A study of the changes in fungal and earthworm activity associated with soil disturbance

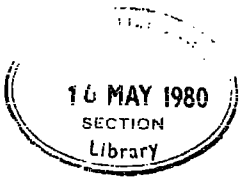
Dunning, A.

A STUDY OF THE CHANGES IN FUNGAL AND EARTHWORM ACTIVITY ASSOCIATED WITH SOIL DISTURBANCE.

by

A. Dunning

being



A dissertation presented to the University of Durham in partial fulfilment of the requirements for the Degree of M. Sc. in Ecology by Advanced Course.

December 1979

CONTENTS

Contents (contd.) Page

1 INTRODUCTION

Soil disturbance is associated with many of mans' activities: regular small disturbances in agricultural cultivations, and much larger long term soil movement and storage in the construction and mining industries. At restoration it is important to reinstate the land in good heart, able to support the growth of food crops or amenity grassland. Much work has already been undertaken on the condition of mineral soil, drainage, and fertiliser requirements for productivity, but soil organisms are often considered to be unimportant and left to undertake their own re-establishment.

As an initial step towards an understanding of complex processes which lead to the restoration of the full compliment of soil organisms, it is necessary to observe changes associated with disturbance, and the functional role of organisms in relation to other species, and in the context of their environment. The investigation of ecosystems has often been facilitated by study during disturbance.

The site chosen for the present study was an open cast coal mining site at Quebec, Durham, with a variety of soils disturbed and then allowed to settle for various periods of time.

The object of the work was to study the changes in earthworm and fungal activity associated with soil disturbance. This study was chosen because earthworms play a major role in the fragmentation and incorporation of litter into soils, and fungi are important decomposers, thus completing the natural pathway in the cycling of plant nutrients. Soil disturbance is likely to break this decomposer cycle, and therefore lead to reinstated soil with a lower intrinsic fertility than its former natural status.

2 LITERATURE REVIEW

2 i The Interaction of Fungi and Earthworms

Fungi appear unaffected by passage through the earthworm gut: Baweja (1939) observed that earthworms disperse spores of harmful fungi, e.g. Pythium. Khambata and Bhatt (1947) found that Fusarium spores were spread by earthworms. Dwarf bunt spores, Tilletia controversa, were viable after passing through earthworms in tests reported by Hoffman and Purdy (1964). Hutchinson and Kamel (1956) also support the theory that the rate of spread of fungi through the soil is greater when earthworms are present. They isolated seventeen species of viable fungi from the guts of ten specimens of Lumbricus terrestris; many of these species had thin walled spores, which suggested that many more would have been digested, and therefore more species of fungi would have been found from a larger sample of earthworms. Some species of fungi were isolated consistently from worms taken from different areas, and at different times of year. Fewer fungi were isolated in midwinter than in late autumn; this corresponds with the seasonal fluctuations in microbial populations in the soil.

The levels of micro-organisms in soil and in earthworm casts have been studied by several workers: Koslovskaya and Zhdannikova (1961), Zhdannikova (1961), and Ghilarov (1963). Generally there are more micro-organisms in casts than in the soil and decomposition of organic matter occurs faster in the casts. This is probably due to the higher levels of nutrients present, including vitamins. Parle (1963a) did not find any micro-organisms which were symbiotic with earthworms, though symbiotic micro-organisms do occur in the gut of some arthropods. Parle (1963b) observed that yeasts and fungi which occured in soil as spores germinated immediately in worm casts, and the greatest density of hyphae was found in casts 15 days old. This is in contrast to oxygen consumption in the cast which falls consistently from the time of excretion. On adding glucose or cellulose, oxygen uptake increased, but older casts continued

to respire at a lower rate than those recently produced. Therefore this effect is not due to the exhaustion of energy supplies, but to the formation of resting spores.

Anstett (1951) used grape husk cultures with and without earthworms to study the effect of earthworms on microbial decomposition. After 5 months the microbial population was four to five times greater in pots with earthworms. The decomposition of the husks was measured by loss on ignition; those with earthworms lost 75% in weight compared to an 86% loss in the controls. This additional 11% decomposition leading to a lower organic content in the residue ignited appeared to result mainly from consumption of substrate by the increased population of micro-organisms, since losses on ignition were the same from pots which contained earthworms throughout the experiment and from pots from which they were removed after 6 weeks. Barley and Jennings (1959) carried out similar trials using soil enriched with grass and clover litter, and dung. Allolobophora caliginosa were added to some of the cultures. The oxygen consumption, and nitrate plus ammonium-nitrogen accumulation was significantly higher in the cultures with earthworms. In the field earthworms would probably modify the microbial environment to a greater extent because temperature, moisture and aeration were optimum in the laboratory for microbial activity. Comparable fieldwork has been carried out by Edwards and Heath (1963), who used litter bags of mesh sizes 7 mm to 0.003 mm in an exclusion experiment. The rate of breakdown in 0.5 mm mesh bags is three times slower than in 7 mm bags, and that in 0.003 mm bags is even slower; micro-organisms have little effect in the absence of soil fauna. These experiments show that the comminuting effect of soil fauna, especially earthworms, appears necessary to facilitate invasion by the microflora.

In addition to fragmentation and incorporation of organic matter into soils undertaken by earthworms, they excrete simple forms of nitrogen, and increase the levels of vitamin B_{12} in soil up to seven

fold, (Atlavinyte and Dačiulyte, 1969). These nutrients are required for growth by some fungi unable to synthesise their own, (Domsch and Gams, 1977).

Van der Bruel (1964) has shown that earthworms can stop the growth of some fungi in cultures; growth began when the earthworms died. This fungistatic activity may prevent fungal attack of earthworms, and probably serves to keep burrows clear. Waid (1960) refers to the fungistatic effect of soil, which prevents the germination of spores when conditions are likely to be unfavourable.

Microbial activity has been found by Satchell and Lowe (1967) to increase palatability of oak litter during the weathering of fresh litter. This increases the utilisation of the litter by earthworms, and therefore the rate of removal, fragmentation and decay. Brocks, Brown and Handley (1963) studied the chemistry of microbial decomposition, and found that white rot fungi produce enzymes capable of oxidising phenols. Penicillium species and Aspergillus niger can decompose unpalatable compounds, e.g. (+)-catechin, which is repellent to L. terrestris. This indicates the need for microbial weathering. Thus the combined effects of the mixing and fragmenting activities of earthworms, with their low assimilation efficiency, (Mason, 1977), and the degradation effects of micro-organisms using a battery of enzymes, are mutually beneficial, and a synergistic decomposition is the result.

2 ii Soil Disturbance

Soil disturbance by cultivation removes the surface insulating layer, and according to Hopp (1946) reduces numbers of earthworms in North American soils. This would probably not occur to the same extent in Britain, which does not have such cold winters. Evans and Guild (1948) discount the effect of mechanical damage by ploughing because there was no change in the size of the earthworm population in grassland ploughed once. However after 5 years of ploughing, the population had decreased

by 70%. This observation was supported by Graff (1953). Zicsi (1958) compared cultivations at different times of the year; those after harvest were most drastic. Zicsi (1969), and Edwards and Lofty (1971) agree that cultivations which loosens the soil can favour earthworms. These reports indicate that stripping and heaping topsoil with associated compaction and reduced aeration is likely to reduce the earthworm population.

An indirect effect of cultivation observed by Barley (1961), is loss of organic matter, especially in modern agricultural systems when organic manure is not used. This reduces earthworm food, and populations decline. This reduction in organic matter can be more important than the effect of the actual cultivations.

An early estimate of colonisation rate of 10 m per year was made by Hamblyn and Dingwall (1945) studying A. caliginosa from inoculation points in recently limed grassland. The main method of dispersal is by cocoons carried on implements, by animals and water.

Dunger (1969) studied the development and succession of decomposer fauna on re-afforested open cast mining dumps. He found that arthropods were important in the early colonisation, i.e. after 3 years, when moder humus had developed, but after 7 - 10 years earthworms were dominant, and the transition from moder to mull-type soil had begun. This colonisation by earthworms seems slow, but was brobably not limited by their rate of advance, but by the rate of change of the environment into one providing the minimum requirements for earthworms.

Rhee (1969) gives 6 m per year for the advance of A. caliginosa and 4 m per year for Allolobophora chlorotica, which are similar to Hamblyn's figure. Rhee worked on a new polder in the Netherlands, and also studied the population increase; rate of spread could be affected by availability of food.

Waid (1960) states that soil disturbance and addition of organic matter reduce the fungistatic effect of the soil. Thus a large number of spores germinate after these changes; cultivations are likely to

increase the rate of decomposition of limited supplies of organic matter.

2 iii The Effect of Earthworms and Fungi on Soil Structure

Evans (1948) estimated that 1.25 to 8.7% of topsoil to a depth of 10 cm passed through earthworms each year. This could account for an improved soil structure, but cast stability was less than one month, and so he assumed the effect to be small. Swaby (1950) found that pasture soil incubated with a suspension of fresh casts became stabilised with fungal hyphae. Kubiena (1953) states that practically all soil aggregates are casts or residues of casts, and Guild (1955) found that worm worked soils were more water stable than those without worms. The stability of casts depends on the availability of nutritive plant remains. This is why the crumb structure is better in soils under pasture, than those bearing straw crops, (Hoeksema, Jongerius and Meer, 1956). These reports indicate that earthworms are desirable, and improve soil structure.

Parle (1963a) found that the stability of casts reached a peak at 15 days, which corresponds closely to the development of fungal hyphae. He found that after 16 days the proportion of large aggregates declined and small aggregates increased, which could be due to the binding effect of hyphae being taken over by the cementing effect of bacterial gums.

Satchell (1967) estimated the effect of earthworms on soil aeration. Up to $\frac{2}{3}$ of the air capacity of some soils can be as burrows. However, those species which cast below ground open up the soil at one point, but consolidate it elsewhere. The total pore space of soils in Western Europe is 40 - 59%. In comparison, casts on the surface can be 0.5 - 6.0% of the top 10 cm of soil; this is only a small proportion, and therefore earthworms can only improve aeration to a limited extent.

In contrast, earthworms have a marked effect on drainage, (Slater and Hopp, 1947). In a laboratory experiment, Guild (1955) found that water passed through sandy soil containing earthworms in 2 days, compared to 8 days for a similar soil with no earthworms. Raw (1959)

calculated the area of natural drainage channels created by L. terrestris as 44 $mm^2 m^{-2}$. This will improve run-off of surface water. Earthworm channels have also been found to facilitate root growth in direct drilled crops, (Edwards and Lofty, 1976). Raw (1962) mentions the striking difference in the soil profiles of two orchards, one with earthworms, and one without due to poisoning by copper sulphate. The former had a mull profile, but the latter had a clearly defined surface mat of undecomposed organic matter.

3 METHODS

3 i Estimation of Fungal Activity

Garrett (1955) makes the important point that it is essential to differentiate between vegetatively active fungi which are contributing to the economy of the ecosystem, and those which are passive dormant propagules. With this aim several workers have developed direct methods of study using soil sections. Their work is reviewed by Warcup (1967). The soil must be fixed to prevent crumbling, and then cut and polished to give a thin slice. These techniques are complex and lengthy.

A slide burial technique was developed by Rossi (1928), and Cholodny (1930). Brown (1958) used a layer of nitrocellulose thinned with amyl acetate to cement mycelium and soil particles in contact with the slide to the glass. Warcup criticises the burying operation which disturbs the natural relationship between the mycelia and soil particles. Waid and Woodman (1957) buried nylon mesh through which mycelium could grow. The main disadvantage of these methods is that the majority of mycelia are sterile. This is also true for immersion tubes designed by Chesters (1940), and for screened immersion plates developed by Mueller and Durell (1957), and MacWithey (1957). Active mycelia are selected by these methods; non-sporing species, e.g. Papulaspora species have been trapped.

Indirect techniques have been developed for the study of soil fungi: Warcup (1960) used a soil dilution plate. He criticises this method because it favours spore forming organisms, e.g. species of Penicillium, Rhizopus, Mucor, Cladosporium, Fusarium, and Acremonium. Warcup (1950 and 1960) assesses his soil plate method, which uses soil particles rather than soil water, and therefore does not discard fragments of mycelia adhering to the larger soil particles, (Warcup, 1955). Warcup (1967) concludes that the soil dilution plate and the soil plate both give a similar picture of the soil fungi, but the soil plate favours the faster

growing species which are present in lower numbers. Griffiths and Siddiqui (1961) do not dismiss the soil dilution plate method: a single quantitative estimate is of little value, but a series at regular intervals would show changes in populations of spores, (not necessarily in fungal activity).

Contamination of fungal cultures by bacteria has been experienced by several workers. Initially bacteria were supressed by using acids: Wakeman (1922), and Jensen (1931) adjusted the acidity of the medium to pH 4 with sulphuric acid. Later rose bengal was used in a concentration of 1:15 000 by Smith and Dawson (1944). Recently antibiotics have been used: Martin (1950) recommended 30 ug cm^{-3} streptomycin or 2 ug cm^{-3} aureomycin. A disadvantage of these bacterial suppressants is that they can affect the fungi too. Warcup (1960) recorded a reduction in fungal colony size due to rose bengal, and in bright light this dye supresses fungi, (Pady, Kramer, and Pathak, 1960). Streptomycin has been found to inhibit some species of fungi, e.g. Phytophthora, (Echert and Tsao, 1962), and Pythium, (Schmittenner and Hine, 1962).

3 ii Earthworm Population Study

Non-chemical methods of sampling, e.g. vibration, (Reynolds, 1973), and electrical methods, (Edwards and Lofty, 1975), have the disadvantage that the area from which the worms are taken cannot be measured accurately. Handsorting and wet sieving as described by Svendsen (1955), and Ladell (1936), can be used on a known volume of soil, and a greater proportion of the earthworms present are extracted than by other methods, (Raw, 1959; Bouché, 1969), but both these methods are time consuming. Svendsen found that hand sorting was most suited to crumbly soils, and Satchell (1967) agrees that no one method is applicable to all species and habitats, e.g. sample size of L. terrestris will be low when handsorting because this species retreats down its burrow to depths of over 1 m when disturbed. Nelson and Satchell (1972) point out that specimens less than 2 cm,

0.2 g live weight, are likely to be overlooked by handsorting. Wet sieving also recovers cocoons, and Raw (1960) found flotation in magnesium sulphate useful for small species in wet matted hill grassland. Alternatively small surface dwellers in matted turf can be expelled by heat extraction described by Edwards and Lofty (1977).

Evans and Guild (1947) used potassium permanganate for earthworm sampling, but Raw (1959) found that it was toxic, and could reduce numbers emerging by killing them in their burrows, (Edwards and Lofty, 1977). The most widely used method of earthworm sampling is formalin extraction. This chemical acts as a skin irritant and is less toxic than potassium permanganate. Edwards and Lofty (1977) compared several methods of extraction and found that the main disadvantage of chemical methods is that they recover species with wide burrows more efficiently, compared to non-burrowing species. Also aestivating worms do not respond to chemicals. The rates of application of formalin vary widely, as shown in table 1.

Table 1 Rates of Application of Formalin

Author	Rate of Application
Raw, 1959	25 cm^3 of 40% formalin in 4.56 l water, i.e. on 0.36 m^2 soil
Satchell, 1969	0.165 to 0.55% formalin: 3 x 9 l of solution on on 0.5 m^2 soil

Raw (1959) compared 0.14% formalin with 0.55% and found that the weaker solution extracted less than 50% of the earthworms which the stronger solution brought to the surface. Satchell (1969) used three strengths: 0.165%, 0.275%, and 0.55%, and found them all equally effective. However he did use large quantities, (3 x 9 l on 0.5 m^2).

Sample area used depends on the method of sampling. Zicsi (1962) compared areas of 0.06, 0.25, 0.5, and 1.0 m^2 for handsorting and found 16 x 0.06 m^2 to a depth of 20 cm adequate for medium sized species. Edwards and Lofty (1977) recommended 0.25 or 0.5 m^2 for formalin extraction.

Earthworms show seasonal activity. Immature A. chlorotica, A. caliginosa, and Allolobophora rosea studied by Evans and Guild (1947) became quiescent when the soil was too dry or too cold. They also observed that production of surface casts showed marked maxima in Spring and Autumn, and could cease completely at other times of the year. This seasonal activity affects the results of sampling carried out at different seasons. Nelson and Satchell (1962) give the optimum soil temperature at 10 cm depth for formaldehyde expellant as 10.5 °C.

When sampling for earthworms, Gerard (1964) recommends 10% formalin as a preservative, and Satchell (1969) used 5 to 10%. Earthworms lose moisture on preservation; Raw (1962) estimated a loss of 15% when he calculated fresh biomass from formalin preserved specimens. Also when estimating biomass, weight of gut contents should be taken into consideration: Satchell (1969) states that they can be up to 20% of the total live weight. He also gives a conversion factor: 1 g dry weight = 5.5 g live weight. Satchell (1970) calculated a correction factor to adjust earthworm numbers appearing in samples to numbers occuring in ideal conditions of soil moisture and temperature; it only applies to L. terrestris.

3 iii Field Sampling Technique

Ten samples were taken at random from each of the five sites from 7th to 29th July, taking one sample per site on each visit during this time. This ensured that each replicate was taken under similar environmental conditions. The time interval taken for the sampling was kept as short as possible to reduce the effects of the seasonal activity of earthworms described by Evans and Guild (1947), Gerard (1967),

and Edwards and Lofty (1977). Each sample involved several proceedures:

3 iii (a) Earthworm Sampling using Formalin

A 0.5 m^2 plot was pegged out, and the diagonals measured with a tape to check that it was a rectangle. A quadrat was not carried because the site was on a gradient and uneven, and water had to be carried too. Standing vegetation, and any surface mat of litter was removed from the plot so that emerging earthworms could be seen easily, and so that the formalin was not soaked up and partly retained above the soil. 8 l of 0.25% formalin, (25 cm^3 of 40% formalin in 8 l of water), was watered evenly onto the plot in three equal doses using a watering can with a rose, allowing 10 mins between applications. As earthworms appeared they were collected and preserved in 10% formalin, and later taken to the laboratory. No attempt was made to remove partially emerged worms; they withdraw into their burrows. The bases of herbage stems were searched carefully for individuals emerging at these points. Collection was stopped 10 mins after the last application of formalin.

3 iii (b) Soil Temperature

A slit was made in the soil to a depth of 10 cm using a trowel, and a thermometer inserted. The surface soil was pressed around the stem of the thermometer so that the effect of atmospheric temperature on the soil temperature at a depth of 10 cm was minimised. The temperature was taken after 30 mins.

3 iii (c) Soil Sampling

Two soil cores 5 cm in diameter and to 10 cm depth were taken adjacent to each 0.5 m^2 plot, and placed in a labelled polythene bag. This sample was used for the soil analyses and fungal cultures carried out in the laboratory.

3 iii (d) Laboratory Pre-treatment of Soil

On return to the laboratory, each soil sample was spread on a tray until air dry, (2 to 5 days). It was then folded in a newspaper, squashed with a brick, and put through a coarse mesh sieve, (2.5 mm approx.), to provide a fine earth sample, ready for the soil analysis.

3 iv Choice of site

Biggin South Site is approximately 7 miles W.N.W. of Durham, Grid Reference: NZ 175 445. It has been opencast mined for coal by the National Coal Board, and provides five top soil treatments on the one site, giving a minimum of environmental variation, for sampling was all carried out on the North facing slope of Weather Hill, in the centre of the site. The five top soils are:

3 iv (a) Undisturbed Soil (UD)

This has been undisturbed pasture for at least 100 years, and has had small low unconsolidated heaps of top soil deposited on some parts of it for a few years during the recent opencast scheme. Sampling was confined to areas alongside undisturbed hedges to avoid top soil which would have been compacted beneath heaps, according to Tandy (1973). This area was re-seeded with a permanent pasture seeds mixture 3 years ago. This site was used as the control treatment in this study as it had experienced the least disturbance.

3 iv (b) Top Soil Reinstated in 1958 (1958)

This small area was permanent pasture before opencast mining; the top soil was put back 11 years ago. It is uneven, and badly drained despite being on a hillside. The vegetation is self seeded rough grasses, (Appendix 1).

3 iv (c) Heaped Top Soil (H)

The two remaining soil banks from the same slope as the other treatments are of top soil removed 3 years ago in 1976. These heaps

contain decomposing turf, because the land the soil came from was rough permanent pasture. The heaps were covered with couch, Agropyron repens L. Sampling was carried out on the top of the soil heaps.

3 iv (d) Top Soil Reinstated in 1977 (2 YO)

This top soil was put back 2 years ago and was reseeded with a perennial ryegrass and white clover ley. This area was cut last summer for hay, but the hay on half the area had not been gathered. Samples were taken alternately from these two sub-treatments.

3 iv (e) Top Soil Reinstated in 1978 (1 YO)

This top soil was put back 1 year ago and had not been re-seeded. Annual weeds, (Appendix 1), were colonising the bare soil, and there was some gulley erosion down the slope. The site was badly drained before opencast mining and the soil was heavy, so the N.C.B. during reinstatement, mixed a proportion of a sandy sub-soil with the original top soil, to open the texture and improve drainage.

The difference in appearance of the five treatments was striking, (photographs 1 - 6). The species of flowering plants occuring on each soil treatment were dissimilar, (Appendix 1), and clearly indicated their different management.

3 v Estimation of Fungal Activity using Cultures

3 v (a) Preparation of Culture Medium

A natural, non-purified potato/sucrose agar was used, to exclude as few species as possible. The ingredients for 1 litre of agar were:

150.0 g potato, washed, peeled and chopped.

2.0 g sucrose.

0.2 g streptomycin sulphate.

20.0 g agar.

The potato was boiled for 20 mins in 0.5 l water. The sucrose and agar were mixed with a little water in a 2 l flask, and the water from the

P.1. View of Site Looking East from Esh

Key to photograph and map on facing page:

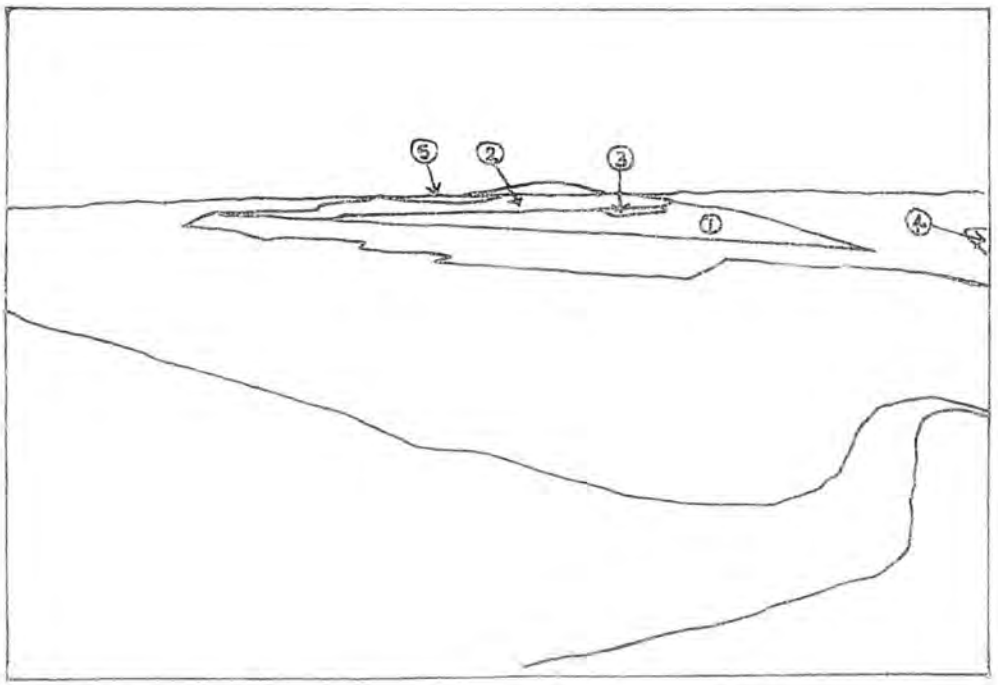
1 Topsoil reinstated in 1978 (1 year old).

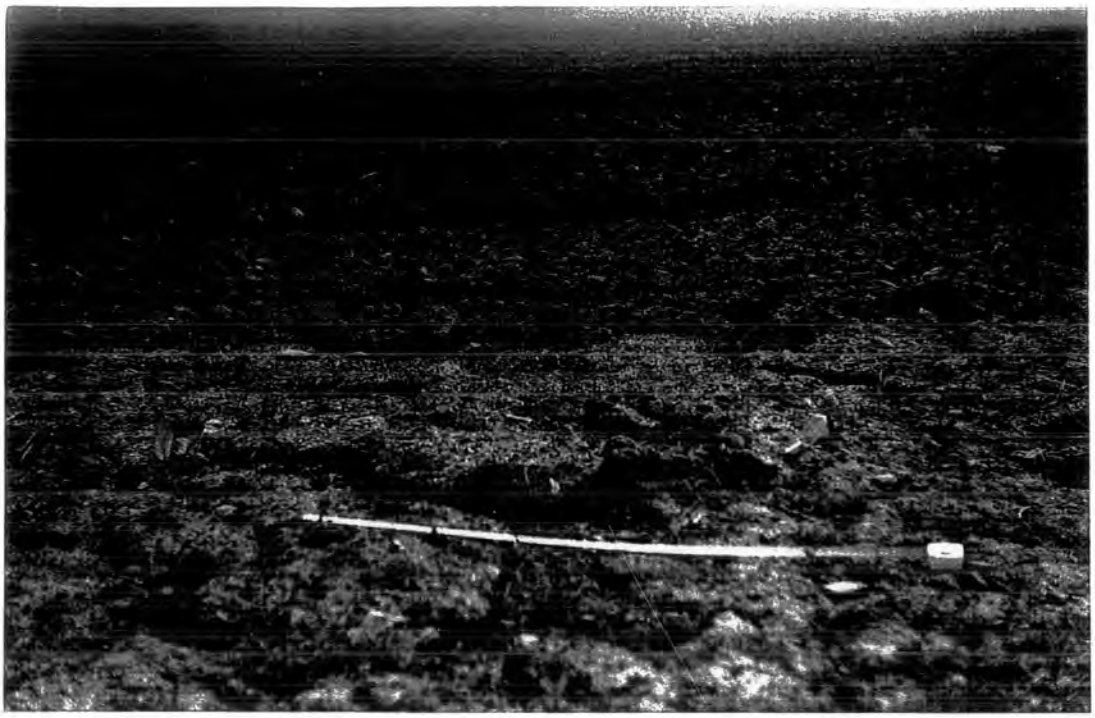
2 Topsoil reinstated in 1977 (2 years old).

3 Topsoil reinstated in 1968 (11 years old).

4 Heaped topsoil (3 years old).

5 Undisturbed topsoil.





P.2. Topsoil Reinstated in 1978 (1 year old)

Tape measures 1 m.

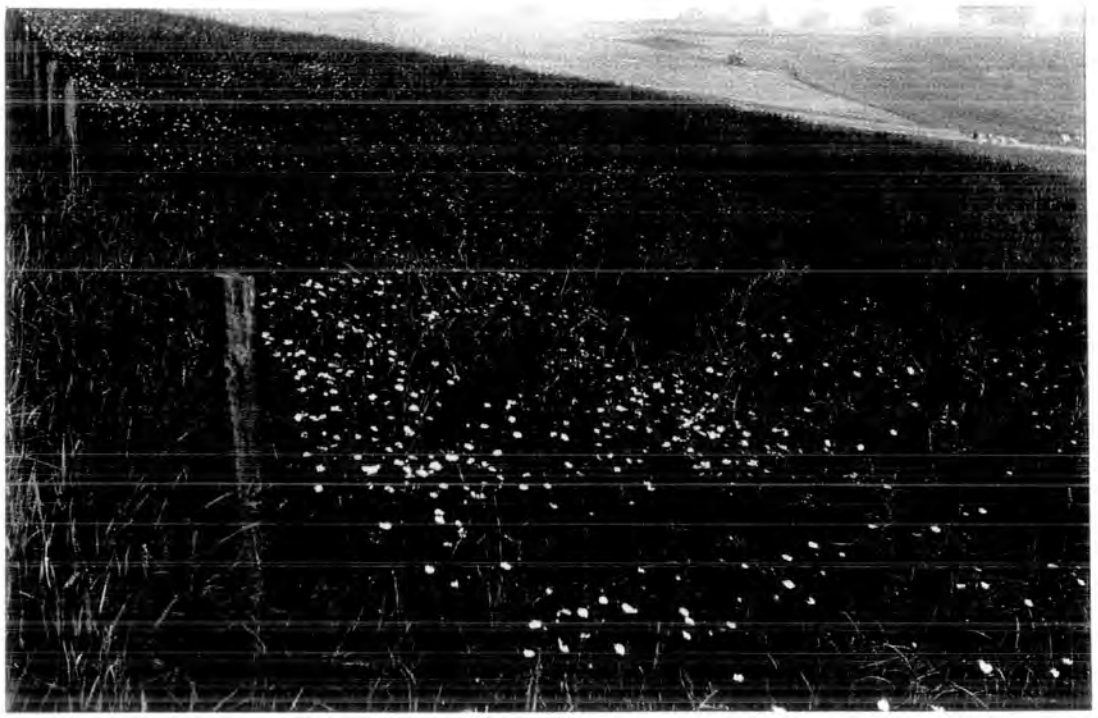
Foreground has not been cultivated, and shows erosion and annual weeds. The more distant area has been cultivated, and stones removed prior to reseeding.

P.3. Topsoil Reinstated in 1977
(2 years old)
Hay crop cut and removed from this area last summer, (1978).
Tape measures 30 cm.

P.4. Topsoil Reinstated in 1977
(2 years old)
Grass cut, but not removed last summer, (1978), and vegetation now growing through decaying hay.
Soil corer handles 50 cm across.

P.5. Soil Heap to West of Site Access Gate

P.6. Soil Heap to East of Site Access Gate



P.7. Topsoil Reinstated in 1958

(11 years old)

Grass tussocks illustrate rough nature of this area.

Fence posts 1.5 m in height.

P.8. Undisturbed Topsoil

(100 years old)

This view of standing crop taken from trimmed 0.5 m^2 earthworm sampling plot shows the dense vegetation.

Tape measures 50 cm.

boiled potatoes added. The volume was made up to 950 cm^3. The flask was stoppered with cotton wool and autoclaved for 15 mins at 15 lb pressure. The streptomycin sulphate was dissolved in 50 cm cool distilled water which had been autoclaved in a stoppered flask, and added to the cooling liquid agar just before pouring. This minimised the destruction of the antibiotic by heat. 30 to 40 plates per litre of agar were poured, at 50 to 60°C, using sterile plastic petri dishes. They were left to cool at room temperature, and then inverted and stored in plastic bags at 0 to 5°C in a refrigerator until used.

3 v (b) Soil Crumb Culture Technique

20 soil crumbs as small as possible were picked out of a few grams of each soil sample, which was spread on a piece of clean paper. The crumbs were arranged on an agar plate using fine forceps, which had been sterilised in a bunsen flame and allowed to cool. The petri dish was replaced and labelled. The cultures were incubated at room temperature, in daylight, but away from strong sunlight, to encourage the development of pigment in some species, e.g. Trichoderma species. The plates were not inverted; condensation was not a problem unless the plates were returned to the fridge after a period at room temperature.

3 v (c) Foil Spread Culture Technique

A small quantity of soil, (about 0.1 g), was taken from each soil sample in turn on a cooled, heat sterilised spatula, and rubbed over a sterilised aluminium foil disc, of diameter 0.5 cm less than the petri dish base. The spatula blade was used to grind the soil into the foil before inverting the disc to remove excess free particles. The inverted foil disc was pressed onto the surface of the agar, peeled off, and discarded. The plate, with lid replaced, was incubated as described above.

The plates were incubated for two days, and then inspected daily for up to approximately 5 days, identifying fungal colonies as they

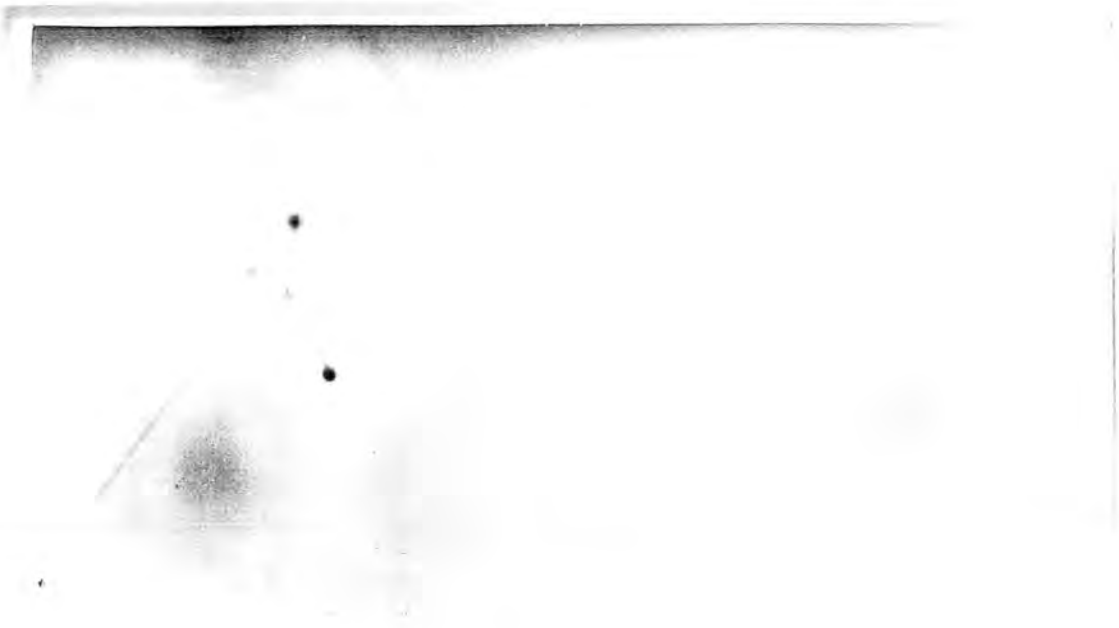
produced spores. Specimens difficult to identify, and slow growing specimens in danger of becoming over-grown by faster growing types such as some Mucoracous species were isolated on to new plates, taking care to record their soil sample of origin. Identification was carried out to the level of genus according to Domsch and Gams (1972), and was aided by preparing a reference collection of genera isolated, (photographs 9 - 23). The number of colonies of each genus grown from each soil sample by both techniques was recorded, (Appendix 2: Original Raw Data).

3 v (d) Identification Methods for Fungi

A Watson Microsystem 70 microscope was used for examination of the cultures, with x 7 eye pieces in the binocular head, and x 4 and x 10 objectives. In most cases light penetration through the agar was sufficient, and colonies were examined in situ, placing the petri dish base on the microscope stage, after first removing the slide holding attachment. The petri dish was tilted for colonies at the edge of the dish; this was an ideal site for spore formation of slow growing specimens, which were prompted to spore by the reduction in nutrient concentration and a check to growth. The higher powered objectives were removed from the rotating head because they were long barrelled, and would make contact with the agar, or the side of the petri dish.

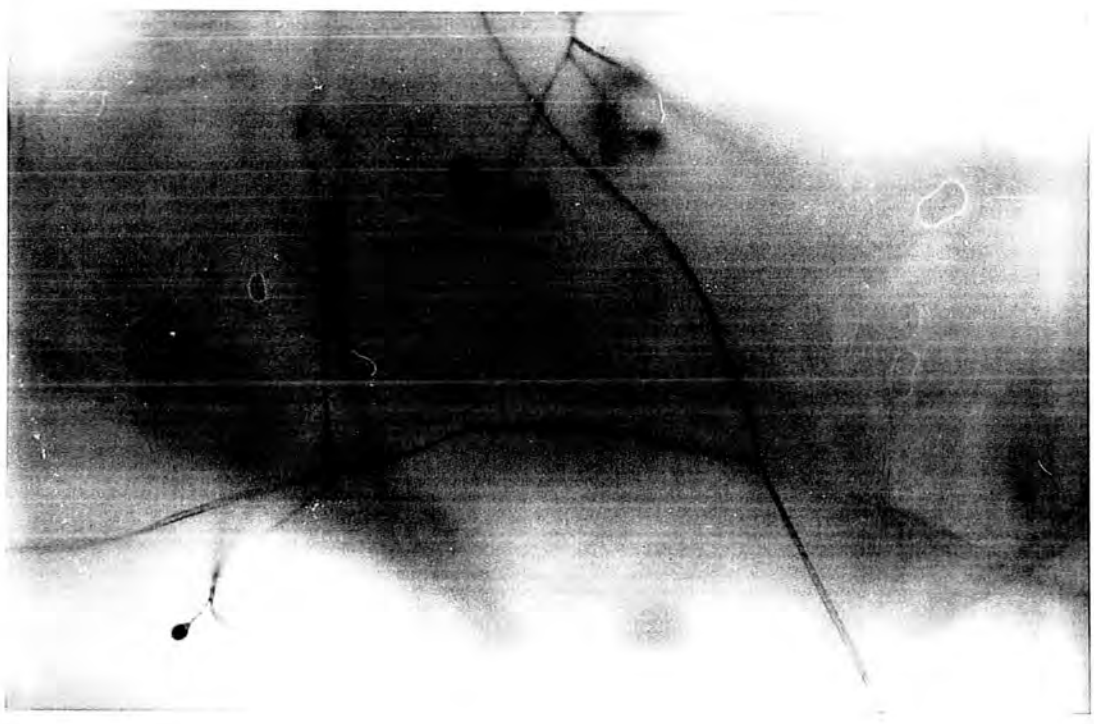
For specimens requiring a more detailed examination, the sticky side of a piece of sellotape was touched onto the mycelium and then stuck onto a slide. Many hyphae and spores are not clear at x40, so a drop of lactophenol and cotton blue stain was added.

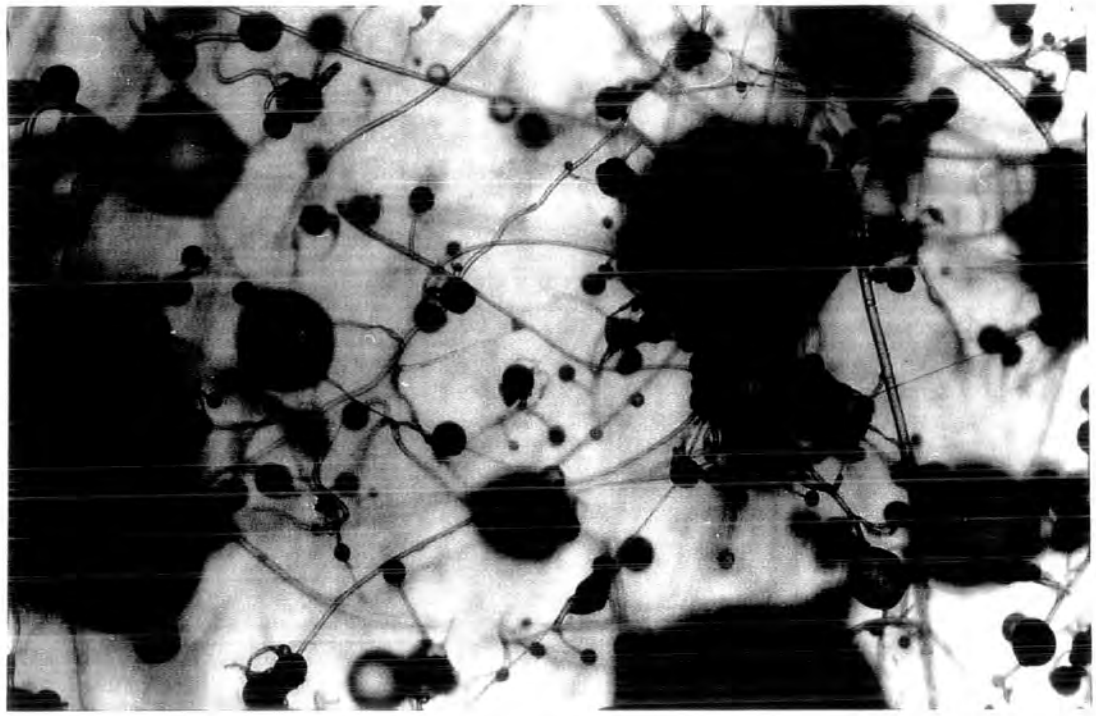
To obtain a side view of the growing mycelium, a wedge shaped section was cut from the agar through the colony, and placed on its side on a slide. After preliminary examination, a drop of stain and a cover slip were added; this improved clarity of fungal structures, but some of the natural growth habit was lost by flattening with the cover slip.



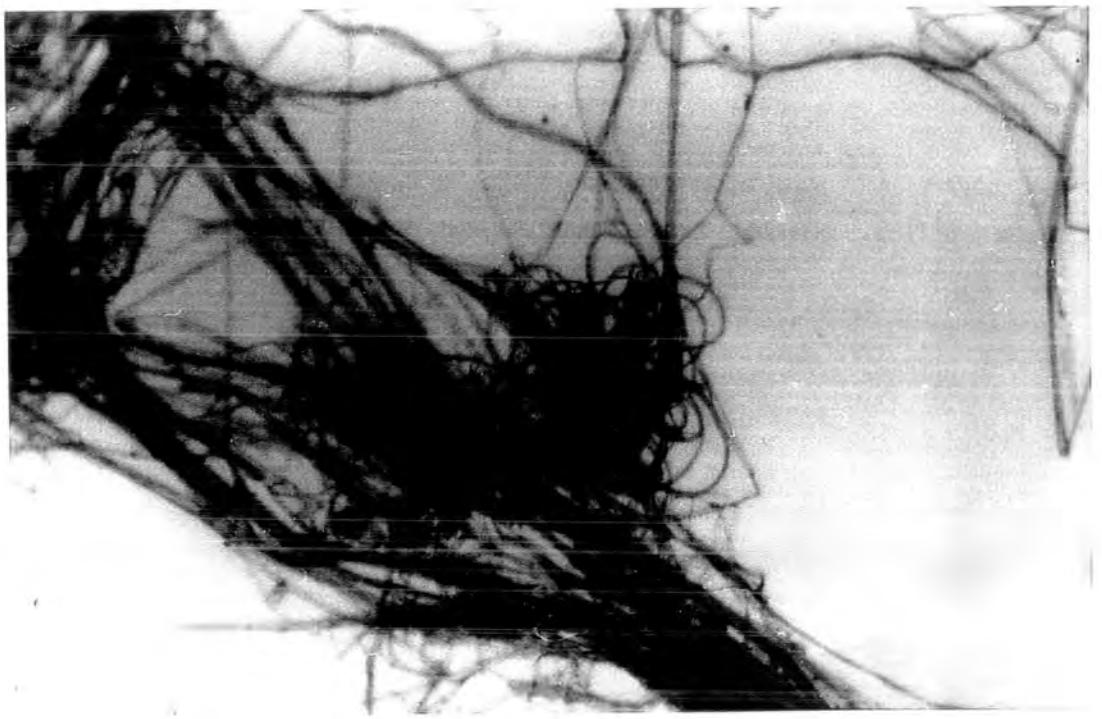
P. 9. Absidia x 100 The arching hypha bears a group of 3 pear-shaped sporangia.

P.10. Thamnidium x 100 showing branched sporangiophore. Also single pear-shaped Absidia sporangium is shown.





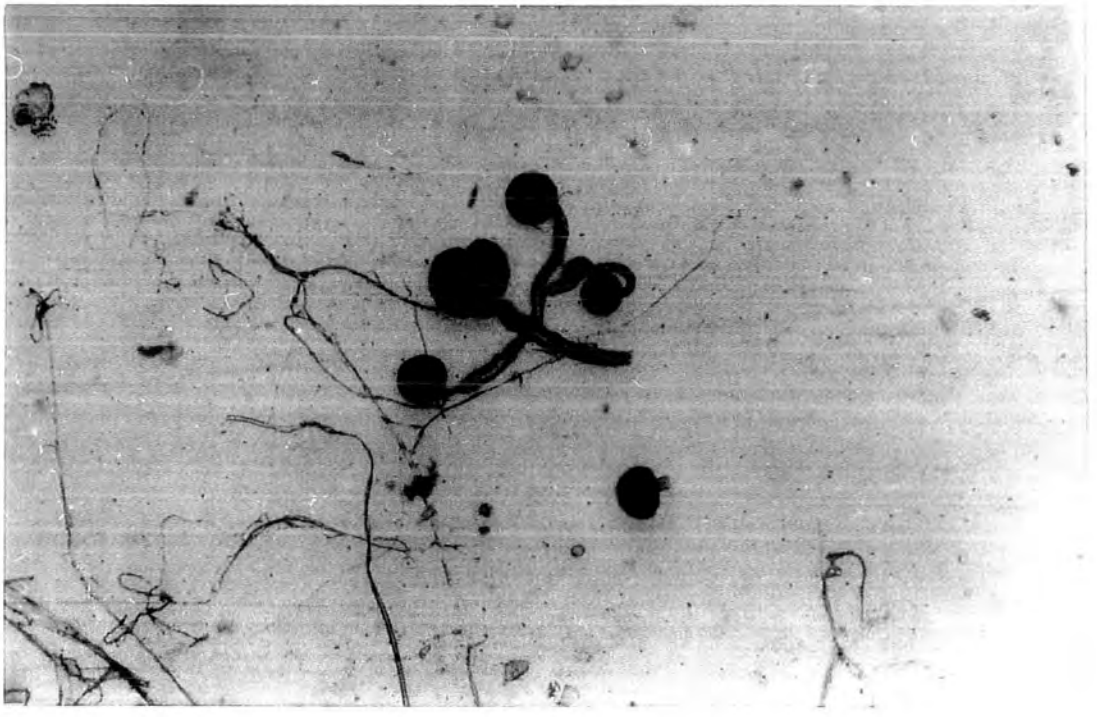
P.11. Zygorynchus x 100 The rough textured zygospores have one large swollen, and one slender suspensor. Large spheres are condensation droplets.

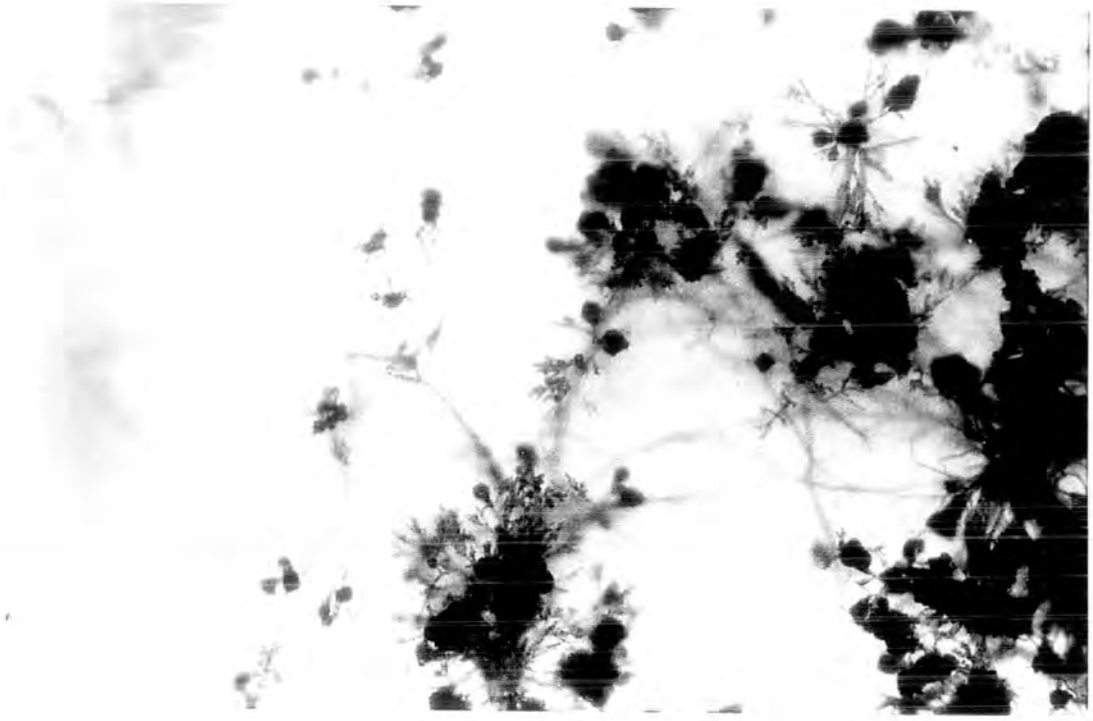


P.12. Absidia x 100 The zygospore is surrounded by curved structures arising from suspensor.

P.13. Mucor x 100 A particularly coarse type with branched sporangiophores.

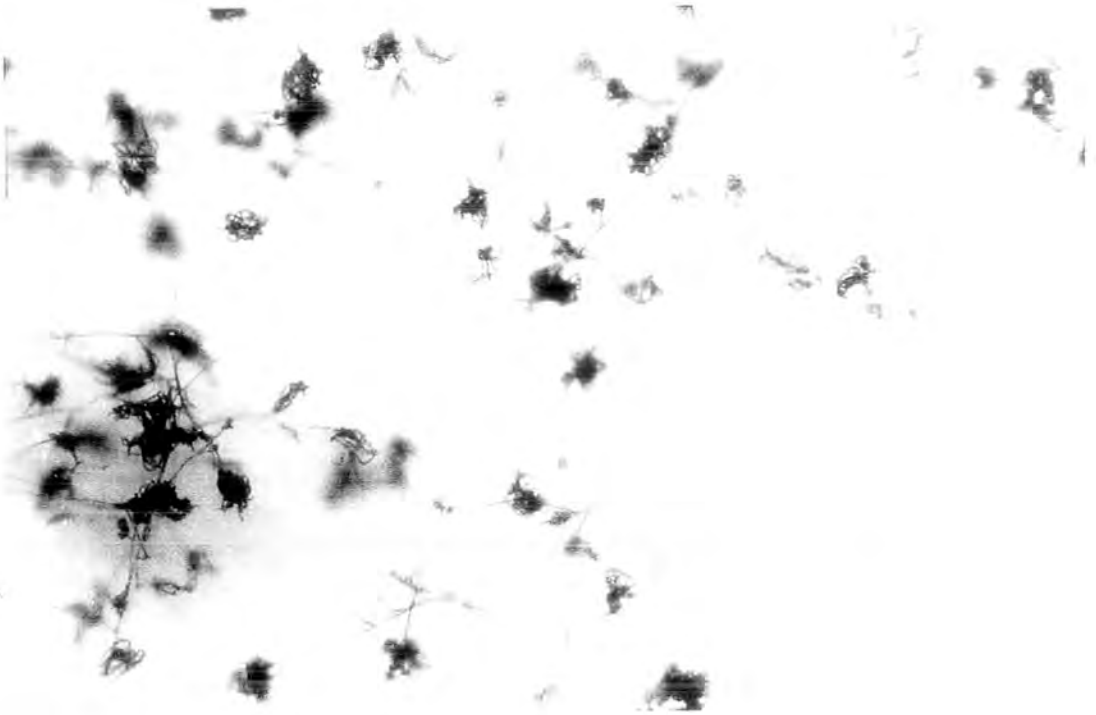
N.B. These two specimens were mounted on slides in glycerine using selotape. All other photographs were taken directly from the culture.





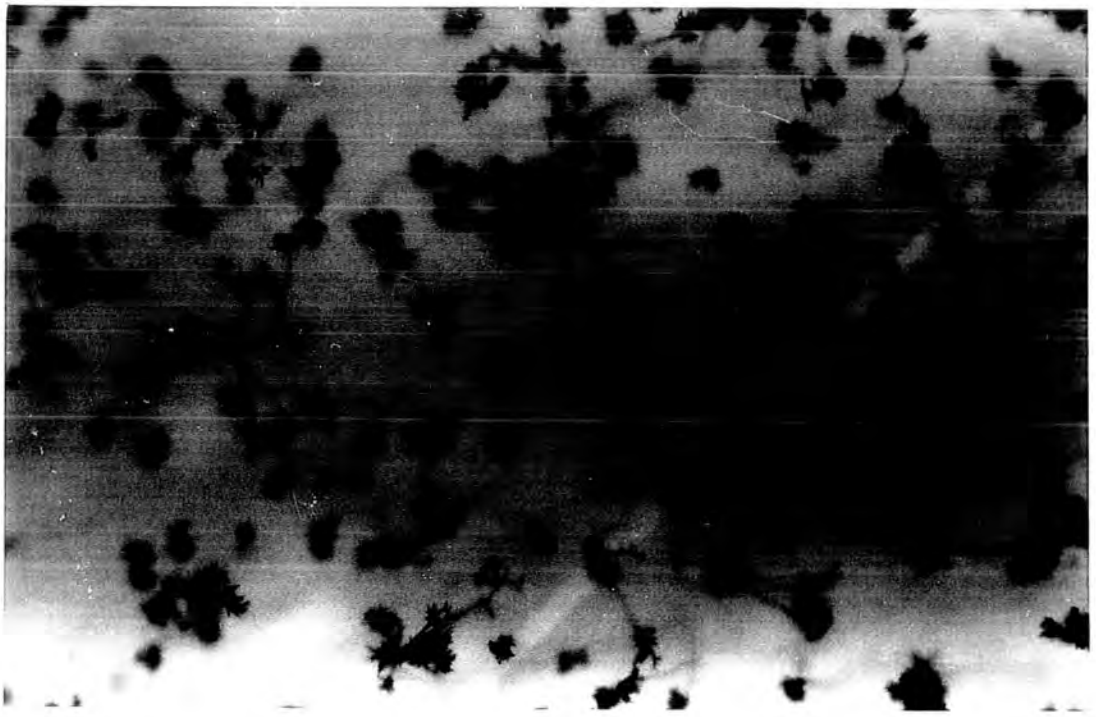
P.14. Scopulariopsis x 100 The branches of the sporangiophores cling together like wet mops. Dark areas are exudation droplets. There is also a little Verticillium present.

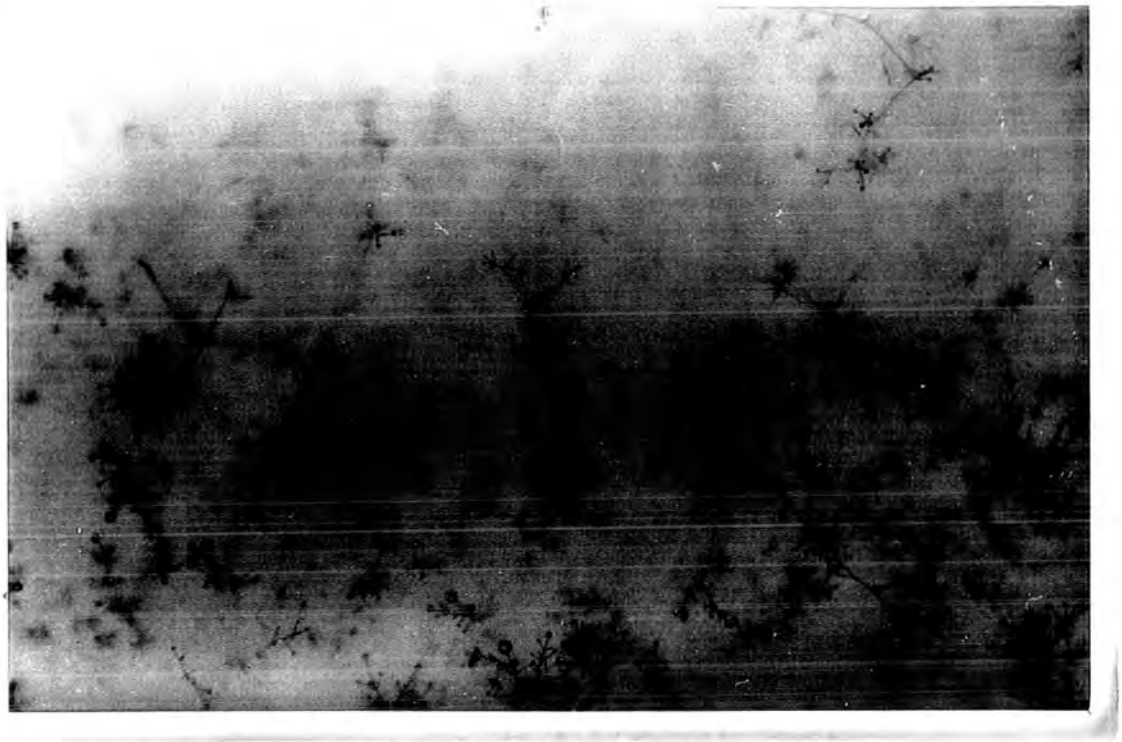
P.15. Botrytis x 100 The dark brown spores are arranged in tight groups like bunches of grapes.



P.16. Penicillium x 100 The long chains of spherical spores on branched ~~sporang~~conid. giophores characterise this genus.

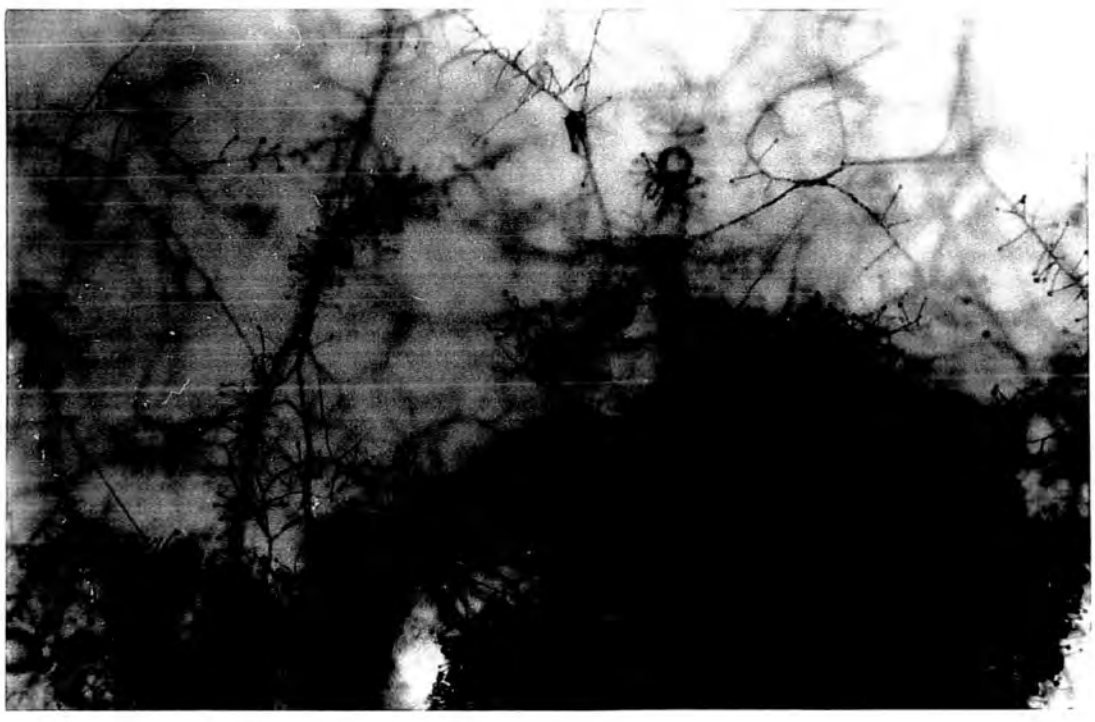
P.17. Cladosporium x 100 Chains of dark brown oval spores on branched ~~sporang~~conid. giophores form khaki-green cushions.

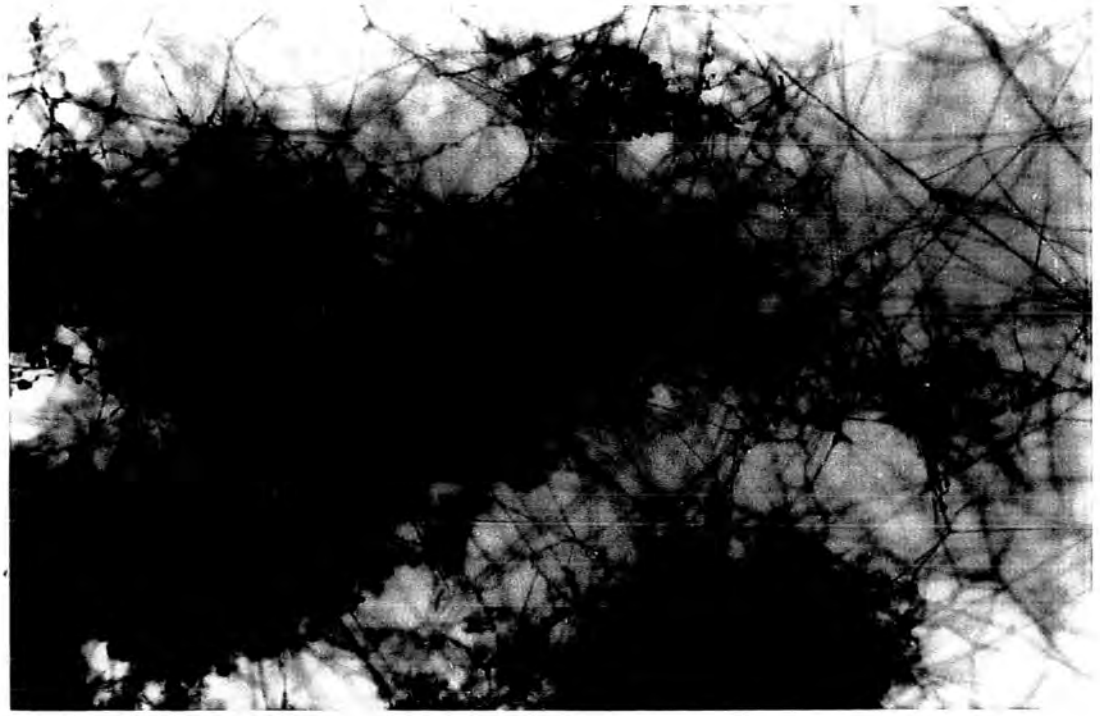




P.18. Verticillium x 100 Single spores at different stages of development (indicated by size) on branched ~~sporang~~conid.giophores.

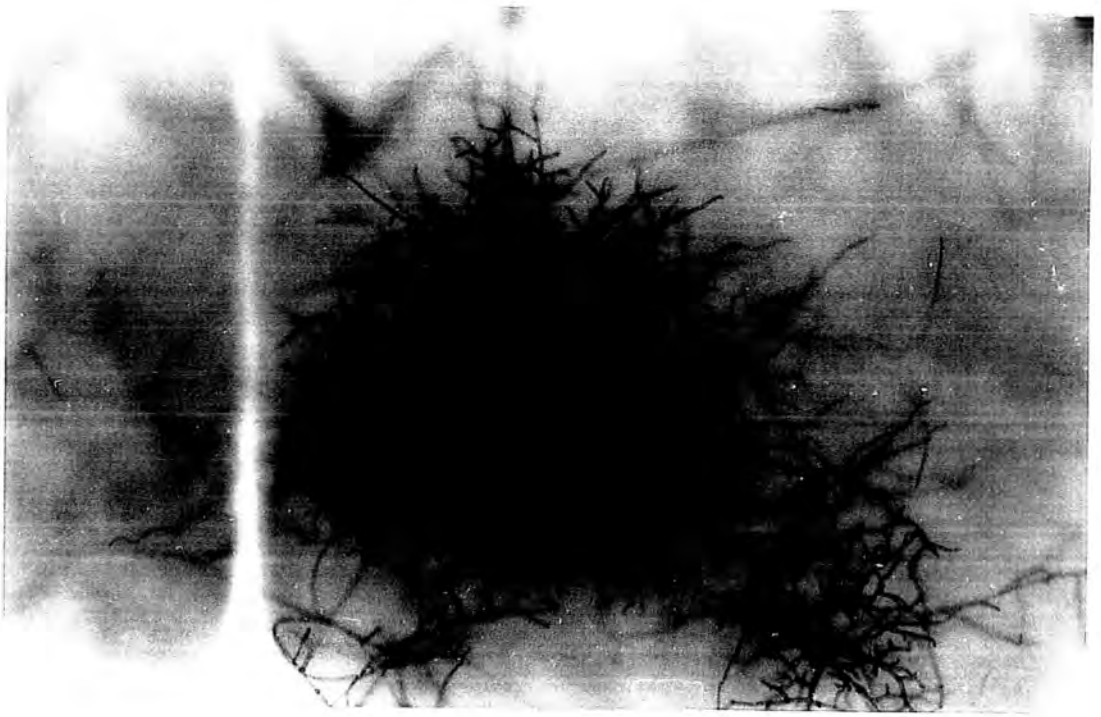
P.19. Acremonium x 100 Single spores on unbranched ~~sporang~~conid.giophores. Basal hyphae are often entwined. Large spheres are exudation droplets.

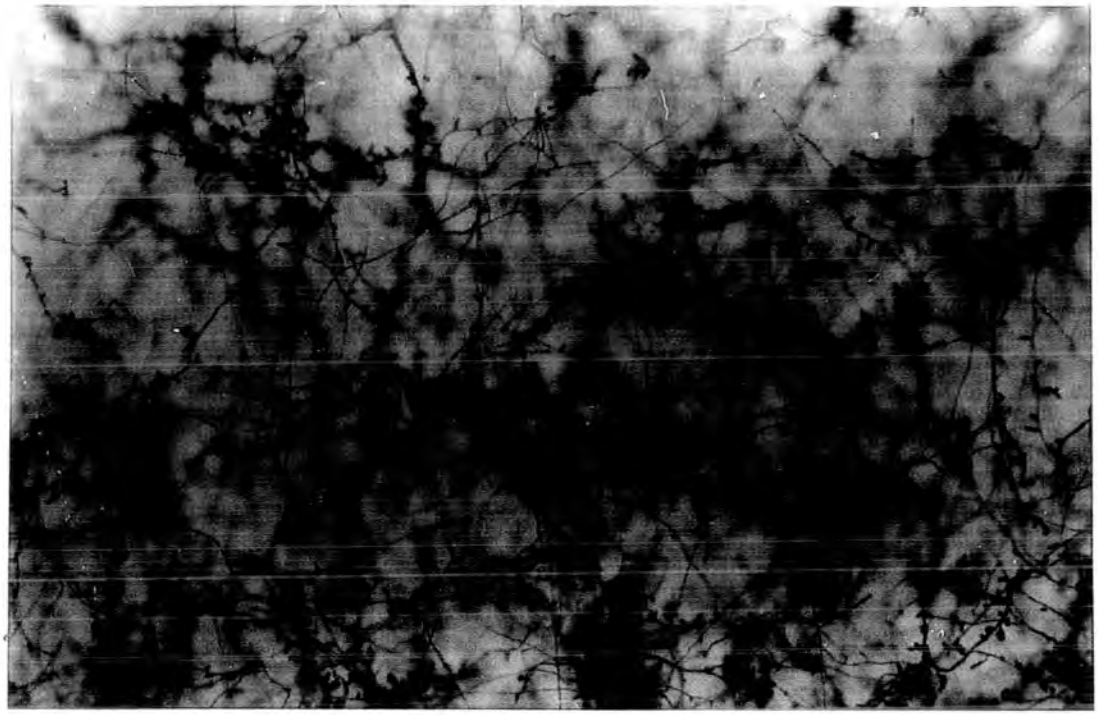




P.20. Trichoderma x 100 Fully developed clumps of spores, which turn green in daylight, are borne on sparse spreading mycelium.

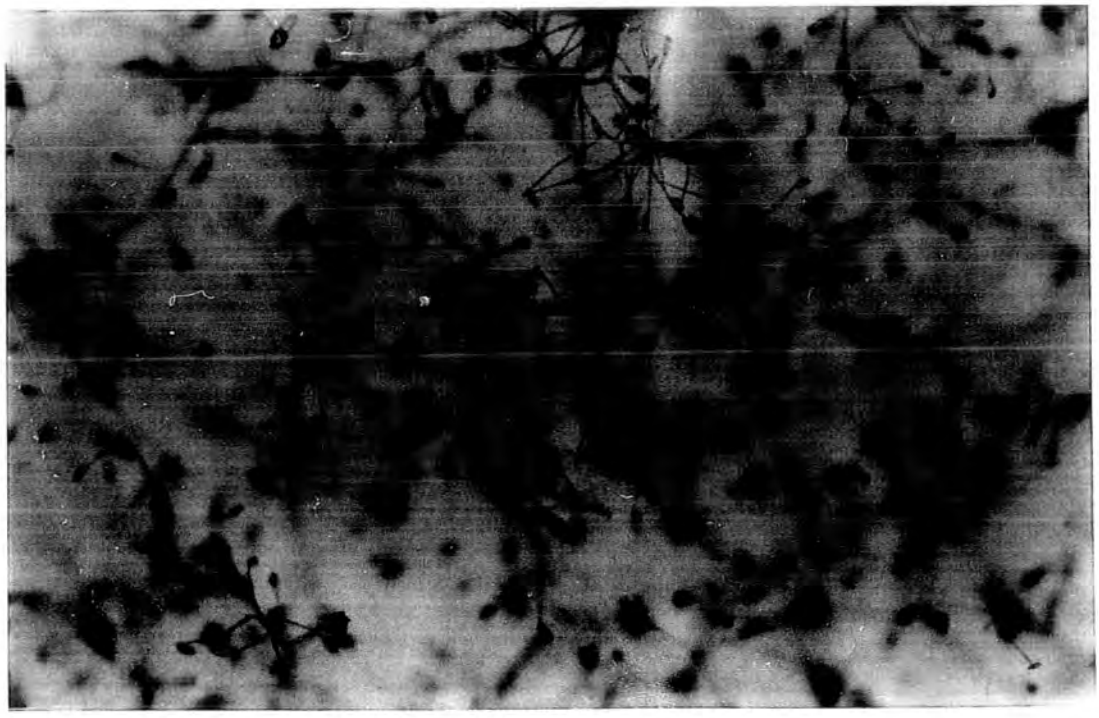
P.21. Trichoderma x 100 Tuft of aerial branching hyphae, prior to spores developing.





P.22. Dendryphion x 100 Chains of brown spores with thich walls in groups of 3 - 4, making them resemble chickens' feet.

P.23. 'Anther' Fungus x 100 This unidentified fungus has spore chains arranged in parallel groups of 2 - 4; thus they resemble the anthers of flowering plants. The spores are like those of Fusarium, with groups of 4 ~~spores~~ cells in a sickle-shaped chain.



3 vi Earthworm Identification and Recording

Preserved earthworms were identified down to species according to Gerard (1964); particular use was made of his 'Key to the ten common species found in good pasture land'. The tabular key produced by Edwards and Lofty (1977) was also used.

The formalin was rinsed off the earthworms from each sample plot in turn; adults were identified first, and then immatures grouped with the adults, and placed in specimen tubes of fresh 10% formalin according to sample site, and species. After this initial grouping, identification was checked when the mature and immature individuals of each species at each site were counted and weighed separately. Adhering water was removed with absorbant tissue before weighing. To obtain dry weights, 5 adult earthworms of each species were taken at random, weighed, and dried to constant weight.

3 vii Soil Texture

In this analysis it was thought necessary to conform to other work, and use a recognised standard method; the soil particle distribution analysis as described by the British Standards Institution was used. This analysis takes one week to complete, and one bulked sample from each of the soil treatments was analysed. Each bulked sample was composed of five sub-samples taken at random from the sites to a depth of 10 cm, using a 5 cm diameter soil corer.

The pre-treatment with hydrogen peroxide is designed to oxidise the humus coating the soil particles, and thus separate the individual mineral grains from the soil crumbs. However the soils from Biggin South Site contained high levels of undecomposed organic matter, especially in the heaped treatment, and in that reinstated two years ago with mown dead grass lying on the soil surface. Therefore a further aliquot of hydrogen peroxide was added to each flask to dissolve more of the dead vegetation; otherwise this material would be weighed in the coarse sand fraction. Despite this modification fibrous fragments of dead vegetation

did appear in the coarse sand fraction; they were removed and weighed separately, and the weight of pre-treated soil adjusted accordingly. Calculations were then carried out as indicated by the method given.

3 viii Nitrogen and Carbon Analyses

Again it was thought necessary to use standard methods of analysis; results will then be easily comparable with other work. Also there are restrictions on the apparatus available. The methods used were those of the Geography Department, Durham University (1978). The nitrogen analysis was based on the Kjeldahl (1883) digestion and distillation, and the organic carbon was based on the method of Walkley and Black (1934).

3 ix Soil pH

The method described in the Geography Department, Durham University Laboratory Handbook (1978) was followed, using a pH meter made by Electronic Instruments Ltd., No. 7020, a glass electrode, (Pye unican probe), and standards of pH 4 and pH 7. Distilled water was used to make the soil suspension. The determination was carried out on each of the fifty air dried soil samples, and was repeated after the suspension had stood for 24 hrs, to allow for carbon dioxide equilibriation with the atmosphere. The average of the two readings on each sample was used in the analysis.

3 x Soil Moisture

This determination was carried out on all 50 samples of soil immediately on return to the laboratory. In each case, 50 to 100 g fresh soil was weighed accurately and left until air-dry, (2 to 5 days). It was then re-weighed, and the percentage moisture content of the fresh soil calculated.

4 RESULTS & DISCUSSION

4 i Age Since Disturbance of Top Soil

The time interval since last disturbance of the top soil does significantly affect the distribution of four species of earthworms, (Lumbricus castaneus, Lumbricus rubellus, Allolobophora longa, and A. chlorotica), total number, and weight of all earthworms, and number of species of earthworms. Total Mucor and Absidia colonies are also affected by age since disturbance, (Table 2).

L. castaneus was absent on 1 and 2 year old sites; its biomass reached a maximum on the 3 year old heaped soil, and declined with increasing age since disturbance up to 100 years. Numbers and biomass of L. rubellus show a similar pattern. The peak on the 3 year old site of heaped soil is probably due to the heaping process collecting surface dwelling species in the top soil bank, which then migrate to the surface of the mound, which is able to support larger numbers because of the dead vegetation incorporated in the mound; this was the standing crop plus surface litter before removal of topsoil, and would be substantial in permanent pasture.

The complete absence of earthworms on the 1 year old site shows that the double disturbance of removal and re-spreading the top soil, together with reduced aeration produced by compaction during heaping, and depth of heap, has killed all earthworms. 2 years after redistribution, L.castaneus and L. rubellus have not re-colonised these soils; A. longa and A. chlorotica are present in low numbers.

The 100 year old undisturbed site probably represented a stable equilibrium situation prior to storage of top soil on it, and subsequent re-seeding. This recent treatment could account for the lower numbers on this site, compared to the 11 year old site.

In all cases, except numbers of A. chlorotica, numbers of earthworms are lower in the 11 year old site, compared to the heaped 3 year old soil. This could be a result of colonisation rate; when

Table 2 Summary of Analyses of Variance to show Variables Significantly Affected by Age since Disturbance of Top Soil

Variable	F Ratio	Level of Significance	Treatment (Age) Means per 0.5 m^2				
			1 year	2 years	3 years	11 years	100 years
L. castaneus Total wt.	9.505	P<0.001	0	0	0.5280 ±0.0967	0.3630 ±0.0972	0.2170 ±0.0973
L. rubellus Total No.	3.857	P<0.01	0	0	0.3000 ±0.1528	0	0
L. rubellus Total wt.	15.507	P<0.001	0	0	0.5060 ±0.0835	0.3470 ±0.1057	0.0120 ±0.0120
A. longa Total No.	3.550	P<0.05	0	0.1000 ±0.1000	4.1000 ±1.6496	2.3000 ±0.6675	3.0000 ±1.1926
A. longa Total wt.	4.213	P<0.01	0	0.2060 ±0.2060	1.7070 ±0.5740	3.1380 ±0.9649	2.1380 ±0.8861
A. chlorotica Total No.	4.631	P<0.01	0	1.9000 ±1.0796	5.4000 ±1.3679	6.2000 ±1.4742	5.3000 ±1.5850
Earthworms Total No.	18.256	P<0.001	0	2.0000 ±1.0750	42.6000 ±7.9235	15.8000 ±2.7960	10.9000 ±2.8962
Earthworms Total wt.	13.135	P<0.001	0	0.8960 ±0.4276	11.7050 ±2,2084	7.5650 ±1.6780	3.6260 ±1.0782
Earthworms No. Species	44.117	P<0.001	0	0.5000 ±0.2236	4.2000 ±0.1333	4.0000 ±0.5164	2.3000 ±0.3000
Total Mucor Colonies	4.011	P<0.01	2.8000 ±0.5735	3.4000 ±0.7180	1.4000 ±0.3712	1.6000 ±0.5416	4.2000 ±0.6960
Total Absidia Colonies	3.605	P<0.05	2.5000 ±0.8199	2.0000 ±0.7888	2.0000 ±0.7746	7.3000 ±2.0712	2.1000 ±1.1101

top soil heaps are re-distributed, only those parts where the surface of the heaped soil is placed will have high numbers of earthworms, and all other areas of the site will have to be re-colonised. A. chlorotica total numbers are highest on the 11 year old site, which is badly drained and covered in coarse vegetation.

Numbers of propagules of Mucor are highest on the 2 and 100 year old sites; Absidia density peaks on the 11 year old site. Mucor substrates include chitin, pectin, urea, fatty acids, and hemicellulose, (Domsch and Gams, 1972), and Absidia substrates are gelatin and pectin. This is consistent with the probability that the 2 and 100 year old sites are at a late stage in decomposition, as compared to the 11 year old site, because more fibrous organic matter is being degraded.

4 ii Physical Factors Influencing the Distribution of Earthworms (Table 3)

4 ii (a) Soil Texture

Medium sand is negatively correlated with total number of earthworms, and fine sand is negatively correlated with total weight of L. rubellus, probably because burrows are less stable in sandy soil. A. chlorotica, however, is positively correlated with sand; this species is a surface dweller, and is not affected by the stability of a permanent burrow system. A. longa is the only deep burrower positively correlated with fine sand.

The number of species of earthworms is negatively correlated with coarse silt, which is not as effective as clay in maintaining burrows.

Multiple regression analysis shows that total numbers of earthworms is related positively to the clay content of the soil, but individual species: L. rubellus, A. longa, and A. chlorotica show a negative association; the clay content of the soil also affects the drainage,which may be the factor affecting earthworms, (and the N.C.B. have adjusted the soil texture on the 1 and 2 year old sites).

Table 3 Significant Pyhsical Environmental Factors in the Multiple Regression Equation for Numbers and Biomass of Earthworms per 0.5 m^2

Factor		No. Spp. worms	Tot. No. worms	Tot. Wt. worms	L.c. Tot. Wt.	L.r. Tot. No.	L.r. Tot. Wt.	A.l. Tot. No.	A.l. Tot. Wt.	A.c. Tot. No.	A.c. Tot. Wt.
Medium Sand	F		6.35							10.06	
	b		-1.70							0.58	
	SE		0.68							0.13	
Fine Sand	F						16.99	8.55			
	b						-0.24	0.99			
	SE						0.01	0.34			
Coarse Silt	F	5.65									
	b	-0.11									
	SE	0.05									
Clay	F		34.67			12.18			33.79	53.65	24.02
	b		2.11			-0.19			-0.28	-0.93	-0.15
	SE		0.36			0.01			0.04	0.13	0.03
pH	F	8.18									
	b	-0.98									
	SE	0.34									
Moisture	F	4.98			3.04						
	b	0.37			-0.80						
	SE	0.02			0.01						
Temp-erature	F				5.50		4.17				
	b				-0.34		0.26				
	SE				0.01		0.01				
Carbon	F		10.84					2.10		15.49	5.56
	b		5.98					-0.80		-2.66	-0.36
	SE		1.82					0.55		0.68	0.15
Nitrogen	F			11.61							
	b			-14.60							
	SE			4.28							
C : N Ratio	F					3.21					5.81
	b					-0.36					-0.24
	SE					0.02					0.10
% Soil Organic Matter	F	17.19					2.30				
	b	0.35					0.48				
	SE	0.08					0.03				
R^2 for Sig. Variables		0.22	0.05	0.02	0.05	0.04	0.16	0.03	0.09	0.22	0.21
Total R^2		0.94	0.96	0.97	0.73	0.89	0.76	0.80	0.83	0.87	0.72

4 ii (b) Acidity

There are more species of earthworms present on acid sites; this may be more of a function of age than pH. Also, within the range of pH found on the five sites, 4.60 to 6.15, earthworms are tolerent of acid conditions, (Laverack, 1961).

4 ii (c) Moisture and Temperature

There are more species of earthworms in the wetter soils. Moisture content of the five soil treatments is determined by two factors in the field: cultivation and age since disturbance, and vegetation cover. The 1 and 2 year old sites were very dry because they were relatively bare, and the 1 year old site was also cultivated several times during the summer, thus increasing water loss. Older sites had a substantial standing crop of vegetation, and a surface mat of dead plant litter, which would shade the soil and reduce evaporation; these soils were also cooler, and therefore earthworms would be active nearer to the surface. Dead litter, standing crop, and dominant species in the vegetation are therefore indicated as additional important variables in earthworm distribution.

4 ii (d) Carbon and Nitrogen

Multiple regression analysis shows that carbon levels had a significant positive correlation with total number of earthworms, and nitrogen levels had a negative relationship with total weight of earthworms. Numbers of L. rubellus and biomass of A. chlorotica were correlated negatively with C : N ratios, indicating an increase in these species as decomposition of dead vegetation proceeded.

4 iii Physical Factors influencing Fungal Activity (Table 4)

4 iii (a) Soil Texture

Total colonies of Absidia showed a significant correlation with medium sand. Mucor was negatively correlated with clay. Fungal

Table 4 Significant Physical Environmental Factors in the Multiple Regression Equation for Numbers of Fungal Colonies of Each Genera

Factor		Mucor	Absidia	Cladosporium	Verticillium
Medium Sand	F		3.30		
	b		-0.50		
	SE		0.27		
Fine Sand	F			5.91	
	b			0.49	
	SE			0.20	
Coarse Silt	F		6.16		3.32
	b		0.36		0.84
	SE		0.14		0.46
Clay	F	13.15			
	b	-0.19			
	SE	0.05			
Moisture	F		10.10		
	b		0.21		
	SE		0.07		
R^2 for Sig. Variables		0.16	0.17	0.13	0.06
Total R^2		0.58	0.80	0.58	0.41

Other physical variables included in the regression equation, but not significant were: coarse sand, medium silt, fine silt, pH, temperature, carbon, nitrogen, C : N ratio, percentage organic matter.

For list of all fungal genera cultured and identified from Biggin South Site see Appendix 3.

propagules tend not to be associated so frequently with the largest and smallest soil mineral particles. However, Cladosporium with fine sand, and Absidia and Verticillium with coarse silt, all showed a significant positive relationship, indicating that propagules of members of these genera are more often associated with the middle range of soil particle sizes. This accounts for the loss of some propagules in soil dilution plates; the propagules are associated with particles which, (apart from the finest ones), settle out from the suspending liquid. The negative relationship between Mucor and clay could be due to the lack of physical crevices on this small mineral particle, which are large enough to accommodate a spore.

4 iii (b) Moisture

Multiple regression analysis showed a positive relationship between Absidia and moisture. This species was the commonest mucoraceous fungus in this study and its growth in culture was more susceptible to drying out than other species which formed closer cushions of mycelium. It did not form exudation droplets to the same extent as other species and might therefore be more dependent on external moisture to provide surface moisture on the hyphae.

4 iv Relationships between Earthworms and Fungi

The number of species of earthworms and the total weight of earthworms per plot are correlated negatively with total Mucor colonies, (Table 5). Mucor probably relies on other agents, e.g. soil water for dispersal. Total Absidia colonies are positively correlated with number of species of earthworms, suggesting that earthworm activity may enhance the activity of this type of fungus; fragmentation of litter may well make more of the substrate accessible to the fungus, though it seems reasonable to suppose that such fragmentation would promote decomposition of vegetable debris by any fungus having appropriate enzyme resources.

Table 5 Significant Correlations between Earthworms and Fungi

	Correlation Coefficients	
	Mucor	Absidia
No. Species Earthworms	-0.387 P < 0.01	0.315 P < 0.05
Total Wt. Earthworms per Plot	-0.358 P < 0.02	-

Table 6 Significant Correlations between Numbers of Earthworms

	L. castaneus Total Wt.
	Correlation Coefficients
L. rubellus Total No.	0.461 P < 0.001
L. rubellus Total Wt.	0.486 P < 0.001
A. chlorotica Total No.	0.497 P < 0.001

Table 7 Significant Correlations between Genera of Fungi

	Correlation Coefficients	
	Mucor	Absidia
Absidia	-0.455 P < 0.001	-
Penicillium	-0.398 P < 0.01	0.674 P < 0.001

The nature of the association is therefore not clear, and it may be a case of both organisms responding to some other characteristic of the soil not measured in this study, but nevertheless may repay further study.

4 v Associations between Species of Earthworms

There were highly significant positive correlations between the biomass of L. castaneus and both number and biomass of L. rubellus, (Table 6), and also between biomass of L. castaneus and number of A. chlorotica. L. castaneus and L. rubellus are litter dwellers, (Gerard, 1964), so this indicates that surface conditions are suitable for both species. A. chlorotica occupies shallow burrows and surface litter, which again supports the idea of suitability of soil conditions near the surface, (Phillipson, 1967).

4 vi Associations between Genera of Fungi

Both Mucor and Absidia, and Mucor and Penicillium are negatively correlated, (Table 7), indicating different substrates. Absidia can break down gelatin and pectin, (Domsch and Gams, 1972), and Penicillium can attack a wide range of substances, (pectin, starch, tannin, cellulose, xylan, and many by-products); it can thus utilise more complex substances than those which Absidia can digest. Mucor substrates include some intermediate in complexity: chitin, pectin, urea, fatty acids, and hemicelluloses. However there is a highly significant correlation between Absidia (simplest substrates), and Penicillium (most complex substrates); it could be that Absidia is active in the same substrate material as Penicillium, by using a fraction of the breakdown products produced by the Penicillium. There is also the possibility of other factors, e.g. Penicillium species may be producing antibiotics, inhibiting Mucor, but which are tolerated by Absidia.

Table 8 Significant Correlations between the Two Methods of Culturing Fungi

Fungus	Correlation Coefficient
Absidia	0.436 P < 0.01
Cladosporium	0.440 P < 0.01
Verticillium	0.360 P < 0.02
Trichoderma	0.391 P < 0.01
Penicillium	0.718 P < 0.001

Table 9 Significant Correlations between Biomass and Numbers of Earthworms per 0.5 m^2 Plot

	Correlation Coefficients	
	A. longa Biomass	A. chlorotica Biomass
A. longa Number	0.768 P < 0.001	
A. chlorotica Number		0.843 P < 0.001

4 vii Comparison of the Two Fungal Culture Methods

Both methods used previously poured plates, and avoided the need for warm liquid agar at each plating out, as is required for the Warcup soil plates. It was thought that the crumb technique would favour growth from fragments of active mycelium adhering to the soil crumbs, and that the foil method would favour fungi forming large numbers of spores. Therefore in the analysis, the numbers of colonies of each genus from the two methods were added together, and this total colony figure used in calculations; this reduced bias from either of the two methods. To see how different the results from the two methods actually were, correlations between the two techniques for each genus were examined: they were significant for Absidia, Cladosporium, Verticillium, Trichoderma, and Penicillium. Only the correlation between the two methods for Mucor was insignificant, (Table 8). Thus in future work, only one method need be used. The crumb method takes longer to plate out, but colonies are easier to identify, because if the soil crumbs are small enough, only one colony grows from each crumb, and slow growing colonies are less likely to be over-grown.

4 viii Relationships between Biomass and Numbers of Earthworms

The results for four species of earthworms were used in the calculations; other species were considered too infrequent. The biomass per plot was correlated with numbers per plot for each of these four species. The correlation between biomass and number was significant for A. longa and A. chlorotica, but not for L. castaneus and L. rubellus, (Table 9). This indicates that in the case of the two Allolobophora species, one measurement would be sufficient. Counting is quicker than weighing, and from this the biomass could be found from an initial sample which were also weighed and used to construct a correlation. In the regression analyses, biomass proved significant more often than numbers of earthworms; this appears to be the measurement which indicates ecological activity most closely.

APPENDIX 1 SPECIES LISTS OF FLOWERING PLANTS

1 i Topsoil Reinstated in 1978 (1 year old site)

Ranunculus acris (L)	Meadow Buttercup
Sinapis arvensis (L)	Charlock
Raphanus raphanistrum (L)	Wild Raddish
Thaspi arvense (L)	Penny Cress
Capsella bursa-pastoris (L)	Shepherds Purse
Viola arvensis (Murr)	Field Pansy
Stellaria holostea (L)	Greater Stitchwort
Stellaria media (L)	Common Chickweed
Spergula arvensis (L)	Corn Spurry
Atriplex patula (L)	Common orache
Ulex europaeus (L)	Gorse
Trifolium pratense (L)	Red Clover
Trifolium repens (L)	White Clover
Trifolium dubium (Sibth)	Lesser Yellow Trefoil
Polygonium aviculare (L)	Knot Grass
Rumex acetosella agg (Meisn)	Sheeps sorrel
Rumex obtusifolius (L)	Broad leaved dock
Myosotis arvensis (L)	Common forget-me-not
Galeopsis speciosa (Mill)	Large flowered hemp nettle
Plantago lanceolata (L)	Ribwort plantain
Senecio vulgaris (L)	Groundsel
Tussilago farfara (L)	Coltsfoot
Bellis perennis (L)	Daisy
Matricaria inodora (L)	Scentless mayweed
Achillea millefolium (L)	Yarrow
Cirsium arvense (L)	Creeping thistle
Cirsium vulgare (Savi)	Spear thistle
Hieracium pilosella (L)	Hawkweed ?
Lolium perenne (L)	Perennial rye grass

1 i Topsoil Reinstated in 1978 (1 year old site) contd.

Poa trivialis (L)	Rough stalked meadow grass
Poa annua (L)	Annual meadow grass
Cynosurus cristatus (L)	Crested dog's tail
Bromus arvensis (L)	Field brome
Agropyron repens (L)	Couch
Anthoxanthum odoratum (L)	Sweet vernal grass
Avena fatua (L)	Oats

1 ii Undisturbed Site (100 years old)

Ranunculus repens (L)	Creeping buttercup
Cerastium vulgatum (L)	Mouse ear chickweed
Trifolium pratense (L)	Red clover
Trifolium arvense (L)	Haresfoot trefoil
Trifolium repens (L)	White clover
Myosotis arvensis (L)	Common forget-me-not
Plantago lanceolata (L)	Ribwort plantain
Taxacarum officinale agg (Dahlst)	Dandelion
Senecio jacobaea (L)	Ragwort
Bellis perennis (L)	Daisy
Dactylis glomerata (L)	Cocksfoot
Cynosurus cristatus (L)	Crested dogs tail
Poa pratensis (L)	Meadow grass
Lolium perenne (L)	Perennial rye grass
Bromus mollis agg (L)	Soft brome grass
Alopecurus geniculatus (L)	Marsh fox tail
Phleum pratense (L)	Timothy

1 iii Topsoil Reinstated in 1958 (11 year old site)

Ranunculus repens (L)	Creeping buttercup
Cerastium vulgatum (L)	Common mouse ear chickweed
Ulex europaeus (L)	Gorse
Vicia cracca (L)	Tufted vetch
Vicia sepum (L)	Bush vetch
Lathyrus pratensis (L)	Meadow pea
Lotus corniculatus (L)	Birds foot trefoil
Trifolium repens (L)	White clover
Lathyrus montanus (L)	Bitter vetch
Veronica chamaedrys (L)	Germander speedwell
Rumex acetosella agg (Meisn)	Sheeps sorrel
Plantago lanceolata (L)	Ribwort plantain
Cirsium arvense (L)	Creeping thistle
Luzula campestris (L)	Field wood rush
Agropyron repens (L)	Couch
Anthoxanthum odoratum (L)	Sweet vernal grass
Cynosurus cristatus (L)	Crested dogs tail
Holcus lanatus (L)	Yorkshire fog
Dactylis glomerata (L)	Cocksfoot
Lolium perenne (L)	Perennial rye grass
Poa trivialis (L)	Rough stalked meadow grass
Festuca ovina agg (L)	Sheeps fescue
Deschampsia caespitosa (L)	Tufted hair grass
Festuca rubra (L)	Creeping fescue

APPENDIX 2 ORIGINAL DATA

2 i Soil Texture Analysis

% Total Mineral Particles in Each Fraction

Soil Treatment (Age since Disturbance) years	Coarse Sand 2.4-0.6 mm	Medium Sand 0.6-0.21 mm	Fine Sand 0.21 - 0.075 mm	Coarse Silt 0.06 - 0.02 mm	Medium Silt 0.02 - 0.006 mm	Fine Silt 0.006-0.002 mm	Clay 0.002 mm
1	5	10	24	25	9	7	20
2	8	9	25	19	13	7	19
3	6	10	21	17	9	9	28
11	6	6	26	21	11	14	16
100	3	10	39	22	6	5	15

2 ii Soil Temperature °C

Date Collected (July 79)	Soil Treatment Age Since Disturbance (years) 1	2	3	11	100
7	13.0	12.5	12.0	10.5	12.0
12	16.5	15.0	14.0	13.5	16.0
14	16.5	13.0	13.0	11.5	12.5
19	16.5	16.0	18.5	16.0	16.5
20	17.0	18.0	18.0	15.0	16.0
21	17.0	17.0	17.0	14.5	15.0
26	10.5	13.0	11.5	11.0	12.0
27	12.5	13.0	11.0	12.0	12.0
28	15.0	14.0	13.5	13.0	13.0
29	15.0	13.5	14.0	13.0	12.5

2 iii % Moisture of Soil Samples

Date Collected (July 79)	Soil Treatment Age Since Disturbance (years) 1	2	3	11	100
7	15.07	37.06	27.72	46.78	16.50
12	19.80	20.37	28.40	23.81	22.82
14	19.30	20.62	33.68	27.60	27.17
19	14.80	17.97	17.05	34.90	18.18
20	5.47	13.41	17.80	31.16	18.03
21	11.61	13.96	13.45	22.78	15.23
26	12.83	17.72	20.28	30.01	24.78
27	13.73	16.37	27.72	36.25	23.63
28	0.88	20.77	22.07	29.82	15.52
29	14.28	18.62	18.97	19.58	16.44

2 iv Soil pH (mean of 2 readings)

Date Collected (July 79)	Soil Treatment Age Since Disturbance (years) 1	2	3	11	100
7	5.70	5.95	5.05	6.15	5.30
12	6.00	5.55	5.05	5.55	5.25
14	5.55	5.80	5.10	5.60	5.60
19	5.75	6.15	5.00	5.20	5.50
20	5.25	5.60	4.90	5.60	5.35
21	5.45	5.95	5.20	5.75	5.70
26	5.65	5.85	4.80	5.25	5.75
27	5.55	5.55	5.05	5.70	5.35
28	5.30	6.00	5.15	5.80	5.55
29	5.30	5.75	4.65	5.25	5.45

2 v % Carbon

Date Collected (July 79)	Soil Treatment Age Since Disturbance (years) 1	2	3	11	100
27	2.03	1.62	3.66	3.74	3.97
28	1.75	1.79	1.52	3.35	3.14
29	2.53	2.34	1.64	3.76	3.84
Average	2.10	1.92	2.27	3.62	3.65

2 vi % Nitrogen

Date Collected (July 79)	Soil Treatment Age Since Disturbance (years) 1	2	3	11	100
27	0.154	0.133	0.280	0.266	0.315
28	0.126	0.175	0.126	0.238	0.280
29	0.210	0.196	0.154	0.364	0.308
Average	0.163	0.156	0.187	0.289	0.301

2 vii Carbon : Nitrogen Ratio

Date Collected (July 79)	Soil Treatment Age Since Disturbance (years) 1	2	3	11	100
27	13.182	12.180	13.071	14.060	12.603
28	13.889	10.229	12.063	14.076	11.214
29	12.048	11.939	10.649	10.330	12.468
Average	13.040	11.449	11.928	12.822	12.095

2 viii % Soil Organic Matter

Date Collected (July 79)	Soil Treatment Age Since Disturbance (years) 1	2	3	11	100
27	3.500	2.793	6.310	6.448	6.845
28	3.017	3.086	2.621	5.776	5.414
29	4.362	4.034	2.828	6.483	6.621
Average	3.626	3.304	3.920	6.236	6.293

2 ix % Moisture Content of Earthworms

Species of Earthworms	Sample Size	Wet Wt. of Preserved Earthworms (g)	Dry Wt. of Earthworms (g)	Wt. Water (g)	% Moisture Content
O. cyaneum	5	1.89	0.57	1.32	69.84
L. terrestris	3	8.10	1.60	6.50	80.25
A. longa	5	7.31	1.57	5.74	78.52
L. castaneus	5	0.84	0.18	0.66	78.57
L. rubellus	5	2.76	0.64	2.12	76.81
A. chlorotica	5	0.96	0.57	0.39	40.63

2 x Earthworms Collected by Formalin Sampling from the Undisturbed Site

Date Collected (July 79)		L.c.	L.r.	A.l.	O.c.	A.c.	Total worms	No. Species
7	No.	6		10		5	21	3
	Wt.	0.91		8.00		0.56	9.47	
12	No.			2	2	7	11	3
	Wt.			1.72	2.10	0.12	4.84	
14	No.	2		9	2	15	28	4
	Wt.	0.50		5.88	1.34	1.58	9.30	
19	No.			5		3	8	2
	Wt.			3.36		0.53	3.89	
20	No.			2		1	3	2
	Wt.			1.77		0.22	1.99	
21	No.	1				1	2	2
	Wt.	0.36				0.26	0.62	
26	No.					2	2	1
	Wt.					0.50	0.50	
27	No.	4					4	1
	Wt.	0.18					0.18	
28	No.		8			12	20	2
	Wt.		2.12			1.56	3.68	
29	No.	1		2		7	10	3
	Wt.	0.22		0.65		0.92	1.79	

Key: L.c. = L.castaneus, L.r. = L. rubellus, A.l. = A. longa,

O.c. = O. cyaneum, A.c. = A. chlorotica.

2 xi Earthworms Collected by Formalin Sampling from the 1958 Site

(Numbers and weights per 0.5 m^2)

Date Collected (July 79)		L.c.	L.r.	L.t.	A.l.	O.c.	A.c.	Tot. worms	No. Species
7	No.	4	2	3	4	1	9	23	6
	Wt.	0.47	1.02	5.06	5.34	2.25	2.14	16.28	
12	No.		4		5	1	8	18	4
	Wt.		1.00		6.39	0.83	2.15	10.91	
14	No.	7	6	3	1	1	11	29	6
	Wt.	0.51	1.41	8.11	1.45	0.55	1.87	13.90	
19	No.				2		2	4	2
	Wt.				2.97		0.25	3.22	
20	No.		2		6		3	11	3
	Wt.		0.81		9.19		0.58	10.58	
21	No.	4						4	1
	Wt.	0.31						0.31	
26	No.	7	1		1	2	15	26	5
	Wt.	0.96	0.71		1.42	2.31	2.36	7.77	
27	No.	3	7		1	3	6	20	5
	Wt.	0.36	3.24		1.97	0.77	0.65	6.99	
28	No.	6	1			1	6	14	4
	Wt.	0.61	0.69			0.32	0.67	2.29	
29	No.	3	1		3		2	9	4
	Wt.	0.41	0.59		2.11		0.29	3.40	

Key: L.c. = L. castaneus, L.r. = L. rubellus, L.t. = L. terrestris,

A.l. = A. longa, O.c. = O. cyaneum, A.c. = A. chlorotica.

2 xii Earthworms Collected by Formalin Sampling from the Heaped Soil Site

(Numbers and weights per 0.5 m^2)

Date Collected (July 79)		L.c.	L.r.	L.t.	A.l.	D.m.	A.c.	Tot. worms	No. Species
7	No.	14	62		3		13	92	4
	Wt.	1.95	17.30		3.49		2.14	24.88	
12	No.	9	29		7		3	48	4
	Wt.	0.55	8.31		1.76		0.40	11.02	
14	No.	5	34		12		9	66	4
	Wt.	0.17	5.22		5.57		1.35	12.31	
19	No.	3	12		2		3	20	4
	Wt.	0.11	2.91		0.35		0.23	3.60	
20	No.	11	11		3		4	29	4
	Wt.	1.14	5.91		0.44		0.29	7.78	
21	No.	6	3	1			2	12	4
	Wt.	0.76	1.63	1.43			0.02	3.89	
26	No.	11	15		3	2	11	42	5
	Wt.	1.47	6.33		1.26	0.12	0.77	9.95	
27	No.	6	12		1		1	20	4
	Wt.	0.75	5.63		0.86		0.17	7.41	
28	No.	5	25	1	2		1	34	5
	Wt.	0.53	10.58	6.89	3.13		0.05	21.18	
29	No.	13	41		2		7	63	4
	Wt.	1.85	12.24		0.21		0.73	15.03	

Key: L.c. = L. castaneus, L.r. = L. rubellus, L.t. = L. terrestris,

A.l. = A. longa, D.m. = D. mammalis, A.c. = A.chlorotica.

2 xiii Earthworms Collected by Formalin Sampling from the 2 Year Old Site

(Numbers and weights per 0.5 m^2)

Date Collected (July 79)		A.l.	A.c.	Tot. worms	No. Species
7	No.	1	1	2	2
	Wt.	2.06	0.12	2.18	
14	No.		6	6	1
	Wt.		2.29	2.29	
28	No.		10	10	1
	Wt.		3.73	3.73	
29	No.		2	2	1
	Wt.		0.76	0.76	

Key: A.l. = A. Longa, A.c. = A. chlorotica.

N.B. There were no earthworms collected from the 1 year old site.

2 xiv Fungal Colonies Cultured by Soil Crumb Method from Samples Collected on Undisturbed Site (100 years old)

Fungal Genera	Date Collected (July 1979)									
	7	12	14	19	20	21	26	27	28	29
Mucor	3	5		3	2	2	5	2	6	4
Rhizopus					1	1		2		
Absidia			4		1					
Mortierella			1							
Zygorynchus										
Thamnidium										
Cunninghamella										
Papulaspora				1						
Scropulariopsis										
Aureobasidium	1					6				
Cladosporium	3	6	10	6	17	1	6	3	3	4
Botrytis										
Acremonium										
Verticillium						1				
Trichoderma			1	4	3			1		3
Paecilomyces										
Penicillium	2	1	1			1			1	1
Fusarium										
Dendryphion										
Pink Sterile Mycelium 'Anther' Fungus	1	1	1				2			1
Grey/Black Sterile Mycelium	2	1	1							

2 xv Fungal Colonies Cultured by Foil Spread Method from Soil Samples Collected on Undisturbed Site (100 years old)

Fungal Genera	Date Collected (July 1979)									
	7	12	14	19	20	21	26	27	28	29
Mucor	1				2	2	2		1	2
Rhizopus								4		
Absidia		1	6	2				7		
Mortierella					1					
Zygorynchus										
Thamnidium										
Cunninghamella										
Papulaspora										
Scropulariopsis						1				
Aureobasidium						3		1		
Cladosporium	8	5	30	5	9	3	10	1	5	3
Botrytis				1	1		1			
Acremonium								1		
Verticillium		2		2	2	2				
Trichoderma				4						1
Paecilomyces										
Penicillium	12	31	20	5	13	1		7		1
Fusarium										
Dendryphion										
Pink Sterile Mycelium 'Anther' Fungus	1			2	1	2			1	
Grey/Black Sterile Mycelium	2	2	1		1					

2 xvi Fungal Colonies Cultured by Soil Crumb Method from Soil Samples Collected on 1958 Site (11 years old)

Fungal Genera	Date Collected (July 1979)									
	7	12	14	19	20	21	26	27	28	29
Mucor					2	3	2	1	1	3
Rhizopus										1
Absidia	11	1	12	7	1	4		7		3
Mortierella										
Zygorynchus				1						
Thamnidium										
Cunninghamella										
Papulaspora										
Scropulariopsis		5								
Aureobasidium						2				
Cladosporium	1		4		1	3	5	2		
Botrytis										
Acremonium										
Verticillium	2	2		1	3		2	1		8
Trichoderma	2	4			4		4	2		
Paecilomyces										
Penicillium	10		10	20	2	1	6	2	1	2
Fusarium										
Dendryphion										
Pink Sterile Mycelium 'Anther' Fungus					3		2		3	
Grey/Black Sterile Mycelium									1	

2 xvii Fungal Colonies Cultured by Foil Spread Method from Soil Samples Collected on 1958 Site (11 years old)

Fungal Genera	Date Collected (July 1979)									
	7	12	14	19	20	21	26	27	28	29
Mucor					1	1	2			
Rhizopus										
Absidia	6	1	6	6		3			5	
Mortierella		1								
Zygorynchus										
Thamnidium	1					1				
Cunninghamella										
Papulaspora										
Scropulariopsis										
Aureobasidium										
Cladosporium	3		30		9		3	1	3	
Botrytis						1				
Acremonium										
Verticillium		1				3		2		
Trichoderma	2	2			1	1	1	2		1
Paecilomyces		1								
Penicillium	69	9	30	60		2	3	5	18	5
Fusarium										
Dendryphion										
Pink Sterile Mycelium	1				1					
'Anther' Fungus							1			
Grey/Black Sterile Mycelium										

2 xviii Fungal Colonies Cultured by Soil Crumb Method from Soil Samples Collected on Heaped Soil Site (3 years old)

Fungal Genera	Date Collected (July 1979)									
	7	12	14	19	20	21	26	27	28	29
Mucor	1	1			1	1	1			1
Rhizopus					1	1	1			
Absidia	2	3		1	1	1				
Mortierella	2				1			1		
Zygorynchus			1		1					
Thamnidium										
Cunninghamella										
Papulaspora				1						
Scropulariopsis										
Aureobasidium										
Cladosporium	3		2	1	2		4	1	3	6
Botrytis										
Acremonium								1		
Verticillium	1							2		
Trichoderma	5			2		1		2		
Paecilomyces										
Penicillium		3	11	7	2	5	5	3	2	
Fusarium									2	
Dendryphion										
Pink Sterile Mycelium		1			3	1	4		3	
'Anther' Fungus								2		
Grey/Black Sterile Mycelium									1	

2 xix Fungal Colonies Cultured by Foil Spread Method from Soil Samples Collected on Heaped Soil Site (3 years old)

Fungal Genera	Date Collected (July 1979)									
	7	12	14	19	20	21	26	27	28	29
Mucor	2	2			1	1	1		1	
Rhizopus					1			2		13
Absidia	1	2	7	2						
Mortierella					1					
Zygorynchus										
Thamnidium										
Cunninghamella										
Papulaspora										
Scropulariopsis										
Aureobasidium	1	1					1			
Cladosporium			3		4	2	8	3		3
Botrytis										
Acremonium										
Verticillium				1			1	1	7	
Trichoderma	2	1		1	1		1			
Paecilomyces								1		
Penicillium	1	7	70	13	8	6	1	1	12	
Fusarium							1			
Dendryphion								2		
Pink Sterile Mycelium 'Anther' Fungus		1			1		1			
Grey/Black Sterile Mycelium		1		1				2		

2 xx Fungal Colonies Cultured by Soil Crumb Method from Soil Samples Collected on Site with Topsoil Reinstated 1977 (2 years old)

Fungal Genera	Date Collected (July 1979)									
	7	12	14	19	20	21	26	27	28	29
Mucor	1	4		2	2	2	5	1	1	7
Rhizopus							1			
Absidia	1		3	1			2	1		
Mortierella	2		1	3						
Zygorynchus										
Thamnidium										
Cunninghamella										
Papulaspora										
Scropulariopsis		1								
Aureobasidium	1				3	1				
Cladosporium		3	4	1	5	10	3	2	3	
Botrytis										
Acremonium										
Verticillium		1	1		1				1	2
Trichoderma			3		2	3	1		3	1
Paecilomyces										
Penicillium	8	3	6	17	1			2	3	3
Fusarium										
Dendryphion										
Pink Sterile Mycelium 'Anther' Fungus		3	1		2		6	1		1
Grey/Black Sterile Mycelium			1							

2 xxi Fungal Colonies Cultured by Foil Spread Method from Soil Samples Collected on Site with Topsoil Reinstated 1977 (2 years old)

Fungal Genera	Date Collected (July 1979)									
	7	12	14	19	20	21	26	27	28	29
Mucor			3		3		2		1	
Rhizopus								1		4
Absidia	6		3	1		1				1
Mortierella								2		
Zygorynchus										
Thamnidium										
Cunninghamella		1								
Papulaspora										
Scropulariopsis										
Aureobasidium	1					2		1		
Cladosporium		1		1	36	8	7	3	4	4
Botrytis					3					
Acremonium									3	1
Verticillium	1					2	20	1	2	
Trichoderma		3		1				1		
Paecilomyces										
Penicillium	21	2	25	31	2			6		1
Fusarium										
Dendryphion										
Pink Sterile Mycelium										
'Anther' Fungus							2			
Grey/Black Sterile Mycelium		2		1						

2 xxii Fungal Colonies Cultured by Soil Crumb Method from Soil Samples Collected on Site with Topsoil Reinstated 1978 (1year old)

Fungal Genera	Date Collected (July 1979)									
	7	12	14	19	20	21	26	27	28	29
Mucor		3	2	2		2	5		1	4
Rhizopus								1	1	
Absidia	1		3				1		2	
Mortierella	2									
Zygorynchus										
Thamnidium										
Cunninghamella										
Papulaspora										
Scropulariopsis										
Aureobasidium										
Cladosporium	1			1	3		4			
Botrytis	1									
Acremonium										
Verticillium	1	4		2		4		1	1	
Trichoderma			1		1		3	4		1
Paecilomyces										
Penicillium	12	8	4	19	2	5	1	3	4	4
Fusarium										
Dendryphion										
Pink Sterile Mycelium 'Anther' Fungus					2	1				5
Grey/Black Sterile Mycelium		1			1					

2 xxiii Fungal Colonies Cultured by Foil Spread Method from Soil Samples Collected on Site with Topsoil Reinstated 1978 (1 year old)

Fungal Genera	Date Collected (July 1979)									
	7	12	14	19	20	21	26	27	28	29
Mucor	2	1			1	1	1		2	1
Rhizopus	1							1		
Absidia	1	3	5	6				1		2
Zygorynchus						2				
Thamnidium										
Cunninghamella										
Papulaspora										
Scropulariopsis										
Aureobasidium		1								
Cladosporium					1	3	9	1		1
Botrytis			1		2					
Acremonium										
Verticillium	1	49			1	30	1			
Trichoderma				4			7	4		2
Paecilomyces										
Penicillium	6	15	18	53	2		8	1	1	20
Fusarium										
Dendryphion										
Pink Sterile Mycelium										3
'Anther' Fungus					1					
Grey/Black Sterile Mycelium	1		2							

APPENDIX 3

List of Fungal Genera Identified, with Authorities

Absidia (van Tieghem)

Rhizopus (Ehrenberg)

Mucor (Micheli)

Zygorhynchus (Vuillemin)

Thamnidium (Link)

Mortierella (Coemans)

Cunninghamella (Matruchot)

Papulaspora (Preuss)

Scopulariopsis (Bainier)

Aureobasidium (Viala et Boyer)

Cladosporium (Link)

Botrytis (Micheli)

Acremonium (Link)

Verticillium (Nees)

Trichoderma (Persoon)

Paecilomyces (Szilvinyi)

Penicillium (Link)

Fusarium (Link)

Dendryphion (Wallroth)

SUMMARY

Introduction

Soil disturbance is associated with many of man's activities, but little is known of its effect on soil organisms and their re-establishment; they are essential for the natural cycling of plant nutrients. The object of this piece of work was to study the changes in earthworm and fungal activity associated with soil disturbance.

Literature Review

1. Previous studies have found that fungi are not affected by passage through the earthworm gut, and there is more microbial activity in casts than in soil.

2. The comminuting effect of soil fauna, especially earthworms, facilitates microbial decay, and provides nutrients necessary for the growth of some fungi.

3. Microbial activity increases palatability of litter for earthworms. Therefore earthworms and micro-organisms are mutually beneficial.

4. Soil disturbance increases drying out and temperature fluctuations in the soil. Decrease in soil organic matter, associated with harvesting crops, and the use of inorganic fertilisers, reduces earthworm food.

5. Migration rates of earthworms in re-colonisation studies are slow, and this retards the rate of soil improvement.

6. Soil disturbance, and the addition of organic matter reduces the fungistatic effect of the soil, and increases fungal activity.

7. Earthworms promote crumb formation, and improve the soil structure, especially in pasture soils. Fungal hyphae and bacterial gums act as binding agents between the soil particles.

8. Earthworms improve aeration of the soil to a limited extent, and facilitate drainage, root growth, and incorporation of surface litter into the mineral horizons of the soil.

Methods

1. The aim was to study active fungi; several direct and indirect techniqies are compared.

2. The control of bacterial contamination is discussed.

3. Methods of sampling for earthworms are assessed, and the seasonal activity of earthworms noted.

4. A method of preserving earthworms, and adjustments for gut content and weight loss in preservative are given.

5. The field sampling techniques for earthworms, soil temperature, soil cores, and the pre-treatment of soil are described.

6. The N.C.B. opencast mining site at Biggin South is described, giving details of the previous management of the five experimental areas used, which vary in age from 1 to 100 years since disturbance.

7. Fungal activity of the five sites is estimated using two soil culture techniques: soil crumbs and a foil spread. The medium used throughout is potato/sucrose agar. Methods employed to identify fungal genera are described. The number of colonies of each genera per plate were counted.

8. The earthworms from each sample were identified, counted, and weighed.

9. Soil analyses included texture, nitrogen, carbon, pH, and moisture.

Results and Discussion

1. The distribution of 4 species of earthworms, and 2 genera of fungi was affected by the time interval since disturbance.

2. Physical factors influencing distribution of earthworms include soil texture, acidity, moisture and temperature, and carbon & nitrogen levels.

3. Physical factors influencing fungal activity include soil texture and moisture.

4. A negative correlation was found between both number of species of earthworms and total weight of earthworms with number of Mucor colonies, but number of Absidia colonies and number of earthworm species showed a positive association.

5. Two litter dwelling species of earthworms were closely correlated; also a shallow burrowing species and a litter dweller were associated. This may indicate favourable surface conditions.

6. Two negative correlations were found between genera of fungi, indicating different substrate preference. One positive association indicates the use of by-products by one genus, or tolerence of antibiotics produced.

7. Bias from either of the fungal culture methods was reduced by summing results from the two methods. Correlations between the results from the two methods were significant, therefore one method only would be sufficient.

8. In the case of the two most common species of Allolobophora found, one measurement, either biomass or number per sample would be sufficient, as these two parameters were closely correlated.

ACKNOWLEDGEMENTS

Acknowledgements are gratefully made to Mr. P.A. Wood, and Mr. R. Bone of the National Coal Board, for allowing sampling on their site, and for providing background information on request.

I would also like to thank Dr. G.H. Banbury, my supervisor, for his valuable assistance with the fungal identification and activity study, and Dr. V. Standen, for her advice on earthworm population studies and computer analyses.

I am also most grateful to Dr. M. Alexander and his technical staff in the Department of Geography for providing facilities for soil analysis, and to the academic and technical staff of the University for their help and support.

BIBLIOGRAPHY

Anstett, M.A. (1951), in Burgess, A. and Raw, F. (1967), Soil Biology. Academic Press Lond. and N.Y.

Atlavinyte, O. and J. Dačiulyte, (1969). The effect of earthworms on the accumulation of vitamin B in soil. Pedobiologia, 9, 165-70.

Barley, K.P., (1961). Adv. Agron. 13, 249-268.

Barley, K.P. and A.C. Jennings, (1959). Aust. J. Agric. Res. 10, 364-70.

Baweja, K.D., (1939). Studies of the soil fauna with special reference to the recolonisation of sterilised soil. Journal of Animal Ecology, 8, 120-61.

Bouché, M.B., (1969). in Edwards, C.A. and J.R. lofty, (1977), Biology of Earthworms, 2nd edition, Chapman & Hall, Lond.

British Standards Institution. Methods of testing soils for civil engineering purposes BS 1377 : 1967. B.S.I. 2 Park St., Lond. W.1.

Brocks, Brown, and Handley, (1963). in Soil Biology, Burgess, A. and F. Raw, (Eds.) (1967). Academic Press, Lond. & N.Y.

Brown, J.C., (1958). in Burgess, A. and F. Raw, (Eds), (1967), Soil Biology. Academic Press, Lond. and N.Y.

Bruel, W.E. van der, (1964). in Edwards, C.A. and J.R. Lofty, (1977), Biology of Earthworms, 2nd edition. Chapman & Hall, Lond.

Chesters, C.G.C., (1940). Trans Br. mycol. Soc., 24, 352-55.

Cholodny, N., (1930). in Burgess, A. and Raw, F., (1967), Soil Biology, Chapter 3. Academic Press, Lond. and N.Y.

Domsch, K.H. and W. Gams, (1972). Fungi in Agricultural Soils. Longman, Lond.

Dunger, W., (1969). Annual Succession. Pedobiologia, 9, 366-71.

Eckert, J.W. and P.H. Tsao, (1962). Phytopathology, 52, 771-77.

Edwards, C.A. and C.W. Heath, (1963). in Doesken, J. and J. van der Drift, (Eds), (1963). North Holland, Amsterdam, pp 76-84.

Edwards, C.A. and J.R. Lofty, (1969). Effects of cultivation on earthworm populations. Rep. Rothamsted exp. Stn. for 1968, 247-8.

Edwards, C.A. and J.R. Lofty, (1976). in Edwards, C.A. and J.R. Lofty, (Eds.), (1977). Biology of Earthworms 2nd edition, Chapman & Hall, Lond.

Edwards, C.A. and J.R. Lofty, (1975). The invertebrate fauna of the Park grass plots. Rep. Rothamstead exp. Stn. for 1974, Pt. 2 pp 133-54.

Edwards, C.A. and J.R. Lofty, (1977). Biology of Earthworms 2nd edition. Chapman & Hall, Lond.

Evans, A.C., (1948). Ann. appl. Biol. 35, 1-13.

Evans, A.C. and W.J.McL. Guild, (1947). Studies on the relationships between earthworms and soil fertility, I Biological studies in the field. Ann. appl. Biol. 34, 307-30.

Evans, A.C. and W.J.McL. Guild, (1948). Studies on the relationships between earthworms and soil fertility, V Field populations. Ann. appl. Biol. 35, 485-93.

Garrett, S.D., (1955). Trans. Br. mycol. Soc., 38, 1 - 9.

Geography, Dept. of, (1978). Fundamentals of Soil Science Laboratory Handbook. University of Durham.

Gerard, B.M., (1964). Synopses of British Fauna (6) Lumbricidae. Linn. Soc. Lond. 55pp.

Gerard, B.M., (1967). Factors affecting earthworms in pastures. J. Anim. Ecol., 36, 235-52.

Ghilarov, M.S., (1963). On the interrelations between soil dwelling invertebrates and soil microorganisms. In Soil Organisms, Doeksen, J. and J. van der Drift, (Eds.) North Holland Publishing Co., Amsterdam, pp 255-9.

Graff, O., (1953). in Edwards, C.A. and J.R. Lofty, (1977). Biology of Earthworms 2nd edition. Chapman & Hall, Lond.

Griffiths, E. and Siddiqi, (1961). in Burgess, A. and F. Raw, (Eds.), (1967), Chapter 3, Soil Biology. Academic Press, Lond. & N.Y.

Guild, W.J.McL., (1955). In Kevan, D.K.McE., (Ed.) Soil Zoology pp 83-91. Butterworths, Lond.

Hamblyn, C.J. and A.R. Dingwall, (1945). Earthworms. N.Z. Jl. Agric. 71, 55-8.

Hine, R.B., (1962). Phytopathology, 52, 736.

Hoeksema, K.J., A. Jongerius, and K. van der Meer, (1956). In Burgess, A. and F. Raw, (Eds.), (1967), Soil Biology pp259-322. Academic Press, Lond. & N.Y.

Hoffman, J.A. and L.H. Purdy, (1964). Germination of Dwarf Bunt (Tilletia controversa) teliospores after ingestion by earthworms. Phytopathology, 54, 878-9.

Hopp, H., (1946). Earthworm fight soil erosion too. Soil Conserv., 11, 252-4.

Hutchinson, S.A. and M. Kamel, (1956). The effect of earthworms on the dispersal of soil fungi. J. Soil Sci., 7 (2) 213-8.

Jensen, H.L., (1931). Soil Sci., 31, 123-58.

Khambata, S.R. and Bhatt, J.V., (1957). In Edwards, C.A. and J.R. Lofty, (1977), Biology of Earthworms, 2nd edition. Chapman & Hall, Lond.

Kozlovskaya, L.S. and E.N. Zhdannikova, (1961). In Edwards, C.A. and Lofty, (1977), Biology of Earthworms 2nd edition. Chapman & Hall, Lond.

Kubiena, W.L., (1953). The Soils of Europe. Murby, Lond.

Ladell, W.R.S., (1936). A new apparatus for separating insects and other arthorpods from the soil. Ann. appl. Biol., 23, 862-79.

Laverack, M.S., (1961). In Burgess, A. and F. Raw, (Eds.), (1967), Soil Biology, pp 259-322. Academic Press, Lond. & N.Y.

MacWithey, H.S., (1957). In Burgess, A. and F. Raw, (Eds.), (1967), Soil Biology. Academic Press, Lond & N.Y.

Martin, J.P., (1950). Soil Sci., 69, 215-232.

Mason, C.F., (1977). Studies in Biology No. 74, Decomposition, p 40. Edward Arnold, Lond.

Mueller, K.E. and Durrell, L.W., (1957). Phytopathology, 47, 243.

Nelson, J.M. and J.E. Satchell, (1962). In Murphy, P.W., (Ed.), Progress in Soil Zoology, pp 294-99. Butterworths, Lond.

Pady, S.M., C.L. Kramer and V.K. Pathak, (1960-. Mycologia, 52, 347-50.

Parle, J.N., (1963a). Micro-organisms in intestines of earthworms. J. Gen. Microbiol., 13, 1-13.

Parle, J.N., (1963b). A microbiological study of earthworm casts. J. Gen.Microbiol., 13, 13-23.

Phillipson, J., P. Abel and S.J.R. Woodell, (1976). Earthworms and the factors governing their distribution in an English beechwood. Pedobiologia, 16, 258-85.

Raw, F., (1959). Estimating earthworm populations using formalin. Nature, 184, 1661-2.

Raw, F., (1960). Earthworm population studies: a comparison of sampling methods. Nature, 187, 257.

Raw, F., (1962). Studies of earthworm populations in orchards: I Leaf burial in apple orchards. Ann appl. Biol., 50, 389-404.

Reynolds, J.W., (1973). In Edwards, C.A. and J.R. Lofty, (1977), Biology of Earthworms, 2nd edition. Chapman & Hall, Lond.

Rhee, J.A. van, (1969). Inoculation of earthworms in a newly-drained polder. Pedobiologia, 9, 128-32.

Rossi, G.M., (1928). In Burgess, A. and F. Raw, (Eds.), (1967). Soil Biology. Academic Press, Lond. & N.Y.

Satchell, J.E., (1967). Lumbricidae. In Burgess, A. and F. Raw, (Eds) Soil Biology, pp259-322. Academic Press, Lond. & N.Y.

Satchell, J.E. and D.G. Lowe, (1967). In Graff, O. and J.E. Satchell, (Eds.), Progress in Soil Biology. Vieweg, Brunswick.

Satchell, J.E., (1969). Studies on methodical and taxonomical questions. Pedobiologia, 9, 20-5.

Satchell, J.E., (1970). Measuring population and energy flow in earthworms. In Phillipson, J., (Ed.), Methods of Study in Soil Ecology UNESCO.

Schmitthenner, A.F., (1962). Phytopathology, 52, 1133-8.

Slater, C.S. and H. Hopp, (1947). Proc. Soil Sci. Soc. Am., 12, 508-11.

Smith and Dawson, (1944). Soil Sci., 58, 467-71.

Svendsen, J.A., (1955). Nature, Lond., 175, 864.

Swaby, J.R., (1950). J. Soil Sci., 1, 195-7.

Tandy, C.R.V., (1973). In Lovejoy, D., (Ed.), Land Use and Landscape Planning, pp 199-233. Leonard Hill Books, Bucks.

Waid, J.S., (1960). The growth of fungi in soil. In Parkinson, D. and J.S. Waid, (Eds.), The Ecology of Soil Fungi: An International Symposium. Liverpool U.P.

Waid, J.S. and M.J. Woodman. (1957). In Burgess, A. and F. Raw,)Eds.), (1967), Soil Biology, Ch. 3. Academic Press, Lond. & N.Y.

Wakeman, S.A., (1922). J. Bact., 7, 339-41.

Warcup, J.H., (1950). Nature, Lond., 166, 117.

Warcup, J.H., (1955). Trans. Br. mycol. Soc., 38, 298-301.

Warcup, J.H., (1960). In Parkinson, D. and J.S. Waid, The Ecology of Soil Fungi, pp 3-21. Liverpool U.P.

Warcup, J.H., (1967). Fungi in soil. In Burgess, A. and F. Raw, (Eds.) Soil Biology. Academic Press, Lond. & N.Y.

Zhdannikova, (1961). In Edwards, C.A. and J.R. Lofty, (1977). Biology of Earthworms 2nd edition. Chapman & Hall, Lond.

Zicsi, A., (1958). In Edwards, C.A. and J.R. Lofty, (Eds.), (1977), Biology of Earthworms, 2nd edition. Chapman & Hall, Lond.

Zicsi, A., (1962). Determination of number and size of sampling unit for estimating Lumbricid populations of arable soils. In Murphy, P.W., (Ed.), Progress in Soil Zoology. Butterworths, Lond.

Zicsi, A., (1969). In Edwards, C.A. and J.R. Lofty, (Eds.), (1977), Biology of Earthworms 2nd edition. Chapman & Hall, Lond.