oxygen uptake measured by the change in pressure of the gas in the reaction vessel.   Carbon dioxide liberation was determined by the "direct" method of Warburg (1926) (Umbreit et al, 1957).

When measurements were to be made, the Warburg apparatus was switched on and left overnight so that the stirred water bath reached the required temperature.   This temperature was $10^{\circ}C$ for the laboratory experiments, and the average daily temperature of the week preceeding each sampling for field experiments.   Thus in the first field experiment this temperature remained at $10^{\circ}C$ for the first three samplings (28 days) but then declined steadily to $2.5^{\circ}C$.   In the second field experiment this temperature fluctuated little and the bath temperature was kept constant at $12^{\circ}C$.   To maintain the low temperatures used in some of the experiments, the Warburg apparatus

was kept in a cold room; final experimental control being made by
the thermostat of the Warburg water bath.

Each sample was placed in a Warburg flask, and 10% NaOH was
pipetted into the centre well of the oxygen uptake flasks.   To
increase the area available for CO_2 absorption, a piece of filter
paper (2 x 2 cm) was fitted into the centre well so that it
protruded into the gas space, (the appropriate weight/volume
allowance was made when calculating the results).   The samples were
allowed $\frac{1}{2}$ hr. to equilibrate, after which the manometer taps were
closed.   Readings were taken at hourly intervals for six hours.

(e) Fungal isolation

Known fungi sporing on the pellet surface were recorded.
Unknown sporing species were isolated on to 2% potato-dextrose agar
(P.D.A.) for later identification.   Because sporing fungi were
infrequently observed in the field, an additional sampling technique
was used.   About 50 isolations/sampling were made by picking pieces
of superficial mycelium from the pellet surface with fine forceps
and growing these on P.D.A.

Careful study of the early growth of cultures made in this way
indicated that developing colonies grew from the "picked" hypha and
not from contaminating spores.

Non-sporing isolates were induced to spore by growing on 2% malt extract agar, 0.5% yeast extract agar, and 2% potato carrot agar. Air-drying, sunlight, and stroking with a hot needle were used also. If these stimuli failed, the isolate was classified as "sterile". The formulae for all media used in experiments may be found in the appendix.

(f) Statistical procedures

Means for oxygen uptake, mycelial lengths and bacterial counts are given ± 95% confidence limits. The latter were calculated using the "differences of squares" formula giving the variance,

$$s^2 = \frac{1}{n-1} \sum (x - \bar{x})^2 \text{ where } s^2 = \text{the variance,}$$

n = the number of observations, $\bar{x}$ = the mean of the

observations, x = each observation.

Since for all samplings, except one, n < 30, the confidence limits for the mean were $\bar{x} \pm \frac{ts}{\sqrt{n}}$, where t with n-1 degrees of freedom was obtained from tables. (Fisher and Yates 1948). For the one sampling where n > 30 confidence limits were $\bar{x} \pm \frac{ds}{\sqrt{n}}$, where d = 1.96.

Any change or fluctuation of a variable discussed in the text is a significant one. The modified form of Student's 't' formula for a small number of samples (Bailey 1959) was used to test the

differences between two means (x_1, x_2, with n_1, n_2 observations).

$$t = \frac{\bar{x}_1 - \bar{x}_2}{s \sqrt{\dfrac{1}{n_1} + \dfrac{1}{n_2}}}$$

$$s = \frac{1}{n_1 + n_2 - 2} \left(n_1 - 1 \, (s_1^{\,2}) + n_2 - 1 \, (s_2^{\,2}) \right)$$

When the variances of the two samples to be tested were significantly different (using the ratio, $F = \dfrac{s1^2}{s_2^{\,2}}$ where $s_1^{\,2}$ is the larger variance, from tables of F distribution (Fisher and Yates 1948), with $n_1 - 1$ and $n_2 - 1$ degrees of freedom), the approximation was used:

$$d = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\dfrac{s_1^{\,2}}{n_1} + \dfrac{s_2^{\,2}}{n_2}}}$$

'd' being distributed approximately like 't' with 'f' degrees of freedom.

$$f = \frac{1}{\dfrac{u^2}{n_1 - 1} + \dfrac{(1 - u)^2}{n_2 - 1}}$$

$$u = \frac{\dfrac{s_1^{\,2}}{n_1}}{\dfrac{s_1^{\,2}}{n_1} \quad \dfrac{s_2^{\,2}}{n_2}}$$

III.   APPROACH AND CHOICE OF TECHNIQUES

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Before starting a large scale experiment a small "pilot" experiment was made to test the suitability of the methods. Sometimes methods were modified and the pilot experiment was repeated before the large scale experiment was begun. The unsuccessful pilot techniques and results are mentioned only where they are of special interest.

One useful approach to ecological research is to adopt a number of techniques all having the same general aim. An overall picture gives results which may also indicate the errors involved in each technique (Nicholas 1962). In the present studies, because many different aspects of <u>Glomeris</u> pellet decomposition were investigated this approach could not be used. Therefore, as far as possible, measurements were made which were complementary to each other and the interrelationships of the different types of results are indicated and discussed.

All the apparatus used was carefully checked for accuracy. The results of the laboratory and two field experiments have been presented together to make comparison easier.

IV. RESULTS

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(1) Microscopic Changes in Pellets

The pellets of <u>Glomeris</u> (Plate 6) are smooth truncated cones approximately 2 x 0.5-1 mm and 0.1-1.0 mg dry wt. In the present investigations they were a mixture of hazel leaf lamina fragments (0.001-0.01 sq. mm), pollen grains, spores, fungal and actinomycete mycelium, algal cells and bacteria.

Fresh pellets (moisture content 70-90%) were used in the laboratory study, and air-dried pellets (moisture content 15%) were used in the field experiments. The air-dry/regained moisture rapidly pellets in the field reaching 70-90% moisture after 24 hours.

In both the laboratory and field studies the appearance of pellets was unchanged until the third day, when superficial hyaline hyphae were observed. Thereafter, in the laboratory samples, fungal colonization of the pellet surface proceeded quickly and many species were observed sporing. Most pellets were completely covered with a mycelial mat after 28 days. After one year the mat was still present and the pellets retained their original shape. By contrast, fungal mycelium was always sparse on the surface of pellets exposed to field conditions and few species were recorded with spores. Hyaline mycelium was
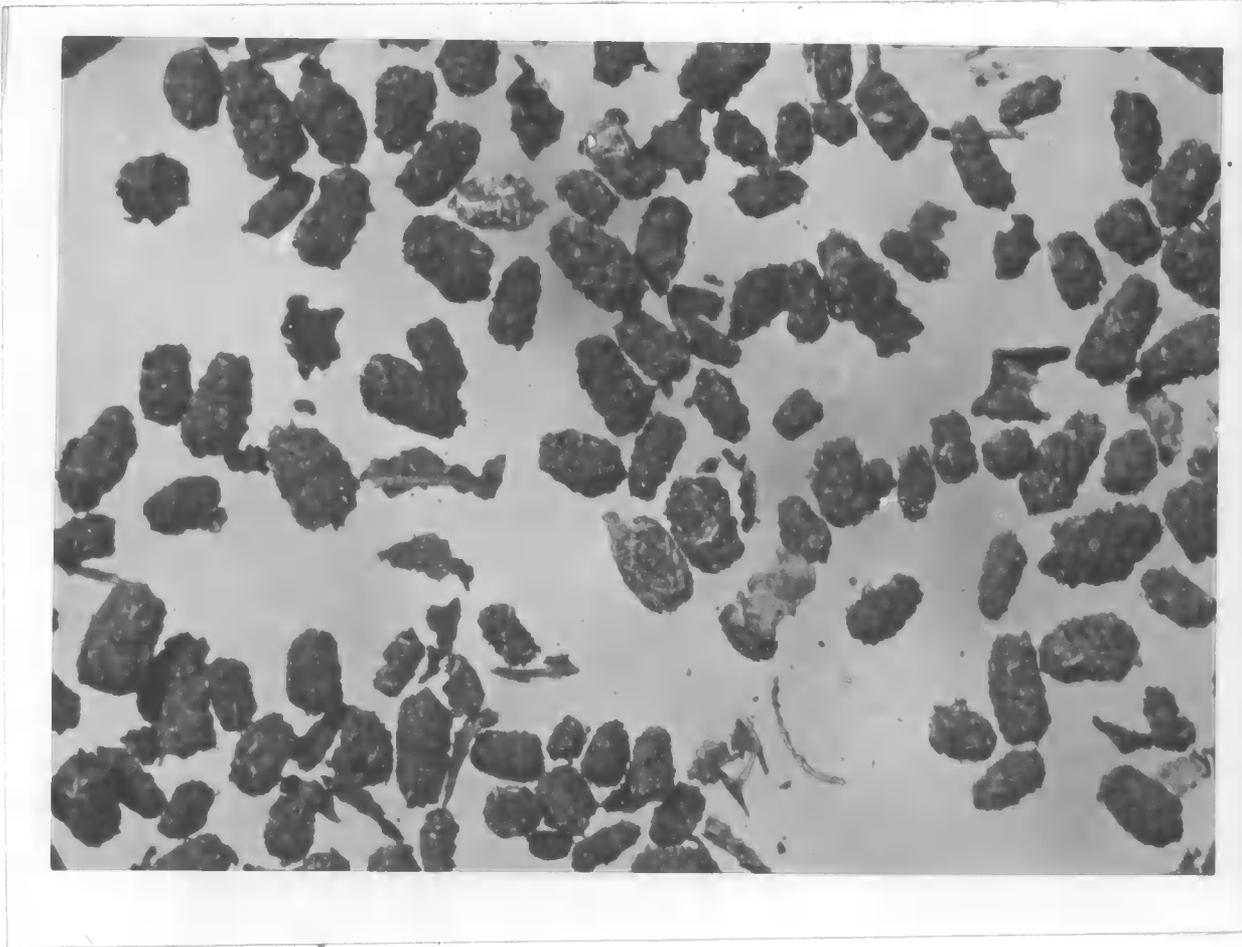
Plate 6. Faecal pellets (x5) produced by _Glomeris_
fed exclusively on hazel litter

common during the first 21 days, after which dark mycelium appeared.
After 42 days dark mycelium was more common on the pellets.
Apparent mechanical damage caused by rainfall was misleading.   After
heavy rainfall, pellets sometimes adhered together in a moist
amorphous mass.   On drying, however, the pellet shape was retained,
although the pellets now adhered in a "crust".   In a number of these
moist samples enchytraeids were observed.

After six months in the field about 50% of the pellets had dis-
integrated, and after one year none were observed intact.   Frequently,
numerous pellets of small animals were found in place of the _Glomeris_
pellets.   Mites, Collembola, isopods and enchytraeids were observed
on the samples.

(2) Fungal succession

There was a marked succession amongst the 45 species of super-
ficial fungi recorded from the decomposing pellets (Fig. 1 and Table 1).
The general pattern was the same in the field and laboratory studies.

Mucoraceous fungi, especially _Mucor hiemalis_ and _M. ramannianus_
were common up to the 14th day of decomposition after which they were
replaced by a wide range of Fungi Imperfecti and sterile forms.
After 14 days _Helminthosporium hyalosperma_ and the Ascomycete
Chaetomium sp. were common on laboratory samples, while in the field the
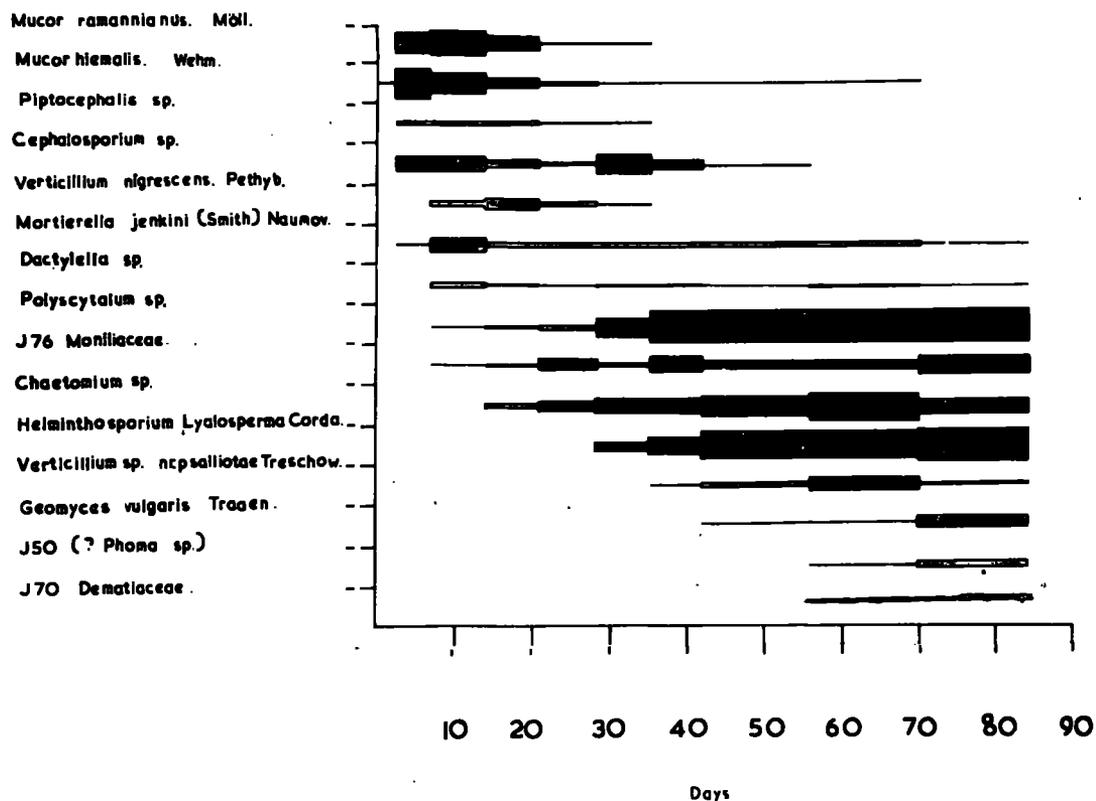common forms were _Geomyces cretaceous_, _Phoma_ sp. and _Trichoderma_

Fig. 1. Succession of fungi on Glomeris pellets decomposing in the laboratory. The depth of each histogram is proportional to the % of samples colonised n (100%) = 30.

Table 1. Fungi on _Glomeris_ pellets decomposing in the field

Fungi Isolated	Sampling period (days)							
	3	7	14	28	42	56	84	140
Cladosporium herbarum Link ex Fr.	-	3	-	1	1	1	-	-
Geomyces cretaceous Traaen	-	-	4	1	1	1	-	1
Mucor hiemalis Wehm.	-	-	3	-	1	-	-	1
Mortierella isabellina Oud.	-	-	1	-	-	2	1	-
Phoma sp.	-	-	-	-	7	4	4	-
Trichoderma sporulosum (Link) Hughes	-	-	-	-	1	2	1	1
Papularia sphaerosperma (Pers.) von Höhn.	-	-	-	-	-	2	-	1
Sterile Fungi	-	1	1	1	1	-	3	4
Named species isolated once only*	-	1	3	2	3	2	5	4
Total no. of successful isolates	-	5	12	5	15	14	14	11
Total no. of isolations (n)	-	14	16	33	50	50	50	22
Fungi Observed								
Mucor ramannianus Möller	-	4	-	-	-	-	-	-
Mucor hiemalis Wehm.	5	4	2	-	-	-	-	-
Geomyces cretaceous Traaen	-	-	-	1	-	-	-	2
Dactylella sp.	-	-	-	-	1	-	6	-
Stachybotrys dichroa Grove	-	-	2	-	1	-	-	1
Polyscytalum sp.	-	-	-	-	2	1	-	-

Results for fungi isolated are shown as the number of identifiable isolates
from each sample date. Fruiting fungi observed directly are expressed as
the number of samples with sporing structures at each sampling date.

* Aureobasidium sp., Cephalosporium sp., Cylindrocarpon didymum (Hartung) Woll., Dactylella sp.,
Diheterospora catenulata, Dothiorella sp., Epicoccum nigrum Link ex Fr., Geomyces sp., Glomerella
cingulata (Stonem.) Spauld and Schrenk, Mucor fragilis Bain., Penicillium sublateritium Biourge.,
P. nigricans Bain., P. frequentans Westling., Phoma sp., Polyscytalum sp., Septonema chaetospira
(Grove) Hughes., Stachybotrys dichroa Grove., Trichoderma viride Pers. ex Fr., Verticillium nigrescens
Pethybr.

sporulosum. On both laboratory and field pellets, Polyscytalum sp.

was common in the later stages.

Helminthosporium hyalosperma is widespread on dead herbaceous

material and decaying wood (Morgan-Jones and Cole 1964). Geomyces

cretaceous and G. vulgaris (see Fig. 1) were first reported from soil

by Traaen (1914). Watling (1963) isolated G. vulgaris and many species

of the related genus Aleurisma from incubated hawk pellets.

Trichoderma sporulosum is a common soil isolate (Hughes 1958).

It was regrettable that many of the isolates could not be

identified to species but this is frequently the case when new

substrates are investigated. Chaetomium sp. could not be induced to

spore in culture. Members of this genus are saprophytes most

frequently isolated from soil and decaying vegetable matter (Ames 1961).

Polyscytalum sp. is a member of a little known genus usually isolated

from decaying plant litter. Mosca (1961) isolated P. roseum from

dead leaves of spanish chestnut, Ginkgo and oak. Brief descriptions of

the important unfamiliar isolates are found in Appendix Note 3.

Culture success using the direct isolation technique was 35%. As

in other studies in which direct isolation techniques have been used

(Warcup 1957) a large number of sterile fungi were isolated. These

occurred mainly in the later stages of decomposition and included at

least two Basidiomycetes.

(3) Quantitative estimates of Fungi and Bacteria

In both the laboratory and field studies the amount of fungal mycelium increased slowly during the period of study (Figs. 2 and 3), whilst bacterial numbers rose to peak values during the first two weeks of decomposition and afterwards decreased to a relatively constant level (Figs. 2 and 4).

Hazel litter contained about 500 m. of mycelium per g. dry weight, whilst fresh pellets contained about 1,000 m/g. In the laboratory a steady increase to 3,500 m/g. at 56 days was recorded as the pellets decomposed. In the first field experiment, mycelium increased significantly (P < 0.01) to 2,500 m/g. at 28 days and subsequently remained at about this value. In the second field experiment, although full results are not available, the length of mycelium was comparable with that in the laboratory, reaching 2,800-4,500 m/g. at 42-56 days.

It is not known why the mycelial length of fresh pellets was higher than that of hazel litter. A careful study of the Glomeris gut fungus Eccrina flexilis Leger Duboscy, showed that excreted hyphae from this source were not the cause. Selection of fungus-rich litter or differences in the techniques of making agar films from leaf litter or pellets are possible explanations.

Fig. 2. Laboratory experiment. Microbial changes in Glomeris faecal pellets decomposing in the laboratory. (a) Fungal mycelium length (m/g dry weight), n = 10; (b) bacterial numbers (individuals/g dry weight), n = 10; (c) oxygen uptake (μl/g dry weight/h), n = 30; and (d) RQ. Means $\pm$ 95% confidence limits given for (a), (b) and (c).

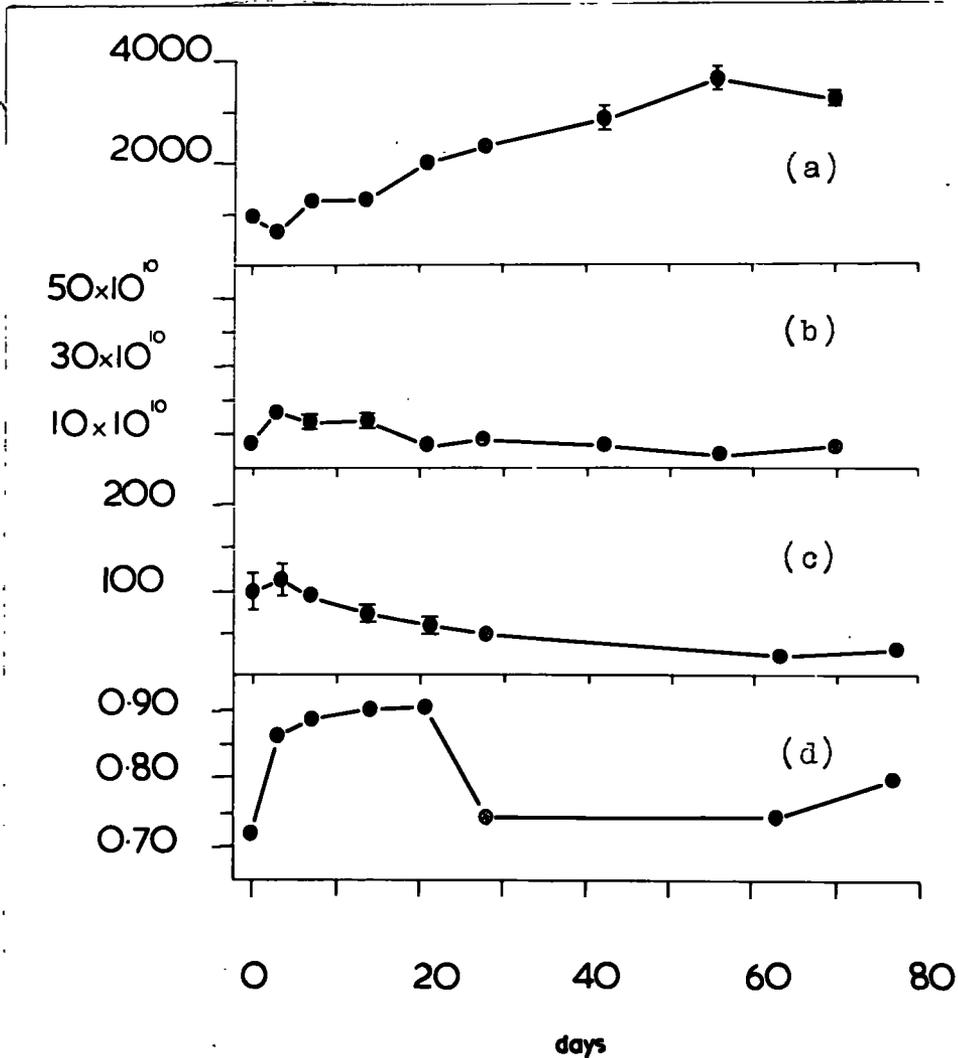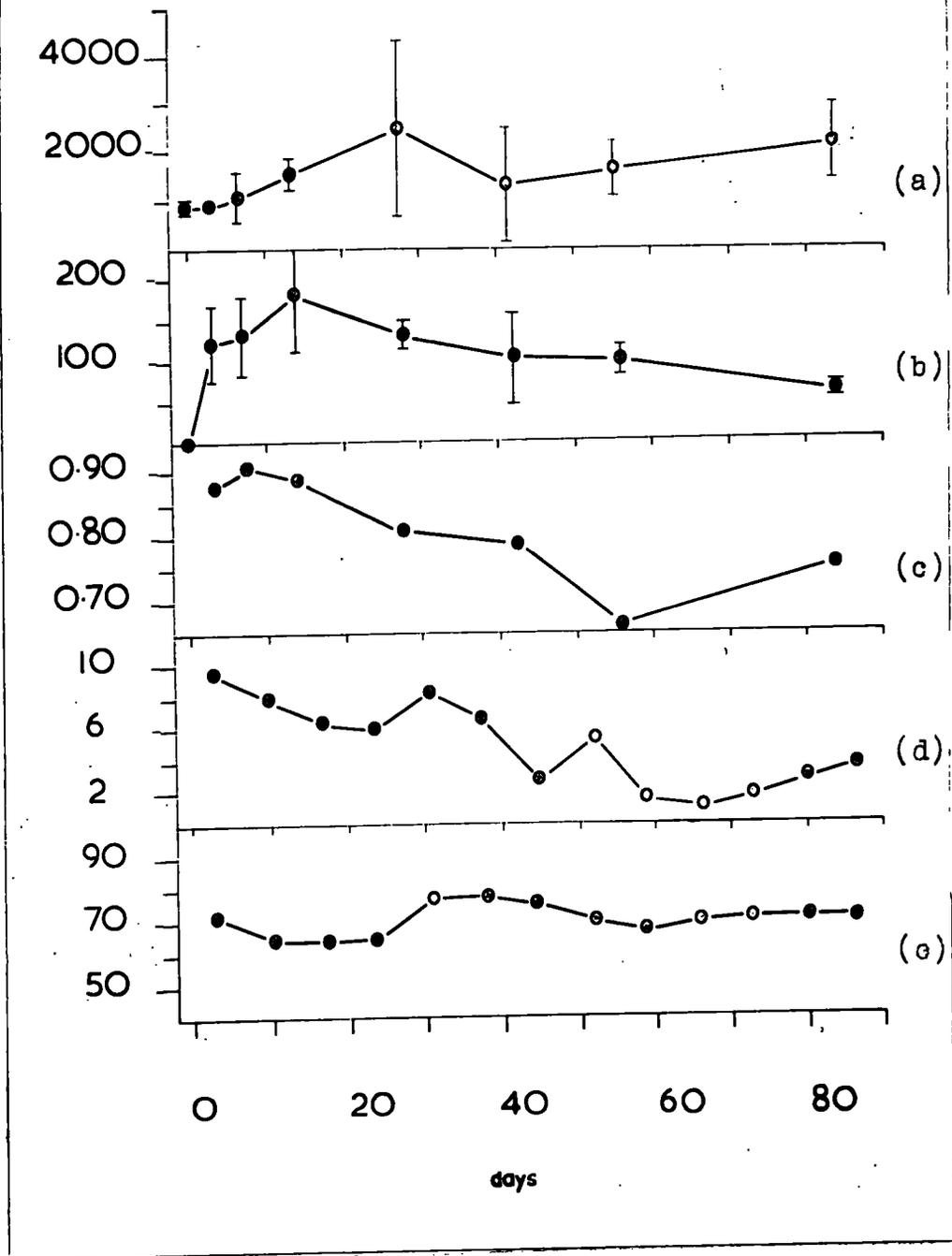Fig. 3. First field experiment. Microbial changes in
Glomeris faecal pellets decomposing in the
field. (a) Fungal mycelium length (m/g dry weight),
n = 2;   (b) oxygen uptake (μl/g dry weight/h),
n = 5;   (c) RQ, n = 5;   (d) field temperature ($^{\circ}$C);
and (e) sample moisture content (% wet weight).
Means $\pm$ 95% confidence limits given for (a) and (b).

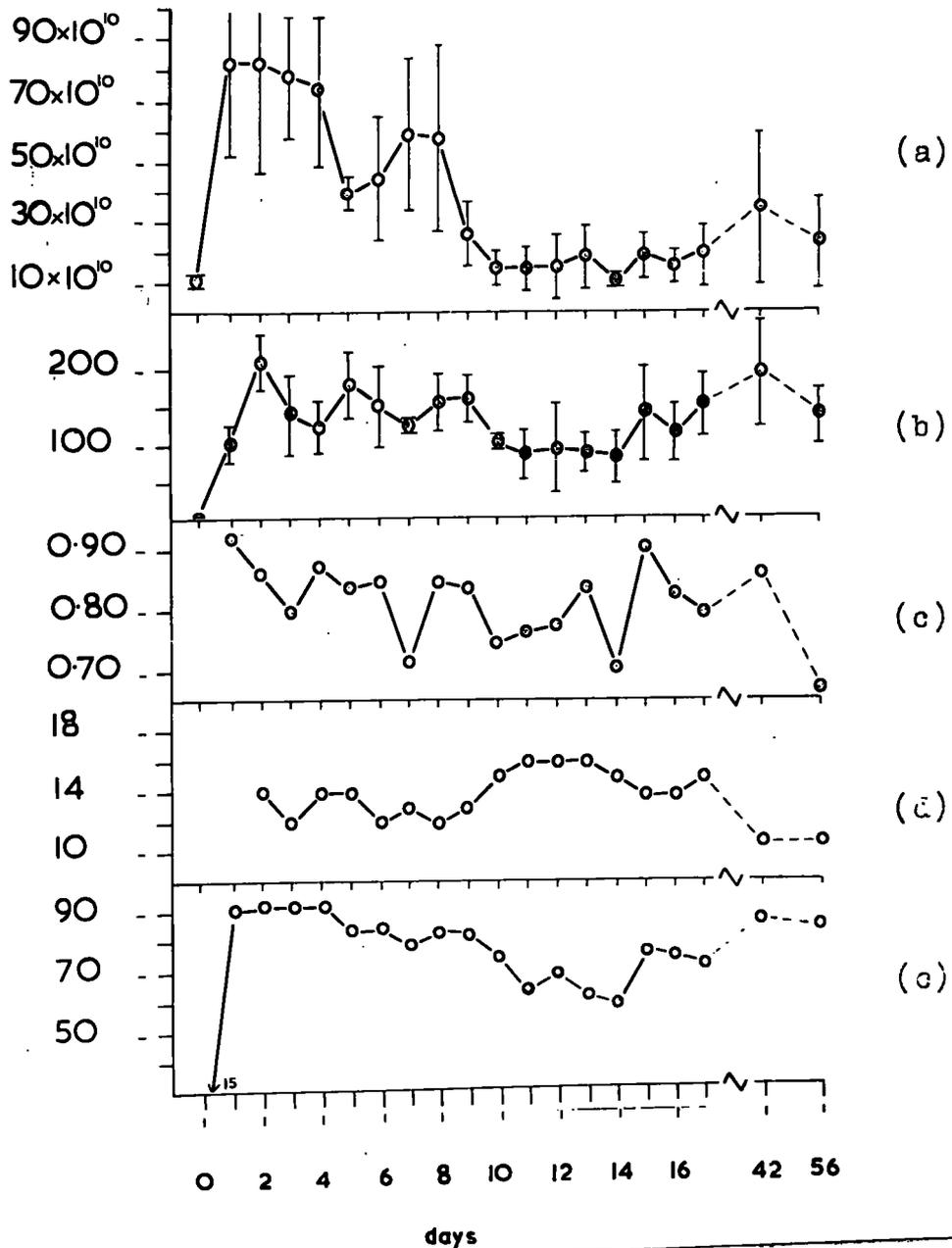Fig. 4.   Second field experiment.   Microbial changes in
Glomeris faecal pellets decomposing in the field.
(a) Bacterial numbers (individuals/g dry weight),
n = 3;   (b) oxygen uptake (μl/g dry weight/h),
n = 6;   (c) RQ, n = 6;   (d) average daily
temperature ($^\circ$C);   and (e) sample moisture
content (% wet weight).   Means $\pm$ 95% confidence
limits given for (a) and (b).

Numbers of bacteria in laboratory samples increased significantly from 7 x 10^{10}/g dry weight in fresh pellets to 17 x 10^{10}/g dry weight at 3 days. High values were maintained at 7 and 14 days, but at 21 days the count had decreased again to 6 x 10^{10}/g dry weight. Counts did not change significantly thereafter. Bacterial counts on field samples rose from 10 x 10^{10}/g dry weight in the initial fresh pellets to 82 x 10^{10}/g dry weight after 1-2 days in the field. Subsequent daily counts decreased until the 10th day when the count had dropped to 15 x 10^{10}/g dry weight, a value from which the last 7 daily counts did not differ significantly. As the pellets dried from the 5th to the 14th day of decomposition the numbers of bacteria decreased.

(4) <u>Respiration Measurements</u>

In the field and laboratory experiments the maximum rate of oxygen uptake occurred during the first 14 days of decomposition (Figs. 2, 3 and 4).

Oxygen consumption of laboratory pellet samples rose from 98 µl/g dry weight/h for fresh material to 109 µl/g/h after 3 days decomposition, then fell steadily to 19 µl/g/h at 63 days. In the first field experiment (Fig. 3) oxygen consumption rose to a peak at 14 days, and then declined. The peak value in the second field

experiment (Fig. 4), in which daily respiratory measurements were made, occurred on the second day (209 μl/g/h). The readings then settled to a level about 150 μl/g/h until the tenth day when there was a sharp drop to 106 μl/g/h. There was a significant increase (P $<$ 0.05) in the oxygen uptake again after the fifteenth day.

Birch (1959) and Stevenson (1956) have shown that re-moistening air-dried soil gave greatly increased microbial activity. The presence of a period of high respiration in the laboratory experiments (Fig. 2) indicates that the rise in oxygen consumption in the field samples (Figs. 3 and 4) is not the result of re-moistening air-dried pellets.

Changes in the respiratory rate were correlated with climatic changes in the field. In the first experiment, the soil temperature dropped from 8°C (November 1964) to 2°C (January 1965) and the temperature of the respirometer water bath was adjusted to the change. Over this period the oxygen uptake decreased by 120 μl/g/h. Bunt and Rovira (1955) have shown that soil respiratory rate is depressed by low temperatures. In the second experiment, the temperature fluctuated irregularly between 12-16°C (August 1965) and all respiratory measurements were made at 12°C. Respiratory changes were not correlated with field temperature changes. During both

field experiments the rainfall was intermittent but large changes

in moisture content and respiratory rate only coincided in the

second experiment. Oxygen uptake declined by 130 μl/g/h from the

second to the fourteenth day while moisture content decreased by 25%.

Similarly a 60 μl/g/h increase in oxygen uptake on the fifteenth day

coincided with a 20% rise in moisture content.

The RQ was highest during the first 3 weeks of decomposition in

the laboratory and in the first field experiment (Figs. 2 and 3).

In the second field experiment, in which the RQ was recorded daily,

wide fluctuations were observed (Fig. 4).

(5) Changes in dry weight and chemical composition

The faecal pellets lost 7.4 $\pm$ 3.2% of their initial dry weight

after 12 weeks laboratory decomposition compared with 23.3 $\pm$ 6.0%

lost in the field. After 52 weeks in the field the pellets had lost

50.6 $\pm$ 7.7% of their initial weight (Fig. 5).

Chemical changes in the pellets were determined after 8 weeks in

the laboratory and after 12 weeks in the field therefore data have

been interpolated in Table 2 to facilitate comparison. The absolute

ash content of the pellets increased in the field, probably as a

result of contamination with mineral soil. An increase also

occurred in the laboratory but the cause is unknown. Another

Table 2.   Chemical changes in _Glomeris_ pellets

All results are expressed as % of dry weight at time O

	Ash	N	C	Holo-cellulose	Soluble Carbohydrate	% Dry wt. remaining
Time O	7.9	1.71	49.4	38	2.3	100
8 Weeks Laboratory	9.7	1.83	46.4	31	2.2	(95.1)
8 Weeks Field	(10.4)	(1.54)	(39.3)	-	(1.8)	(85.5)
12 Weeks Laboratory	-	-	-	-	-	92.6
12 Weeks Field	11.7	1.46	34.2	-	1.6	76.7

( )   Interpolated result

unexplained result is the 10% loss in nitrogen in the field compared with a gain of 7% in the laboratory.

Loss in % carbon was higher in the field than the laboratory (Table 3). After 8 weeks laboratory decomposition the pellets lost 3.0% carbon compared with 10.1% in the field, similarly the loss of carbon in respiration was greater than in the field. These differences may be explained by differences in environment, and will be considered in detail in the discussion.

A loss of 7% in the holocellulose fraction after 8 weeks laboratory decomposition was not associated with an increase in soluble carbohydrate. This indicates that any carbohydrates which were produced by the breakdown of holocellulose were utilised immediately.

The high RQ values obtained during the early stages of pellet decomposition in all experiments indicated that carbohydrates were being utilised. A supplementary experiment showed that there was a significant loss ($P < 0.05$) of $1.4 \pm 1.1\%$ soluble carbohydrate during the first week, but there was no significant difference between the amounts of soluble carbohydrates in pellets at 1 week and 4 weeks old.

Table 3. Loss of carbon from Gloméris pellets
          calculated from (a) respiration rate
          (b) analysis (cf. Table 2)

Results are expressed as % of initial dry wt.

	a	b
8 Weeks Laboratory	4.4	3.0
8 Weeks Field	6.9	(10.1)
12 Weeks Field	10.6	15.2

( ) Interpolated result

V. DISCUSSION

V. DISCUSSION

(1) Methods

Harper (1962) modified "Tullgren" funnels for the collection of rabbit pellets for a mycological laboratory experiment. The *Glomeris* pellet collection apparatus was much smaller than that of Harper (1962) but was fundamentally the same. One advantage of this technique is that the pellets can be collected in the eventual decomposition damp chamber, avoiding undue handling, contamination and damage. The apparatus could be adapted for use with many of the larger soil arthropods, the chief problem being the prevention of desiccation.

When a good recovery of test samples of organic matter placed on the soil is required, most workers employ some method of artificially enclosing or labelling the samples (Hering 1965, Frankland 1966). In the present studies, pilot experiments showed that after decomposition for one week *Glomeris* pellets could not be recovered to satisfactory accuracy either after placing then unenclosed in small heaps on the soil, or after placing the heaps on to discs of glass fibre filter paper. The terylene mesh bags gave a good recovery of test samples but as the experiments progressed they became increasingly contaminated with adhering soil particles and needed

careful cleaning. Towards the end of the experiments it was difficult to distinguish adhering soil from the pellet samples, and this is a possible source of error. The use of the terylene bags is also subject to criticism because the mesh size of the bag artificially determines which soil animals have access to the samples. For this reason, in the present studies, the effect of the larger invertebrates (especially earthworms) on the decomposition of _Glomeris_ pellets must remain unknown.

The hyphal isolation technique used to isolate fungi from field samples is similar to that employed by Warcup (1957). Small pieces of superficial mycelium were picked off the pellets and plated on to P.D.A. Unfortunately there was some danger of contamination from spores adhering to the hyphae and it might have been wise to incorporate a washing or surface sterilizing technique before the plate preparation. Probably my hyphal isolation technique is not so difficult as that of Warcup (1957) for hyphae were easily seen interlacing the pellets, whereas soil tends to be featureless and free hyphae are often difficult to find. Warcup (1957) pointed out that such techniques may be selective for (1) large hyphae (2) mycelia that do not fragment readily (3) dark hyphae. With my technique there is the additional danger that some fungi will not produce aerial

mycelium and are therefore missed altogether. However, because the faecal pellets are made of finely divided material and have a high spore content, the isolation technique used was perhaps more suitable than the familiar dilution counts, soil plates (Warcup 1950) and soil washing techniques (Harley and Waid 1955; Parkinson and Williams 1961). Further as Warcup (1957) showed, hyphal isolation allows many fungi to be isolated that are not recorded by plating techniques.

(2) Comparison of field and laboratory results

The pattern of changes in pellets ageing in the laboratory and the field was the same, but changes in the field tended tobe more vigorous and pronounced. Thus dry weight loss was greater in the field and correspondingly chemical changes were in general more marked. Respiration reached and maintained higher values in the field. After 56 days oxygen uptake in the field was 80-85% of values recorded after three days compared with c.25% in the laboratory. Similarly, bacterial counts were far higher in the field than in the laboratory. By contrast, fungal counts were of the same order. This may be connected with the strong growth of superficial mycelium and sporing structures recorded in the laboratory. Why superficial fungal growth was weak on field samples is unknown, but it is an occurrence familiar to mycologists. Indeed, it is a standard method

for field samples to be brought into the laboratory where sporing structures soon grow under controlled conditions of humidity, temperature and air movement and without animal interference.

The reasons for the differences between field and laboratory results may be connected with differences in environment. Field samples are exposed to a more varied microclimate and microfauna, to greater animal interference and contamination by soil. Thus, in the field, changes in both respiratory rate and bacterial count are correlated with climatic changes, whilst visual evidence of the activities of the microfauna are plentiful (Appendix Note 1). A comparison of percentage carbon lost calculated from respiratory values and chemical analysis shows that respiration accounts for all carbon loss in the laboratory but only 68% of the loss in the field (i.e.) 32% may be attributed to the activities of the microfauna and to removal by leaching and fragmentation. A richer mycoflora in the field and differences in the most frequent isolates in field and laboratory indicates contamination by soil. In conclusion, whilst the field and laboratory results are qualitatively similar, quantitatively they are very different. Thus great care must be taken when quantitative data obtained from laboratory experiments are extrapolated to a field situation.

(3)   Comparisons with other work and conclusions

Van der Drift and Witcamp (1959) found that the faecal pellets of a soil arthropod were a more favourable habitat for litter micro-organisms than whole leaf litter.   Several workers have recorded greater microbial activity or higher numbers of decomposer micro-organisms in the freshly produced faeces of soil animals than in comparable food material (Van der Drift and Witkamp 1959, Kozlovskaya and Zhdannikova 1963, Parle 1963).   On the basis of this evidence and similar arguments put forward by Macfadyen (1961, 1963) and Franz and Leitenburger (1948), it can be concluded that the overall rate of decomposition of plant debris is increased when this material is converted to faeces.

Contrary to this conclusion, in the present studies there was no indication of a higher overall rate of decomposition in the faeces than in litter (Bocock,1964, Hering 1965); for there was a marked similarity between the rates of disappearance of pellets on a mull-like rendsina soil and hazel litter on mull and moder soils from nylon net bags exposed on the soil surface (Fig. 5).   It could be argued that the soils of the moder and mull sites were not strictly comparable with the soil used in the pellet experiments which is best classed as an intermediate between the moder and mull extremes.   However, both litter and pellets were exposed for decomposition in the same locality
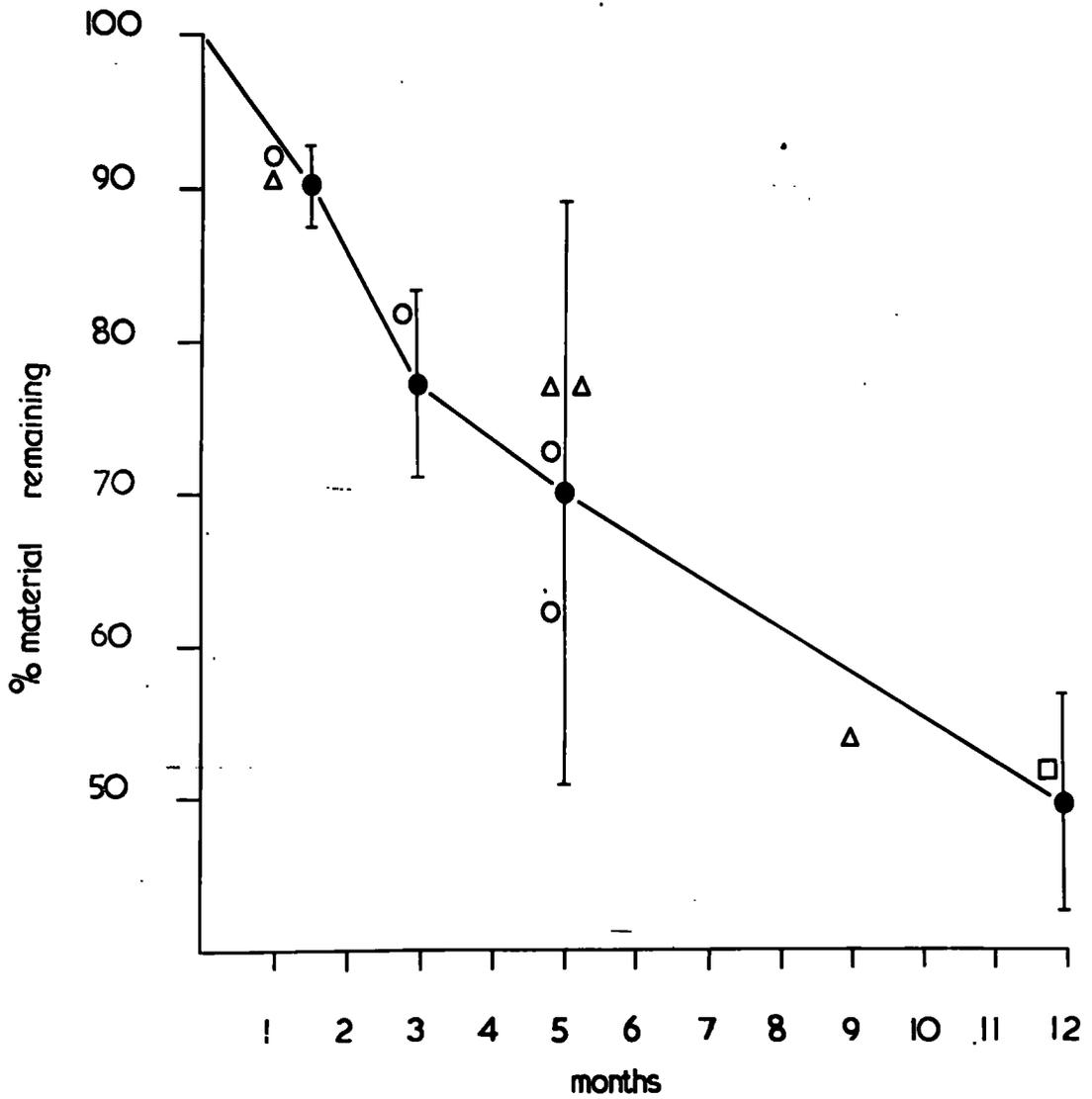
Fig. 5.   Rates of disappearance of <u>Glomeris</u> pellets
on a mull-like rendzina site (●) and of
hazel litter on moder sites (▲, Bocock 1964;
◻ , Hering 1965) and on a mull site
(○, Bocock 1964).   Means for the pellets
± 95% confidence limits;   n = 20, 20, 10 and
37 at 1½, 3, 5 and 12 months respectively.

and under very similar physical conditions. It can be concluded that Glomeris pellets and hazel litter disappear at about the same rate in the first year of decomposition.

In this discussion, care has been taken to distinguish between disappearance and decomposition of exposed samples. Bocock (1964) compared the total dry weight loss of ash litter from the soil surface with the loss when the larger soil invertebrates (especially earthworms) were excluded. He found that, after five months, 40% loss in dry matter on a mull site and 10% on a moder site could be attributed to the activities of the larger soil animals. What proportion of the dry weight loss of exposed samples of Glomeris pellets and hazel litter was the result of soil invertebrate activity is not known.

In all my studies and in the experiments of Van der Drift and Witkamp (1959) and Parle (1963), respiratory activity was initially high. The differences which occurred in the timing and size of the peak values in all these studies could result from differences in experimental material, temperature and moisture content of the samples, and frequency of sampling.

Although it is generally considered that changes in soil respiratory rate are related to microbial activity, there have been

few attempts to correlate changes in soil respiratory rate and microbial numbers. Stevenson (1956) and Smith and Humfeldt (1931) found a positive correlation between changes in bacterial numbers from plate counts and respiratory rate, whilst Robinson and McDonald (1964) found soil respiratory changes correlated with direct bacterial counts. Jensen (1936) found correlations between changes in bacterial and fungal counts and respiration. By contrast, Vandecaveye and Allan (1935), Vandecaveye and Baker (1938), and Parle (1963) found no correlation between any of these measurements. Stevenson (1956) observed that there was a time lag between the peak respiratory values and peak bacterial counts. The suggestion that this lag can extend over periods of weeks as in the results of Vandecaveye and Allan (1935) seems unlikely however, because the viable bacterial population can fluctuate greatly even over two-hour periods (Thornton and Gray 1930, Taylor 1936). Clearly, this problem needs further research.

In the present experiments there was a positive correlation between changes in oxygen uptake of decomposing _Glomeris_ pellets and changes in direct bacterial count, but not in changes of length of fungal hyphae. In the laboratory experiments, no lag occurred, but in the field experiments bacterial numbers were high on the first day whilst respiratory values reached a peak on the second day. This

is the reverse of the sequence outlined by Stevenson (1956), who found that the respiratory maximum occurred before the bacterial maximum.

Because no distinction is made in direct microbial counts between viable and non-viable tissue, all interpretations connecting direct counts and activity are suspect. However, with this proviso, these results indicate that bacteria were more active in the decomposition of Glomeris pellets than fungi, especially in the first 14 days. An interesting result in support of this conclusion is found by converting the fungal and bacterial count data into relative weights of tissue (allowing 285,000 a mycelium/g mycelium for fungi, and sp. gr. 1, and average diam. 0.75 for bacteria. See Appendix Note 4). At the peak of laboratory decomposition, the relative weight ratio was 13:1 for bacteria; fungi. By contrast this ratio was 0.6:1 after 56 days. In the field this ratio was 64:1 on the first day and 5:1 after 42 days. Thus the dominant position of bacteria in terms of total mass during the first few weeks is strongly indicated.

When easily assimilable carbohydrates are added to a soil a burst of microbial activity occurs (Lees 1949, Robinson and McDonald 1964). The peak of microbial activity which occurs in Glomeris faeces during the early stages of decomposition may also be associated

with the presence of easily assimilable carbohydrates.   In all
experiments, RQ readings were high during the first 14 days.   This
indicates that a mixture of nutrients was being oxidized containing
a large percentage of carbohydrates.   The strong growth of
Phycomycetes over this period, fungi that rely principally on soluble
carbohydrates for their metabolism (Jenson 1931, Thayson and
Bunker 1927, Reese and Levinson 1952) is additional evidence.
Finally, from analysis, there was a significant loss of soluble
carbohydrates during the first seven days of decomposition, after
which this percentage did not change.   Clearly, soluble carbohydrates
must be an important contributary factor to the production of the
early respiratory peak.

   The fungal succession observed on Glomeris pellets was
fundamentally the same as that reported for the faeces of a
trichopteran larva and rabbit dung (Van der Drift and Witcamp 1959,
Harper 1962).   There was an initial growth of Phycomycetes but after
14 days these were replaced by Fungi Imperfecti and Ascomycetes.
Burges (1958) suggests that this succession is widespread even if not
of universal occurrence, although on fallen leaves the "phycomycetes
phase" may only occupy a few days, and be undetectable except
by histological examination.   Hering (1965) investigated the fungal
succession of hazel leaf litter but did not make histological

examination of the fresh material and possibly this phase was missed. Even so, the main isolates of hazel litter and _Glomeris_ pellets were markedly different. From the available evidence, hazel litter and _Glomeris_ pellets in the same locality and under similar conditions, disappear at the same rate, but have different fungal successions. This is all the more surprising when it is remembered that gross chemical analysis had indicated that the two substrates are very little different (Van der Drift 1951, Bocock 1963).

VI. SUMMARY

VI. <u>SUMMARY</u>

1. The decomposition of the faecal pellets of <u>Glomeris</u> <u>marginata</u> (Villers) was studied in the laboratory and field.

2. The pellets used in the experiments were obtained from cultures of <u>Glomeris</u>. A "Tullgren" funnel apparatus was modified for the culture of <u>Glomeris</u> and the delivery of faecal pellets. The animals were starved for 24 hours after which they were fed with moistened hazel litter (<u>Corylus</u> <u>avellana</u> L).

3. For laboratory experiments, the pellets were allowed to collect in glass jars containing moistened filter paper. The jars were sealed and their contents allowed to decompose at $10-13^{O}C$.

4. The site chosen for the field experiments was a deciduous woodland in the grounds of Merlewood Research Station (Nat. Grid Ref. SD(34)409796). Weighed samples of air-dried <u>Glomeris</u> pellets in narrow mesh terylene bags were placed directly on to the soil.

5. Samplings of both laboratory and field experiments were made regularly for 12 weeks and after six months and one year for the field experiments.

6. In both laboratory and field the appearance of the pellets was unchanged until the third day when superficial hyaline hyphae were observed. On the laboratory samples many species were observed sporing whilst on field samples mycelium was sparse and

few species spored.   After one year in the field all the pellets had disintegrated or had been converted to the pellets of other animals.

7. In both laboratory and field experiments Mucoraceous fungi were common up to the fourteenth day of decomposition, after which
    and
    they were replaced by Fungi Imperfecti/Ascomycetes.

8. In both laboratory and field, fungal counts increased steadily as the experiments progressed.   Bacterial counts reached peak values after two-three days and then declined.

9. In both laboratory and field, oxygen consumption reached peak values after 2-14 days and then declined.   There was evidence that fluctuations in respiratory rate in the field were correlated with climatic changes.   The RQ was highest during the first three weeks of decomposition.

10. Laboratory pellet samples lost 7.4 $\pm$ 3.2% of their initial dry weight after 12 weeks decomposition compared with 23.3 $\pm$ 6.0% lost in the field.   After one year field samples lost 50.6 $\pm$ 7.7%.

11. Loss in percentage carbon was 10.1% in the field compared with 3% in the laboratory.   Ash content increased in both field and laboratory but there was a loss of 10% nitrogen in the field compared with a gain of 7% in the laboratory.

12. Laboratory pellet samples lost 1.4 ± 1.1% of their soluble carbohydrate content after one week's decomposition but there was no significant loss thereafter.

13. Some of the methods used are discussed and compared with other work.

14. The laboratory and field results are compared. The pattern of changes was the same in laboratory and field samples but quantitatively the results were very different. This indicates that care should be taken when results obtained in the laboratory are extrapolated to a field situation.

15. The results were compared with those of other workers and some conclusions were reached.

16. Contrary to current opinion there was no evidence to suggest that _Glomeris_ pellets disappeared at a faster rate than the food leaf litter exposed under similar circumstances.

17. An early period of peak respiratory values for excrements has been recorded by other workers. There was a correlation between the bacterial counts and the respiratory results but because there is controversy in the literature this result is difficult to interpret.

18. It is suggested that soluble carbohydrates contributed to microbial activity peak values.

19. The fungal succession was similar to that recorded on other faeces substrates but was unlike that recorded for the food litter.

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APPENDIX

APPENDIX

Note 1

Some animals were frequently seen on <u>Glomeris</u> pellets and the surrounding soil. The following were identified:-

Collembola: <u>Tomocerus</u> <u>minor</u> (Lubbock)

<u>Lepidocyrtus</u> <u>lignorum</u> F.

<u>Folsomia</u> <u>multiseta</u> Stach.

Acari: Pthyracaridae (<u>Oppia</u> sp. or <u>Belba</u> sp.)

Mesostigmatidae (2 spp.)

<u>Pergamasus</u> sp.

<u>Veigaia</u> sp.

Isopoda: <u>Trichoniscus</u> sp.

Gastropoda: <u>Punctum</u> sp. (possibly <u>P</u>. <u>pygmaeum</u>)

Note 2

1)   2.5% Water agar.   25 g. agar was dissolved in 1000 ml water at $105^{O}C$.

2)   2% Potato Dextrose agar (P.D.A.).   20 g. skinned potatoes were boiled $\frac{3}{4}$-1 hr., allowed to cool, then homogenised and made up to 1000 ml.   2 g. Dextrose and 15 g. agar were added and dissolved by melting at $105^{O}C$.   When a liquid medium was required the agar was not added.   (pH 5.8).

3) 2% Potato Carrot agar (P.C.A.).   20 g. carrots + 20 g.

potatoes were treated as for P.D.A.   Dextrose was not

added.   (pH 5.8).

4) 2% Malt Extract agar.   20 g. Malt extract (obtained from

Oxo Ltd.).+ 12 g. agar were dissolved in 1000 ml distilled

water.   (pH 6.0).

5) 0.4% Yeast Extract agar (Y.E.A.).   4 g. Yeast extract

(Difco Ltd. from Gallenkamp Ltd., Widnes, Lancs.) + 15 g.

agar were dissolved in 1000 ml distilled water.   (pH 6.6).

All media were sterilised by autoclaving at 15 lb/sq. in. for
20 min.   Only Oxoid No. 3 agar (Oxo Ltd., London, S.E.1.) was used.

Note 3

Descriptions of unfamiliar fungi frequently isolated from
Glomeris pellets in the experiments.

1) J 76 (Moniliaeceae).   Grew very poorly in culture rarely lasting
more than 2-3 weeks.   A loose white flocose growth on P.D.A. with
uncoloured reverse.   On the pellet substrate produced chains of
spores (110-150μ) sometimes simply branched.   Spores: fusiform,
4 celled, 16-18μ x 2-2.5μ at the distal end and 2 celled, 10-14μ
x 2.5-3μ at the base.

2) <u>Polyscytalum</u> sp. Grew well in culture, attaining a diameter of 5 cm after 14 days on P.D.A. Low sparse grey mycelium interspersed with white sporing velvety patches. A dark purplish pigment was always produced in the medium. Spores: cylindrical, 1 celled, $6-8\mu$ x $1-1.5\mu$, plentiful. On the pellet substrate only the white sporing patches ($50-200\mu$ high) were observed.

3) <u>Cephalosporium</u> sp. Grew moderately well in culture attaining 3 cm in 14 days on P.D.A. A close white mycelial turf with occasional coarse aerial strands. Spores: oblong with obtuse ends ($4-6\mu$ x $1-1.5\mu$), plentiful.

4) <u>Verticillium</u> sp. <u>nr. psalliotae Treschow</u>. Failed to produce blue colour in colony reverse as <u>Verticillium</u> <u>psalliotae</u> Treschow. Grew moderately well on P.D.A. reaching 3.5 cm in 14 days. Short white flocose growth. Spores: allantoid/falcate, 1 celled, $4-6\mu$ x $2-3\mu$, sparingly produced.

5) <u>Geomyces cretaceous Traen</u>. Grew well in culture attaining 4 cm in 14 days. Characteristic low "floury" appearance; white but with grey/brown patches, dark brown reverse. Conidiophores complexly branched and varying in length. Conidia sometimes sessile on the main branch and laterals, but usually on a short (1 x 1μ) conidiophore. Conidia: sub-globose, 1 celled, $2-3\mu$ diam., plentiful.

6)   <u>Dactylella</u> sp.   Grew very poorly on P.D.A.   Sparse, white floccose growth, reverse uncoloured.   No spores in culture.   On the pellet substrate produced numerous small groups of conidiophores, 50-80μ high, bearing single, ventricose, 3-4 celled conidia, 35-60μ x 20-30μ .   This fungus closely resembled the predaceous <u>Dactylella</u> <u>megalospora</u> Dresch. isolated by Frankland (1966).   Numerous erect nematodes attached to pellets or hyaline fungal hyphae in damp chambers indicated the presence of a predaceous fungus.

7)   <u>J 70 Dematiaceae.</u>   Grew very poorly in culture and soon died. Easily recognised on pellets by the grey/brown mycelium giving rise to chains of spores, tightly coiled into balls 18-25μ diam. which did not break up readily.   Spores: subglobose, 1 celled, 1-2μ diam.

8)   <u>J 50, probably Phoma</u> sp.   A thick floccose, dark brown mycelium in culture producing a black/purple pigment in the medium (P.D.A.).   Pycnidia: readily produced in culture but infrequently observed on the pellets; thin-walled, non-ostiolate, dark brown, 100-150μ diam. Pycnidiospores: irregularly cylindrical, hyaline biguttulate, 6-8μ x 2-3 μ .

9)   <u>Chaetomium</u> sp.   Grew to 3-4 cm in 14 days on P.D.A.   A thick, dark green/brown floccose mycelium which did not produce perithecia in culture.   On pellets, the perithecia were brown, 130-200μ x 200-400μ , with a neck 50-80μ x 40-100μ , to the ostiole; covered in light

green unbranched septate hairs, 20-40μ long at perithecium base but extending to 180-200μ in a dense tuft round the ostiole. Asci: 8 spored, club shaped, deliquescing before the ascospores were mature. Ascospores: lemon-shaped, dark brown, 7-8 x 6-7μ, adhering in tall columns above perithecium.

Note 4

Fungal mycelium density. The Jones and Mollison film results for fungi are given as metres mycelium/g. dry soil. To convert these figures to g. mycelium/g. dry soil, the ratio metre mycelium: g. mycelium was required. The Jones and Mollison method was again used. Five plates each of <u>Trichoderma viride</u>, <u>Mucor hiamalis</u>, <u>Penicillium</u> sp. and <u>Phoma</u> sp. were grown in liquid culture (P.D.A. without agar) and incubated for 28 days. The liquid medium was filtered off and the residue mycelia were thoroughly mixed and then dried in the standard way. 0.15 g. was homogenised in 10 ml water for 20 min. using a laboratory blender. Four 5 ml quantities were pipetted off and separately made up to 50 ml with 2.5% water agar. Jones and Mollison films were prepared from these and examined as described earlier.

Result: 286.4 $\pm$ 49.4 kilometres of fungal mycelium: 1 g. dry wt.