THE ROLE OF EARTHWORMS IN NITROGEN RELEASE FROM HERBAGE RESIDUES

A Thesis presented in partial fulfilment of the requirements for the degree of Master of Agricultural Science in Soil Science at Massey University

Belarmino Emilio Ruz Jerez

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ABSTRACT

Decomposition and nutrient release from pasture litter were examined in two biotic systems; either with or without large organisms ("macrobes"). Earthworms were the test macrobe and nitrogen (N) the test nutrient.

This experiment addressed the hypothesis that consumption of herbage residues by macrobes, as opposed to microbes, should result in more of the contained N becoming available for uptake by plants or for loss processes, because macrobes oxidise a greater proportion of the contained carbon (C) by energetics.

Earthworms influenced both soil metabolism and mineral N availability, irrespective of litter type (ryegrass or clover) and temperature (15 or 22.5 C). Carbon dioxide evolution and oxygen consumption increased by 26% and 39%, respectively, in the presence of earthworms. After an 11-week incubation about 50% more mineral N was recorded in the soils containing earthworms. Moreover, less microbial biomass was recorded in the presence of worms.

This influence of macrobes carried over into a subsequent, exhaustive cropping experiment, using ryegrass as the test plant. Where soils had been previously influenced by earthworms, there was a significant increase in plant growth and N uptake.

Carbon dioxide evolved during incubation was highly correlated with soil mineral N ($r= 0.84^{**}$) present at the conclusion of incubation, and also with subsequent plant dry matter yield ($r= 0.75^{**}$) and plant N yield ($r=0.85^{**}$).

The link between elaborated C and contained N has long been recognised as providing stability to organic residues in soils. In the design of this experiment, other influences of macrobes (e.g.

mixing or structural influences) were largely obviated, so one can conclude that nitrogen availability was increased primarily through carbon respiration by the macrobial population. These results offer a fresh perspective on the balance between mineralisation and immobilisation in the soil-plant complex and, hence, on the dynamics of nutrients contained in organic matter. Better understanding of these relationships may allow improved management of the dynamics of soil organic matter in temperate grassland ecosystems.

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CHAPTER 1

GENERAL INTRODUCTION

"Biological" soil fertility involves nutrient release by decomposition of pasture litter and mineralisation of resident organic matter. Consideration of this complex topic, as it relates to well-developed grass-clover pastures in New Zealand is the central focus of this study.

In spite of the comparative success of clover-based pastures in facilitating a high level of production from ruminant animals (Levy, 1970) most NZ soils in their improved state still exhibit a considerable degree of nitrogen (N) deficiency, at least seasonally (Field and Ball, 1978; Steele, 1982). To overcome this problem, fertiliser N may be introduced periodically (Ball and Field, 1982). However, the escalating cost of fertilisers, increased transport and spreading costs, the potential for suppressed exploitation of symbiotic N-fixation (Ball and Crush, 1986) and the risk of contaminating freshwaters (Burden, 1982) have all drawn attention to studies into improving the efficiency with which N is cycled in pasture soils (and also other nutrients contained in organic matter, notably P and S), and the prospect for exploitation of biological fertility.

Nutrient cycling in grassland ecosystems is of major importance to the build-up and maintenance of soil fertility. This is because only a small fraction of net primary production is consumed and respired by domestic herbivores; the greater proportion is transferred to the soil system. Thus, the soil decomposers are responsible for processing most of the primary biomass production within the ecosystem. However, this pronounced accumulation of organic matter in

grassland soils does not neceassarily imply any immediate improvement in supply of nutrients to plants, since the nutrients are substantially stabilised with C in elaborated organic compounds. Jackman (1960) highlighted the substantial quantities of N, P and S immobilised in soil organic matter during the early phases of pasture development; for N some 110 kg N/ha/yr. Therefore, it is important to consider how organic residues are incorporated by the decomposer network and how the subsequent activities of organisms effect C oxidation and hence mineralisation of essential elements, so releasing them for recycling within the ecosystem.

It has often been assumed that nutrient mineralisation is mainly a result of the activity of microflora (bacteria and fungi), while the soil fauna is considered to have only a minor direct influence (Barley, 1961; Golebiouska et al., 1977). Most attempts to quantify the role of the soil fauna in organic matter decomposition have concentrated on measures of respiratory metabolism. On this basis the macrobes (larger organisms) have been classed as insignificant in effect, and their ecological role has tended to be obscured. While it is known that metabolic activity is a key feature in determining mineralisation rates, it is still not possible to relate metabolism rates to nutrient mineralisation rates in quantitative terms, for all the major components of a soil population. It should not be assumed that a unit of respired energy from microbes is equivalent to the same unit of energy respired by heterotrophic invertebrates in terms of an absolute rate of mineralisation. One technique for evaluating the effect of macrobes in plant litter decomposition is to compare the release of plant nutrients following the mineralisation of plant materials.

O'Neill and De Angelis (1981) presented a general analysis of the role of macrobes in litter decomposition, and they suggested that the biomass of macrobes expressed as a proportion of primary production is inversely related to accumulated organic matter. This indicates that despite the small contribution of macrobes, directly, to decomposition, they either stimulate microbial decomposition (Anderson et al., 1983) or are correlated with it. The outcome is a more rapid organic matter turnover and nutrient cycling where the faunal contribution is maximal (Heal et al., 1984).

Among macrobes, the largest decomposer dominating mull soils in temperate grasslands are earthworms. Densities of the order of 5 to 7 million individuals per hectare have been recorded in Europe by Satchell (1967) and in NZ by Sears et al. (1953). The size of earthworm populations has been closely related to pasture productivity in NZ (Sears et al., 1953; Waters, 1955; Stockdill 1966). By contrast, in soils without earthworms Stockdill (1966) concluded that much of the fertility was locked-up in the peaty layer of dung and dead plant material that accumulates at the soil surface. Decomposition became very slow and there was a definite break in the "fertility cycle".

The purpose of this study was to evaluate decomposition and nutrient release from pasture litter in two biotic systems; either with or without large organisms ("macrobes"). Earthworms were the test macrobe and N the test nutrient.

This project addressed the following hypothesis:

> "That consumption of herbage residues by macrobes, as opposed to microbes, should result in more of the N contained in litter becoming available for uptake by plants or for loss processes, because macrobes oxidise a greater proportion of the contained C by energetics."

CHAPTER 2

CHAPTER 2

REVIEW OF LITERATURE

2.1 Soil Fertility in Managed Temperate Grasslands of NZ

2.1.1 Natural and managed grasslands

Natural grassland can be regarded as the climax vegetation developed under natural conditions in regions where the annual rainfall is insufficient to support forest growth, but adequate to prevent the development of desert. In contrast, "Managed Grassland" is produced when interference by man causes succession of the natural vegetation to be arrested before the climax is reached (Wallwork, 1976). Such interference may involve clearing and burning to remove woodland, ploughing, fertilising, seeding and intensive grazing by domestic animals (Levy, 1970). Managed grassland has been the common pattern that characterises most NZ pastures. The different phases of grassland development from low fertility and productivity conditions to high fertility and productivity, have been outlined by Sears (1962).

A most important characteristic of managed grassland derived by reducing a forest ecosystem, is that it is maintained only by the activities of grazing herbivores (Wallwork, 1976). It may not be possible to farm it at low or moderate fertility levels, because such grassland would revert to scrub species or even woodland (Scott et al., 1985).

2.1.2 Evolution of soil fertility in NZ pastures

During early development of New Zealand's pastures after land clearence and forest burns, good pastures were established, fed by minerals in ash and nutrients released as soil organic matter broke down. Inevitably, nutrients, especially N which had accumulated in forest soils over the preceding millenia became depleted, and introduced European pasture species gave way to less productive grasses and weeds that could tolerate lower fertility conditions (Levy, 1970).

Restoration of soil fertility required heavy applications of nutrients, mainly P and S and in some cases also Mo, to raise natural soil fertility to a level capable of encouraging and supporting introduced legumes (Gillingham et al., 1984), which increased soil N availability in farmed areas (Ball et al., 1985). But, because of its origin, this pastoral farming operates within a basically unstable system, and a certain amount of fertiliser (mainly phosphate) is continually required to maintain near-constant production from well-developed pastures (Sinclair et al., 1984).

During the course of pasture development, the correction of nutrient deficiencies results initially in a continual increase in the N content of the soil, through symbiotic N fixation (O'Connor, 1984), but as soil N accumulates grasses dominate the sward, and progressively clover yield and N fixation decline (Ball and Crush, 1985). At the same time the outflow of N is increased, induced by increasing numbers of grazing ruminants (Ball and Ryden, 1984).

Thus, in developed, high-producing pastures, within the potential imposed by climate and soils, N relationships determine the upper limits to productivity from otherwise well managed pastures (Ball, 1982). It has been established that this deficiency limits annual

production in some of the best NZ pastures by 25 to 35 % (Ball and Field, 1986).

2.2 Biological and Mineralogical Fertility

The large accumulation of organic matter in grassland soils does not necessarily imply a large supply of nutrients to pasture plants, since the nutrients are substantially immobilised in the organic compounds constituting the nutrient "capital" of the system (Laird et al., 1981; Goh et al., 1985). It is only after mineralisation that nutrients are available to plants or loss processes. Soil N availability is particularly dependent on these biological transformations. Henzell and Ross (1973) indicate that if it were not for this "bottle-neck" to N flow, highly productive pasture ecosystems could function on a small quantity of actively cycling N, with inputs being required only to balance losses. A further explanation of this concept was developed by Maldague cited by Lee (1985). He distinguished between inherent "mineralogical fertility" of soil, which derives from the chemical composition of the parent material, and "biological fertility", which derives from the rate of decomposition of plant litter and consequent release of its contained nutrients for circulation in the soil-plant-animal system.

Both kinds of fertility-promoting processes are exhibited simultaneously in all soils at any time, but the balance may vary with time and place. For instance, in the early stages of pasture development in NZ, it would seem that "mineralogical fertility" was more important, but when the nutrient deficiencies (other than N) had been corrected by continual fertiliser topdressing, "biological fertility" became more notable, especially in governing N relationships. Then, as was pointed out by Ball (1982), "N relationships imposed an upper limit to pasture productivity". None

of this is meant to imply that either aspect of soil fertility, mineralogical or biological, is unimportant in any terrestrial ecosystem; both are vitally important and interactive, but the emphasis changes.

2.3 The Role of Herbivores in Determining Biological Fertility

In NZ, estimates of the production of plant biomass in a developed pasture are about 20 t DM/ha/yr, which comprises about 16 t herbage yield, and 4 t roots (Stout et al., 1976; Field and Ball, 1982). Both ruminants and soil organisms utilise plant materials to obtain energy and nutrients. Herbage utilisation by ruminants, in an intensive system, is about 70% of measured herbage yield. However as only a small proportion of nutrients contained in herbage is retained in animal products (Wilkinson, 1973), the larger proportion is returned in excreta to the soil. There, together with plant litter it provides substrate for the soil decomposer network, and some eventually joins the soil organic matter in modified form.

The influence of ruminants in affecting "soil biological fertility" has been recognised in relation to herbage utilisation (Ball, 1979; Hoglund, 1985), return of nutrients in animal excreta (Whitehead, 1970; Wilkinson, 1973) and animal-related losses (Ball et al., 1983). However, the quantitative importance of consumption by soil decomposers of so large a fraction of plant biomass has been more or less ignored in NZ pastures (Ball and Field, 1986). These authors also point out that the complex of decomposers is just as important as ruminants in determining the fate of plant N in the soil, especially in high-producing, grazed pastures.

2.4 Interactions among Domesticated Grazing Animals, Soil Decomposers and the Dynamics of Soil Organic Matter

The way in which energy and nutrients pass through the soil-plant-animal ecosystem is clearly very important to soil fertility. If it were possible to control parts of the pathways, either by suppressing or encouraging them, then it might be possible to increase the productivity of the system (Gray, 1971). So far, little real success has been achieved in this direction. Recommendations for accummulation of mull-type organic matter and its contained nutrients under pasture, their depletion during a cropping phase, and subsequent reinstatement under pasture are well documented (e.g. Sears, 1962; Levy, 1970), but such technology is relatively unrefined. Very substantial losses of soil N can occur during a single cropping sequence out of improved pasture (Jackman, 1964). However, in the last decade, advances in the understanding of organic matter dynamics indicate that the initial approach to management of "soil biological fertility" would be to regulate the level of organic matter accumulation, and hence the potential mineralisable N in the organic matter. From a soil fertility point of view this is of major importance, since it supplies the N available for growth of grass or crop plants (Campbell et al., 1976; Carran, 1983; Keeney, 1985).

In a "steady-state" system, the soil organic matter level reflects the equilibrium between inputs of fresh organic residues and oxidation of resident soil organic matter. In cropping systems the balance is broken by enhancing the mineralisation of resident soil organic matter; the short-term advantage results in declining soil fertility (Sears et al., 1965b; Van Veen et al., 1981; Voroney et al., 1981; Campbell et al., 1982). In contrast, in grassland, the tendency is to accumulate organic matter due to the large return of plant residues to the soil, and the low rate of decomposition of these

residues (Sears et al., 1965a; Steele, 1982).

Recent research has shown that in some circumstances organic matter can decrease in pasture soils when inputs of plant residues become insufficient to compensate for concurrent oxidation (Ball, 1979). Crush et al. (1983) found that soil total N levels declined from 0.42% to 0.39% in 5 years under intensive grazing at a high level of herbage utilisation. A similar tendency had been reported by Ball (1979) under conditions of intensive pasture utilisation in both cut and grazing systems.

Thus, despite the fact that the pattern of dynamics for organic matter in grassland is substantially different from that in a cropping system, the effect on soil fertility in the long term may be similar if exploitive grazing practices were continued. This concept has been supported by Hoglund (1985). In his work, several levels of herbage utilization were maintained in sheep-grazed pastures for three years. Lax grazing allowed organic matter and total N to accumulate (1020 kg C/ha and 86 kg N/ha in the top 100mm for every extra 100 kg residual DM), but close grazing resulted in a significant depletion of these pools, as well as a reduction in pasture production. Clearly in the most intensive system, sheep were harvesting so high a proportion of plant biomass that insufficient residues were going back through the decomposer network to sustain the initial soil organic matter level, or to provide a carbonaceous subtrate for stabilising the excess of nitrogenous compounds in urine patches. Hoglund's studies suggest that maximum pasture and animal production may be achieved from permanent grasslands by adjusting management to ensure that adequate litter is returned to the soil to maintain an appropriate level of soil organic matter.

Understanding emerging from this work (Hoglund, 1985) provided testimony to the need to include soil organic matter dynamics within our concepts for management of pastures, not just within NZ but throughout the temperate zone.

2.5 Soil Decomposer Network

In general, only a small fraction of net primary production in natural grassland systems is consumed and respired by herbivores, and at least 90% is transferred to the decomposer system (Heal et al., 1984). Therefore, it is important to consider how this material is incorporated into the decomposer network and how the subsequent activities of organisms effect C oxidation and mineralisation of other essential nutrients. According to Elliot et al. (1984), because of their enormous importance, the living organisms in below-ground ecosystems should not be considered as a "black box" group of decomposers, but rather their interactions must be mechanistically analysed.

The soil microflora has been traditionally considered responsible for nutrient mineralisation (Satchell, 1974; Alexander, 1977), while soil macrobes have been thought to exert a physical effect and to have only an insignificant direct influence in the decomposition of organic residues *per se* (Barley, 1961; Golabiouska et al., 1977). Recently, however, there has been a heightened interest in the complicated series of balanced interactions among macrobes, microbes and higher plants (Elliot, et al., 1984; Ingham et al., 1985; Lee, 1985), any of which may contribute to, withdraw from, or temporarily immobilise nutrient elements from the inorganic pool, and thus influence their availability to the roots of higher plants (Macfadyen, 1978).

The soil ecosystem consists of a large number of micro- and macroorganisms strongly controlled by the physical and chemical restraints of the soil medium (Richard, 1974). Organic matter is used by primary consumers to produce new tissue which is subsequently used at higher trophic levels. Energy is lost and C is released to the atmosphere at each step in this process. Also, other elements are released into the soil in mineral form when organic materials are oxidized to carbon dioxide (Elliot et al., 1984). Therefore, any organism or process which accelerates the oxidation of C compounds, necessarily contributes to soil fertilily and an increase in the activity of the ecosystem as a whole, at least in the short term.

An important consideration in the microbe-macrobe interaction is direct population control. That is, the size and turnover rate of the decomposer population is directly influenced by the intensity of feeding upon them (Ingham et al., 1985). This control can be exerted at even higher levels in the food chain, resulting ultimately in changes in the decomposition rate. This point has a direct bearing on soil fertility considerations, because microbes themselves can be direct competitors with higher plants for nutrients. So a high level of decomposer activity may deprive higher plants of nutrients rather than increase the supply. Lack of grazing, over-grazing, and optimal grazing are all ways cited by Macfadyen (1978), in which microbial activities can be modified. In this regard, Paul and Voroney (1984) indicate that differential management of agricultural residues has been shown to affect the microbial-feeding, faunal population. They also recognise the potential for significantly altering nutrient cycles, as well as the possibility of increasing efficiency of use of fertilisers in soils through management of the biomass by cultivation, cropping practices, and even by faunal feeding.

When soil fauna and microflora have been experimentally manipulated, interesting results have been achieved. For example, Malone and Reichle (1973), found that after soil fauna were removed, the result was a very rapid decomposition and mineral leaching. In the view of Ausmus et al. (1976), this phenomenon can be interpreted as arising from a bloom of microflora with rapid decomposition of organic substrates, but retention of elements in microbial biomass until organic substrates were exhausted; thereafter periodic cycles of wetting and drying resulted in lysis and rapid loss of elements from the system. So it would appear that the presence of fauna grazing upon microflora serves to regulate the rate of decomposition, such that a more controlled linear release occurs throughout the growing season. In the context of an ecosystem, the advantages of a controlled, continuous release of nutrients from detritus, available for plant uptake, versus a rapid flush and loss from the system is indisputable (Reichle, 1977).

In the mutual interaction between microbes and macrobes one of the most important mechanisms mentioned in the literature is that immobile bacteria soon exhaust their substrate and become inactive; but according to Witkamps and Ausmus (1976), their activity can be restored when they or their surroundings are disturbed by movements of soil animals. Moreover, Madsen (1972) and Hargraves (1976) postulate that soil animals assimilate much microbial protoplasm, despite the fact that they pass relatively large amounts of dead plant matter of high C:N ratio through their gut. The animals in comminuting this material, produce a medium which supports a greatly enhanced microbial flora and, because they consume such large quantities of organic material, members of the soil fauna frequently re-consume the same pieces of what were once plant tissues (Hargraves, 1976).

The review and synthesis of the functional and structural aspects of soil animal populations by Peterson and Luxton (1982), provides much information on sampling theory and substrate utilisation. They conclude that although soil animals (in general) may account for only about 5 % of total soil respiration, their indirect role as "catalysts" for nutrient circulation is considerable.

The individual influence of some soil organisms on the decomposition processes has been demonstrated. For instance, Stout (1980) assessed the effect of protozoa on nutrient cycles. He showed evidence for enhancement of such cycling by increasing the bacterial turnover rate and releasing nutrients immobilised in baterial tissue by these smallest forms of the soil fauna. Clarholm (1981) makes an even stronger case for the importance of protozoa in the decomposition process. The impact of soil nematodes in terrestrial ecosystems has likewise been reviewed by Yeates (1979), and his conclusions were similar to those of Peterson and Luxton (1982); that is, although their biomass is small their impact is quite large.

2.5.1 Effects of earthworms on decomposition processes and nutrient flows

In temperate, deciduous forest and grassland, earthworms are the dominant group of the large decomposers (Wallwork, 1976). Because this invertebrate was used as a test organism in the present study, some of its relevant characteristics as a member of the soil biota are described in this review.

The influences of earthworms on the physical and chemical properties of soil materials have been well documented since the pioneering work of Darwin (1904). The enrichment of earthworm casts with plant nutrients such as P, N, K, Ca and Mg (Parle, 1963; Sharpley and Syers, 1976) and the importance of earthworms in the incorporation

of plant residues in both cropping and pasture systems (Barley and Kleining, 1964; Stockdill, 1966; Mackay and Kladivko, 1985) and in forest ecosystems (Edward and Heath, 1963; Vimmerstedt and Finney, 1973) have been extensively studied.

Syers and Springett (1983; 1984) and Lee (1985) presented comprehensive discussions of the influence of earthworms on soil fertility in temperate grasslands, emphasising the positive effects of earthworms on two soil-related aspects of agricultural production: the soil physical limitation to plant growth and the efficiency of fertiliser use. They indicate, however, that there is still a need for research on the mechanisms by which earthworms bring about improvements in soil fertility.

Earthworms, by feeding on organic materials, help to lower the C:N ratio in residues through their metabolism. As organic matter is digested and a very small fraction is assimilated into the earthworms' tissues (Lee, 1985), some C is removed through respiration, thus leaving the C:N ratio lower when the larger proportion of ingested material is excreted (Laird et al 1981). The extent to which earthworms lower the C:N ratio is not known exactly because of difficulty in measuring the amount of C consumed and the carbon dioxide respired. Present knowledge indicates that the amount of C consumed by earthworms in pasture is between 8 and 12% of the total consumed by decomposers (Satchell, 1967; Coleman et al., 1978). Alone this amount would not be sufficient to lower the C:N ratio sufficiently to greatly influence N availabilty to plants but, according to Laird and Koger (1981), when considered with the amount of C consumed by soil microorganisms, and their interactive effect, this amount is quite significant. In this regard, Bouche (1982) questioned the concept of basing energy flow studies solely on a budget of oxygen uptake or C dioxide output, or differences in energy

content between food and faeces, especially when the calculations are based on measurements over short periods in laboratory conditions. He also suggested other pathways of energy flow through earthworms, which include the loss of metabolically-elaborated materials, especially in the forms of :

i) Ammonia and urea in urine.

ii) Excreted calcium carbonate, which may in some species account for much respiratory C that would not be included in measurements made in respirometers.

iii) Cocoons.

iv) Proteins in mucus secreted onto the body surface or into the intestine.

v) Excess annual biomass production, commonly two to five times the mean annual biomass.

Bouche (1982) also pointed out the need for more quantitative work in this area, and implied that earthworms may play a more significant role in decomposition processes than is generally supposed.

Earthworms are an important component in the "fast N cycle" described by Coleman et al. (1983). In the fast cycle a small proportion of the total soil N is cycled through food chains that involve microorganisms and soil animals, and is returned to the soil in waste products, principally as ammonium, nitrate or in amino acids from dead tissues or mucus, which are readily taken up by plants or microorganisms and recycled (Lee et al., 1984). Nitrogen in the fast cycle may circulate through the plant-soil-decomposer system eight to ten times per year (Coleman et al., 1983). Therefore according to Lee (1985), it is not practicable to take gross levels of N use by earthworms, compared with the annual C flux in ecosystems, as a basis for assessing the role of earthworms in ecosystems.

In natural ecosystems Maldague (1979) concluded that fertility results from the maintenance of soil conditions such that the decomposition process operates at a level adequate to release plant nutrients from the litter at a rate that will sustain optimum growth. However, to achieve Maldague's objectives in a pasture ecosystem, it is interesting to consider a conclusion of Stockdill (1966). He concluded that without earthworms soil fertility is "locked up" in the organic matter at the soil surface and there is a definite break in the "fertility cycle". This point was corroborated in NZ by Keogh, (1979) when he estimated an annual N turnover from organic to mineral N via earthworms to be of the order of 109 to 147 kg N/ha.

2.6 Links between Organic Carbon and Nutrient Flows during Decomposition

The amount of organic matter lying at one time on the surface of, and incorporated into, the soil is the result of a balance between supply and removal, but the level of each of these processes in any particular system is itself determined by a great diversity of variables, some of which are related by circular chains (Trudinger et al., 1979). For example, and to oversimplify, the rate of litter supply is linked to primary production of plant material, itself determined by plant nutrient availability, which is in turn partly determined by the rate of litter breakdown. In this process, according to Van Veen et al. (1985) the cycling of nutrients in the soil is highly dependent on the energy supply of the soil biota. Thus C and nutrient cycles are closely linked, with C metabolism as the driving force to operate the system (Trudinger et al., 1979).

It is generally accepted that the concept of C:N ratio of organic material is important, because mineral N does not become available for assimilation by plants unless the C:N ratio is in the vicinity of 20:1

or lower (Whitehead, 1970; Laird et al., 1981). During the early stages of decomposition of the most freshly fallen plant litter, available N generally becomes immobilised in the protoplasm of decomposers, while C is respired and given off in gaseous form. The net outcome in the substrate is a progressive reduction in its C:N ratio, until it reaches a point similar to that of the decomposers themselves. Then mineralised N can become available for assimilation by living plants (Forbes, 1974).

The C:N ratio alone however has some problems in predicting N availability. For a long time N in soil was considered as the principal factor limiting the rate of decomposition of plant residues (Marshall and Alexander, 1960). More recently however, Powelson (1966) suggested that C would be a limiting factor in certain circumstances, and therefore the C:N ratio would not always give an appropriate indication about the fate of N in organic residues. Moreover, Parr and Papendick (1978) attributed the limitation of C:N ratio for assessing the N immobilisation potential and decomposition rate of a substrate, to the lack of adequate characterisation of the availability of contained C and N to soil organisms. In this regard, more conclusive data has recently been presented by Reinertsen et al. (1984). They concluded that the overall rate of residue decomposition in the early stages is dictated by the size of the soluble C pool. These results imply that the amount of biomass production, and thus the amount of N immobilisation that occurs during residue decomposition, is dependent on the amount of available C present in the residue.

In relation to other nutrients, Trudinger et al. (1979) consider that N, S and P cycles are clearly interdependent with C and oxygen, and any change in one cycle will, in the long term, have a profound influence on the operation of the other four. Comparing C, N, S and P

cycling through organic matter led McGill and Cole (1981), to propose that element cycling can best be interpreted within the framework of a dichotomous system. In this system, elements stabilised as a result of covalent bonding with C (C-N and C-S) are released from soil organisms as waste products during their search for energy, which is obtained through oxidation of C. In contrast, those elements existing as esters (C-O-S) and (C-O-P) are stabilised through reaction of esters with soil components. These elements may be mineralised by the need of organisms for a specific element. All cycles interact during plant uptake of nutrients and growth, and during decomposition processes in soil. Information on interactions between cycles is limited, although individual cycling of N (Paul et al., 1980; McGill et al., 1981), P (Chahuan et al., 1979; Cole et al., 1977) and S (Freney et al., 1983; Saggar et al., 1981) in relation to C flow have received more attention.

2.7 Conclusions

i) In managed grasslands, where the climax is rain-forest, it is probably not possible to farm at low or moderate fertility levels. Productivity and high stock numbers are needed to prevent the ingress of shrubby weeds into pastures.

ii) In well managed pastures N relationships determine the upper limits to pasture productivity.

iii) Grassland soils accumulate substantial quantities of nutrients in organic matter. Only after mineralisation are these nutrients available to plants.

iv) Biological fertility becomes important in developed pastures, especially in governing N relationships.

v) Biological fertility in soils is affected by grazing ruminants, principally by the level of herbage utilisation and return of nutrients.

vi) Soil decomposers are responsible for processing most of the primary biomass, and among them soil microbes (bacteria and fungi) have been considered most important.

vii) Recently there has been a heightened interest in the separated role of organisms, as well as their interactions, because of large differences in their production and assimilation efficency.

viii) During the oxidation of C other elements contained in organic matter are released in mineral form into the soil, where they are available for uptake or loss processes.

ix) In temperate ecosystems earthworms are the dominant group among the large decomposers (macrobes). Their importance in physical and chemical properties of soils has been well documented. However, there is still a need for research into the mechanisms by which earthworms influence soil fertility.

x) The direct control of microbial populations by macrobes is an important interaction which regulates the rate of decomposition of organic matter. The outcome is a more controlled, linear type of release as opposed to a rapid flush-like release, of contained nutrients.

xi) Carbon and nutrient cycling are closely linked, with carbon metabolism the driving force to operate the system.

xii) The C:N ratio is important in determinig rates of organic matter decomposition, but it does not always give an appropriate indication because of inadequate characterisation of the availability

CHAPTER 3

CHAPTER 3

SOIL INCUBATION EXPERIMENT

3.1 Introduction

The incorporation and degradation of a large proportion of plant and animal residues in temperate grassland is a critical requirement for maintaining desirable soil fertility and plentiful pasture production (Waters, 1951; Stockdill, 1959, 1966, 1982; Lee, 1985).

The causal agents of "biological fertility" are the soil organisms (Lee, 1985). Like herbivores that consume part of the herbage above ground, decomposers ingest plant and animal residues as an energy base and nutrient source. It is only during this dissipation of energy that nutrients bound in the organic form are mineralised and made available to the plant (Richard, 1974; Anderson et al., 1981; Coleman et al., 1983), or to immobilisation by soil organisms (Alexander, 1977; Fenchel, 1979), or become available to the loss processes (Russell, 1973; Hausenbuiller, 1980). Consequently, any group of organisms or processes which accelerate the oxidation of C compounds and the liberation of associated plant nutrients (N, P, S) would be contributing to soil fertility and increasing the activity of the ecosystem as a whole (Macfadyen, 1978).

Because of their importance to organic matter dynamics, the current view is that soil organisms should not be considered *en masse* as decomposers but, as was indicated by Elliot et al. (1984) their functional interaction must be mechanistically considered. This increasing interest in examining such a complex decomposer network is being expressed in different ways by a number of different scientific groups. For instance, Ball and Field (1986) pointed out that soil

decomposers are just as important as herbivores such as sheep or cattle in determining the fate of plant N in well managed, temperate grasslands. Alternatively Coleman and co-workers (1983) emphasised the importance of biotic interactions, principally microbe-plant, microbe-microbe, and microbe-fauna, while Petersen and Luxton (1982) assessing the contribution of the various organisms in the ecology of decomposition, established interest in discriminating between the true decomposers and those which merely catalyse the rate of decay. It seems that interest in examining the "black box" of decomposers is being stimulated by the large variation among soil organisms, especially differences in their production rate and assimilation efficiency (i.e. mineralisation-immobilisation relationships) Reichle, 1977). There is an inherent complexity among the decomposition processes, and a lack of simple correspondence between the oxidation of organic compounds and the liberation of plant nutrients (Macfadyen, 1978). Therefore, it is important to give more attention to whether residues are consumed by one group of organisms or another.

One of the broader comparisons is that between microbes and macrobes in relation to their participation in soil metabolism and nutrient flow. The soil microflora have traditionally been considered responsible for nutrient mineralisation (Satchell, 1974; Alexander, 1977), while soil macrobes have been considered to have only but a significant indirect role (Barley, 1961; Golebiowska et al., 1977). However, rarely have the activities of macrobes as grazers been included in the nutrient cycling process or separated from the activities of soil microflora (Coleman et al., 1983). Evidence is now accumulating to the effect that faunal grazers may be responsible for a significant fraction of the mineralisation previously attributed to microflora (Elliot et al., 1984; Ingham et al., 1985; Lee, 1985).

In NZ earthworms are the dominant group of large decomposers in intensively managed, grazed pastures, with densities of the order of 6 to 7 million individuals per hectare (Sears, 1953; Stout et al., 1976). Generally pasture improvement has been recognised as being associated with earthworm activity during all phases of pasture development (Sears, 1962; Stockdill, 1959; 1966; 1982). However, their direct influence on litter decomposition and nutrient availability has not been examined in detail.

In the present study, an attempt was made to examine the decomposition process and nutrient release (N as the test nutrient) from pasture litter in two soil biotic systems, with and without macrobes (using earthworms as the test macrobe).

Two approches were used to differentiate the role of the two biotic systems in litter decomposition. The first approach involved the measurement of soil respiratory activity over time in an incubation experiment. The second involved further evaluation of soil mineral N, soil biomass, and litter disappearence at the end of the incubation.

3.2 Materials and Methods

3.2.1 Introduction

A study of decomposition of pasture residues, under two soil biotic systems (with and without earthworms), two types of litter (grass and clover) and two temperatures (15 and 22.5 C), was carried out using an incubation technique for a period of 11 weeks. Soil biological activity was measured in metabolic terms, viz oxygen uptake and carbon dioxide evolution, while the effects of the treatments on some chemical properties of the soil were determined at the end of the incubation period. The changes in mineral N imposed by the treatments

during the present study were later tested in a second experiment (exhaustive cropping in a glasshouse), which is considered in Chapter IV.

The research was conducted at Grasslands Division of the Department of Scientific and Industrial Research at Palmerston North.

3.2.2 Development of the techniques

Prior to commencing the main experiment, preliminary information on rates of oxygen consumption and carbon dioxide evolution was required to determine the quantity of soil medium, the proportion of head space in the respirometers, the number of earthworms, the rate of litter to be added, the volume and concentration of trapping compounds and the frequency of measurements. After obtaining this information it was possible to build an appropriate respirometer, which was tested for 3 weeks. At this stage, the procedural details were checked and the preliminary results obtained were used to estimate variance and, hence, to decide the number of replicates for the definitive experiment.

3.2.3 Soil type and sampling

The soil used in this experiment was obtained from a terrace adjacent to the Manawatu River. In terms of the NZ classification (Taylor and Pohlen 1968) the soil used in this experiment is a Manawatu fine sandy loam; a slowly accumulating, weakly leached recent soil from quartzo-feldespathic alluvium; and according to the "Soil Taxonomy" classification (soil survey Staff U.S D.A.) corresponds to a Dystric eutrochrept, mixed mesic. (R.H. Wilde, pers. comm.). This soil was under a productive grass-clover sward, and had been intensively dairy-farmed for many years.

A bulk soil sample from the top 10 cm was collected in March 1986. It then was crumbled by hand and coarse roots were removed. Heavy rates of P, K, S and Mg (100, 90, 40, 50 kg/ha respectively) were applied at this stage, to provide these nutrients in excess. This study concentrated on C-N relationships.

3.2.4 Grass and clover litter

A supply of grass and clover residues was accumulated from swards grown in a glasshouse. These were separated into grass and clover by hand-dissection, then dried and stored. This provided bulk samples of two types of residues, and allowed them to be compared. The residue samples were a mixture of senescing and wilted material.

Chemical analysis of grass and clover litter, and analytical data for other materials used in the incubation study, are shown in Table 3.1.

3.2.5 Earthworm species

Two earthworm species were used in this experiment, and compared at temperatures of 15 and 22.5 C. Lumbricus rubellus Hoff. from pasture has an optimum temperature range of 15 to 18 C (Graff, 1953; cited by Lee, 1985) and Eisenia fetida (Savigny) from compost has an optimum temperature range of 20 to 29 C (Kaplan el al., 1980). Both species had to provide a sustainable population active throughout the period April-August, under controlled environmental conditions. A sample of earthworm tissue from each species was dried at 65 C for 24 hours, then C and N were determined by similar methods as were used for soil analyses.

Table 3.1 Analytical data (on a DM basis) for the materials used in the incubation study

Material	%C	%N	C/N	%P	%S
Soil	2.3	0.28	8.21	0.07	
Grass litter	44.0	1.4	31.4	0.38	0.57
Clover litter	44.0	2.8	15.7	0.27	0.26
Earthworms:					
pasture sp.	36.3	7.6	4.8	0.75	
compost sp.	40.6	7.2	5.6	0.60	

3.2.6 Experimental procedure

3.2.6.1 Incubation

The incubation vessels were adapted from "Agee" glass preserving jars, having an internal diameter of 110 mm and a capacity of two litres (Fig. 3.1 and Plate 3.1). Approximately half-filled with 1 kg dry soil, they provided sufficient medium for an earthworm's habitat, and for final measurements and exhaustive cropping with ryegrass on completion of the incubation. The head-space was modified by connecting two balloons, approximately 75 cc each when flaccid, to the lids of the jars. Thus, the balloons provided a variable volume to avoid significant pressure changes in the respirometers, as a consequence of oxygen consumption. The lids were also fitted with a 'terumo venoject' rubber inset, to permit the introduction of a syringe needle for replenshing the oxygen consumed, as well as a hypodermic needle leading to a manometer for monitoring internal pressure.

The respirometers were placed in separate water baths to maintain temperatures of 15 and 22.5 C. Additional units containing earthworms were included for destructive sampling during the experiment, to check on the survival of earthworms.

On completion of the incubation the soil was removed from each jar, the earthworms were counted, washed, blotted and weighed, and then dried at 65 C to prepare samples for tissue analysis. The soil was mixed throughly using a soil divider, and appropriate samples were separated for chemical analysis. Most of the remaining soil was subsequently used in the exhaustive cropping experiment.

3.2.6.2 Soil medium

Jars were filled with 1000 g of air dry soil, autoclaved at 121 C for 45 minutes, then vented for 1 week and inoculated with a

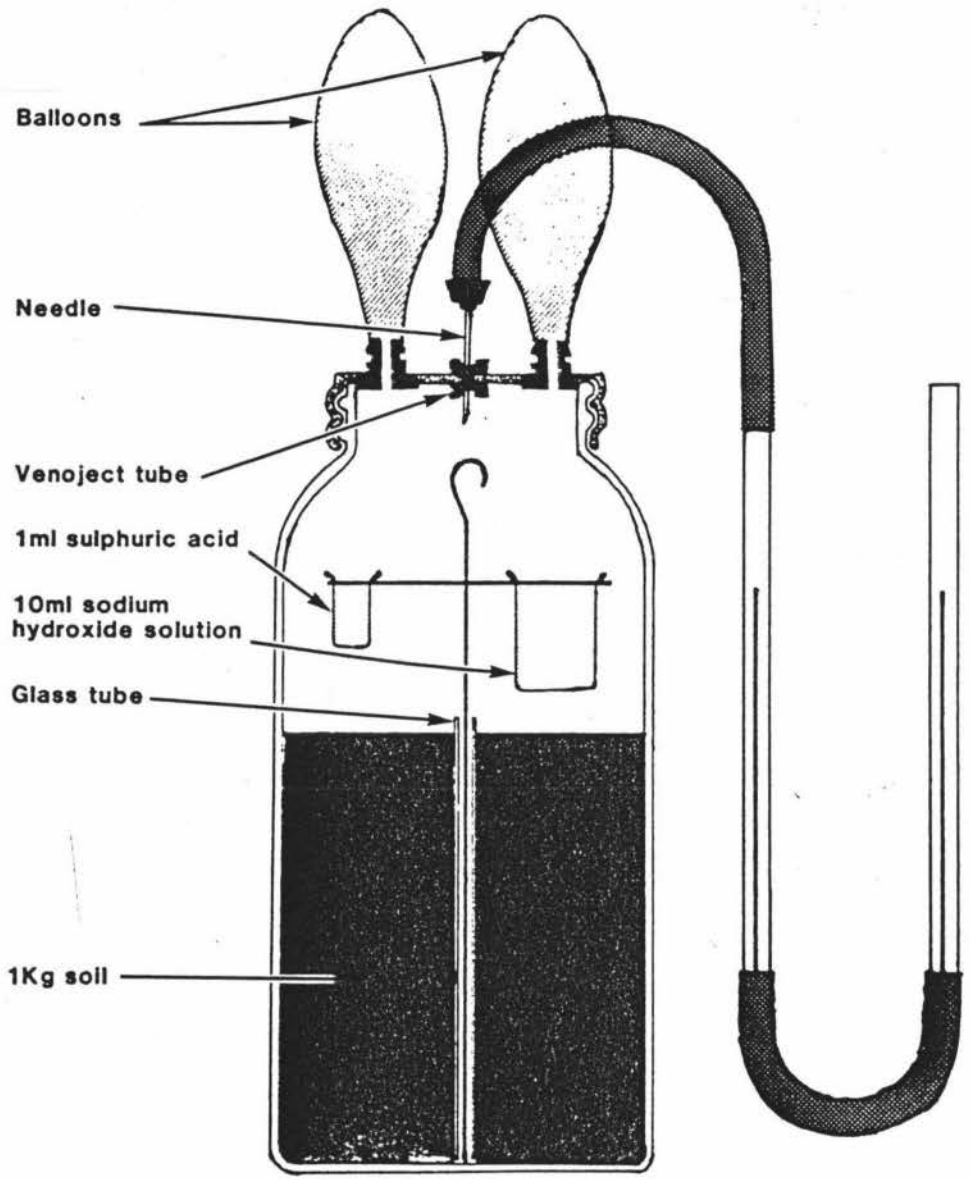

Figure 3.1 The respirometry apparatus

Plate 3.1 Respirometers in a water bath

upper: after inyection of oxygen
lower: early morning prior to inyection

suspension of the microbial fractions from the same unsterilised soil (Parkinson et al., 1971) and the two media in which the earthworms had been contained. The microbial inoculum was obtained by filtering soil slurry through a 100 um mesh sieve. It was inspected microscopically, and considered to be free of large organisms and their reproductive structures. This provided a soil medium free of macrobes.

3.2.6.3 Addition of litter and earthworms

To avoid the possibility of introducing large organisms or viable seeds during litter addition, the bulk samples were treated in a microwave oven for 10 minutes at 200 C. Then an initial input of 700 kg DM/ha of litter was added, moistened and worked into the surface 1 cm of the soil. After that, appropriate treatments were inoculated with 10 earthworms per vessel. Subsequent addition of litter to the soil surface (weekly) was at the rate of 50 kg DM/ha/day, which approximates the maximum rate of dry matter deposition observed in pastures (Hunt, 1971).

3.2.6.4 Soil moisture level

The soil moisture content was raised to 90% of field capacity (40% w/w) prior to enclosing vessels for each week's incubation. The amount of water to be added was determined by weight. It replaced that lost by evaporation during the three days that the vessels were open each week, and comprised about 15 ml/vessel/week.

3.2.6.5 Carbon dioxide evolution

Carbon dioxide evolved was trapped in 10 ml of 3M NaOH, then the C was measured weekly in a solution of barium chloride (Anderson, 1982). Blanks were included to provide both an estimate of the ingoing CO_2 associated with opening and closing the vessels, and also an estimate of analytical error, including reagent

contamination.

3.2.6.6 Oxygen consumption

The rate of oxygen consumption was determined by measuring the volume of oxygen, added daily, to reach the internal pressure previously established in the jars (20 mm water). A calibrated syringe and manometer were used to transfer a known volume of oxygen and to establish the appropriate pressure in the jars. The oxygen was maintained in a rubber football bladder, within each water bath, thus maintaining the temperature of the gas close to that of the incubation environment.

The oxygen consumption in moles of oxygen/day was computed from the volume of oxygen introduced using the following relationship:

$$n = PV/RT$$ where:

n= number of moles of oxygen consumed in the specified interval

P= Atmospheric pressure (atm) (obtained daily from Palmerston North airport).

V= Oxygen volume (cm3)

R= Gas constant (82.053 cm3 atm)/(K,mol)

T= Temperature (K)

3.2.6.7 Ammonia volatilisation

The amount of ammonia volatilised was estimated by the absorption of this gas in a trap containing 1 ml 0.1 N sulphuric acid (Cheng and Bremner, 1965). The ammonium formed was determined colourimetrically using a Technicon Auto-Analyzer.

Gas exchange was recorded for only 4 days every week. Vessels were closed on Monday morning, measured daily through to Friday and then left open for the ensuing 3 days. Leaving the vessels open to allow an element of evaporation is considered to be important, as it provided for regular addition of water to the soil surface. Pasture earthworms, at least, appear to benefit from this procedure, which prevents a build up of soluble salts or other biostatic materials in the burrow linings (R.G. Keogh, pers. comm.). Watering each week also allowed the freshly-added residues to be dampened before the vessels were closed.

3.2.7 Analytical methods

Chemical and other analyses of soil and herbage followed standard practices within the Analytical Laboratory at Grasslands Division.

3.2.7.1 Soil moisture content

Soil moisture content was determined by the gravimetric method (Gardner, 1965). Moist samples in duplicate were weighed, then dried at 105 to 110 C for 24 h. The sample was then re-weighed to determine the amount of water removed. Water content was expressed as a percentage of oven dry soil basis.

3.2.7.2 Soil mineral nitrogen

Triplicate moist samples, each equivalent to 10 g dry soil, were extracted for one hour by shaking with 100 ml 2 M KCl containing phenyl mercuric acetate (Douglas and Bremner, 1970). The extract was filtered through a Whatman No.42 paper. Ammonium and NO_3 -N were determined colourimetrically using a Technicon Auto-Analyzer (Crooke et al., 1971).

3.2.7.3 Soil total nitrogen

To determine total N 1.4 g finely-ground soil was digested by the Kjeldahl method (Bremner 1965) and N was determined colorimetrically on the diluted centrifuged digest, using a Technicon Auto-Analyzer and the hypochlorite-phenol reaction with NH_3 (Twine and Williams, 1971).

3.2.7.4 Soil organic carbon

Soil organic C was determined on 1.5 g of finely ground soil by chromic acid digestion (Barkoff, 1954).

3.2.7.5 Soil microbial biomass

Biomass C was calculated from the formula provided by Jenkinson and Powlson, (1976): [(CO_2 -C evolved by fumigated soil during the 0-10 days period)-(CO_2 -evolved by the unfumigated soil during the 10-20 days period)]/0.41.

3.2.7.6 Litter disappearance

To determine the % litter disappearance, soil from each jar was thoroughly mixed and a sub-sample of 30 g was sieved (<8 mm). The residue was recovered by washing the subsample on a 0.4 mm mesh sieve. The weight of residue recovered was calculated from the amount of residue recovered from the soil to which grass or clover litter had been added, minus the amount of residues recovered from untreated soil ("check"). All weights were on a dry material basis.

3.2.7.7 Herbage analyses

i) Herbage nitrogen and phosphorus

To determine the total herbage N 0.14 g sub-samples were digested by the Kjeldahl method with modification to include NO_3 by the addition of salicylic acid (Bremmer, 1965), following which

both N and P were determined colourimetrically in the same digest on a Technicon Auto-Analyzer.

ii) Herbage nitrate

Herbage nitrate levels were determined colourimetrically in water extracts (0.2 g plant material in 200 ml), following hydrazine reduction to NO_2 by the sulphanilamide-naphthyl ethylene diamide method (Henzell et al., 1968).

iii) Herbage carbon

A 9:1 pure silica sand and herbage mixture was used to determine herbage C by chromic acid digestion (Barkoff, 1954). A silica sand blank was included.

3.2.7.8 Soil pH

Soil pH was measured in distilled water at a soil:solution ratio of 1:2.5, after a 24 h equilibration period.

3.2.8 Design and statistical procedure

The experiment required 24 respirometer units, and included 8 treatments replicated 3 times, arranged in a completely randomised block design. In addition, two "check" treatments receiving no litter were included. The treatments used in the experiment are given in table 3.2.

The statistical analysis was carried out using a "Genstat" program, for a 3 factor experiment. Checks 1 and 2 (Table 3.2) were excluded from the ANOVA, providing a balanced design with the following factors:

Factors - Earthworms: no earthworms, earthworms

- Litter: clover residue, grass residue
- Temperature: 15 C, 22.5 C

The reason for including Checks 1 and 2 was to provide an estimation of litter disappearance. However, in some instances they provided extra information of value to improving the understanding of some effects (although of secondary importance to the hypothesis under study. In those particular cases the comparisons were made between individual treatments by one way variance analysis using the "Minitab" statistical package. The variation of respiration between two week means was tested using t-test.

3.2.8.1 Statistical limitations of the procedure

Because of the practical difficulty in obtaining 6 controlled temperature water baths (3 for 15 C and 3 for 22.5 C) to provide the appropriate temperature treatments only one bath per temperature was used. To minimise any inadequacy, both water baths were carefully calibrated before the experiment began, and incubation vessels were regularly re-randomised within the baths. During the trial period the temperatures were monitored and no significant deviation from the settings was registered.

An additional limitation is that earthworm species and incubation temperatures were confounded. This was inevitable, as no one earthworm species would be expected to thrive over the range of temperatures examined. Nevertheless, this confounding places considerable importance on the significance of any interactions between earthworms and temperature. The general absence of such interactions from the results of the statistical tests indicates that both earthworm species exerted similar effects on soil metabolism and N mineralisation, the parameters of central interest to this study.

Table 3.2 List of treatments

Treatment	Description
1	Soil + microbes + grass litter at 15 C
2	Soil + microbes + clover litter at 15 C
3	Soil + microbes + grass litter + earthworms 15 C
4	Soli + microbes + clover litter + earthworms 15 C
5	Soil + microbes + grass litter at 22.5 C
6	Soil + microbes + clover litter at 22.5 C
7	Soil + microbes + grass litter + earthworms 22.5 C
8	Soil + microbes + clover litter + earthworms 22.5 C
Check 1	Soil + microbes at 15.0 C
Check 2	Soil + microbes at 22.5 C

3.3 Results and Discussion

The earthworm populations affected soil metabolism during the 11-weeks' incubation and mineral N availability, measured at the end of the study. This effect varied in magnitude with the source of litter, and temperature.

3.3.1 Soil metabolism during incubation

3.3.1.1 Dynamics of earthworm populations

Earthworms numbers and weights at the beginning and at the end of the experiment, are given in Table 3.3. Pasture earthworms numbers (incubated at 15 C) remained constant throughout the trial period. On the other hand, compost earthworms (incubated at 22.5 C) fell by one unit. However, their biomass increased, so an element of compensation occurred in this respect. On average earthworm biomass increased by 9% fresh weight (FW) and 7% dry weight (DW).

As the average N content of earthworm tissues was 7.5% of DM, the positive variation in biomass immobilised an amount of N from the soil. The results of these transformations appear in Table 3.4. Pasture earthworms immobilised 3.8 and 4.9 mg N/kg soil under grass and clover litter, respectively. Compost earthworms immobilised 3.0 and 3.4 ug N/g soil; a similar quantity whether grass or clover litter was supplied during the incubation.

3.3.1.2 Effect of earthworms on litter disappearance

Recovery of residues was calculated from the amount of litter (>0.4 mm) recovered from the soil to which grass and clover litter had been added, minus the amount of litter recovered from the "check" vessels. The results are given in Table 3.5.

Table 3.3 Earthworm numbers and weights (g) at the start and finish of the incubation study.

Earthworm	Litter	Temp. (C)	Incubaton period (weeks) 0		11	
			No.	weight	No.	weight
Pasture	grass	15	10	6.21 (0.86)	10	6.60 (0.91)
	clover	15	10	6.03 (0.83)	10	6.54 (0.89)
mean				0.61 (0.08)		0.66 (0.09)
Compost	grass	22.5	10	4.20 (0.68)	9	4.68 (0.72)
	clover	22.5	10	4.19 (0.68)	9	4.70 (0.73)
mean				0.42 (0.07)		0.52 (0.08)
Overall mean				5.16 (0.76)		5.63 (0.81)

Dry weight ( )

Table 3.4 Changes in amount of nitrogen contained in earthworm tissues

Treatments	N (%DW)	Biomass changes DW (g)	N immobilisation (mg N/kg of soil)
Grass + W (15 C)	7.53	0.05	3.8
Clover + W "	8.19	0.06	4.9
Grass + W (22.5 C)	7.51	0.04	3.0
Clover + W "	6.77	0.05	3.4
average	7.5	0.05	3.8

(W = earthworms)

Table 3.5 Influence of earthworms, temperature and litter type on litter disappearance.

Treatments	Litter recovery (% of litter added)
Grass (15 C)	13.1
Clover "	10.7
Grass + earthworms (15 C)	8.9
Clover + " "	0.0
Grass (22.5 C)	14.3
Clover "	11.9
Grass + earthworms (22.5 C)	9.1
Clover + " "	1.2
Lsd (P=0.05)	1.3

In the absence of earthworms, of the total herbage litter added to each vessel during incubation (2.52 g, equivalent to 4550 kg DM/ha), 13 and 14.1% was recovered from soils treated with grass, compared with 10.7 and 11.9% from soils treated with clover, at temperatures of 15 C and 22.5 C, respectively. The rate of disappearance of litter from the surface of soil, was considerably greater in the presence of earthworms. This is reflected in the % recovery figures on conclusion of the incubation (Table 3.5); considerably lower for grass in the presence of earthworms, and only trace amaunts remained for clover litter. These results are consistent with the finding of Raw (1962), Edward and Heath (1963), Stockdill (1982) and Mackay and Kladviko (1985). The more pronounced effect of earthworms with clover rather than grass litter might be explained by the different structure and composition of the two plant tissues (Spedding et al., 1972; Holmes, 1980), including a different proportion of those compounds more resistant to decomposition processes (Smith, 1982). These characteristics can determine a relative preference by earthworms for certain types of litter over others (Lofty, 1974). In addition, Satchell and Lowe (1967) and Satchell (1967) found that among factors that contribute towards litter palatability, N and carbohydrate content must be included, as well as the presence or absence of a number of polyphenolic substances, particularly tannins. Experiments on food selection by such earthworm species as L.terrestris and L.rubellus (Wallwork,1976) have demonstrated that leaf material with a lower C:N ratio is more readily accepted than material with a high C:N ratio. Thus the low C:N ratio of clover litter (15.7) compared with grass (32.1) could be an important factor influencing disappearance of the two types of litter.

This study has also confirmed the importance of earthworms in the incorporation and mixing of surface-applied materials into the soil, as has been described by Barley and Kleinig (1964), Stockdill (1966), Syers et al. (1984), Lee (1985) and many others. This effect was visually apparent throughout this experiment and is illustrated in Plate 3.2. This plate shows the surface of the soil in two incubation vessels, a week after litter was added. In spite of the heavy rate of litter addition (50 kg DM/ha/day), the almost complete incorporation of residues in the treatment containing earthworms is apparent. This attribute of earthworms is of special importance for nutrient turnover in high-producing pastures, because of the large proportion of plant and animal residues returned to the surface of soils. For instance, Keogh (1979) estimated that approximately 7.7 t DM/ha/yr of residues were returned to the soil in an intensively farmed grass-clover sward.

3.3.1.3 Soil microbial biomass

The main effects of earthworms, litter, and temperarture on soil microbial biomass at the conclusion of the incubation are presented in Fig. 3.2, and the individual effect of each treatment are summarized in Table 3.6. No significant difference in soil microbial biomass was found between the two types of litter or between the two temperatures. In contrast, the soil microbial biomass was markedly smaller in the presence of earthworms.

The smaller microbial biomass observed where earthworms were present must be associated with the activity of earthworms, although it is difficult to be precise about the mechanisms. Earthworms may have depleted the microorganisms by reducing the availability of carbonaceous substrates (energy). Also, it is likely that during their feeding activities earthworms consumed a significant proportion of microbes. This mechanism might have especially affected those fungal populations which colonise the litter at the soil surface.

Plate 3.2 Soil surfaces displayed a week after litter was added

left: without earthworms
right: with earthworms

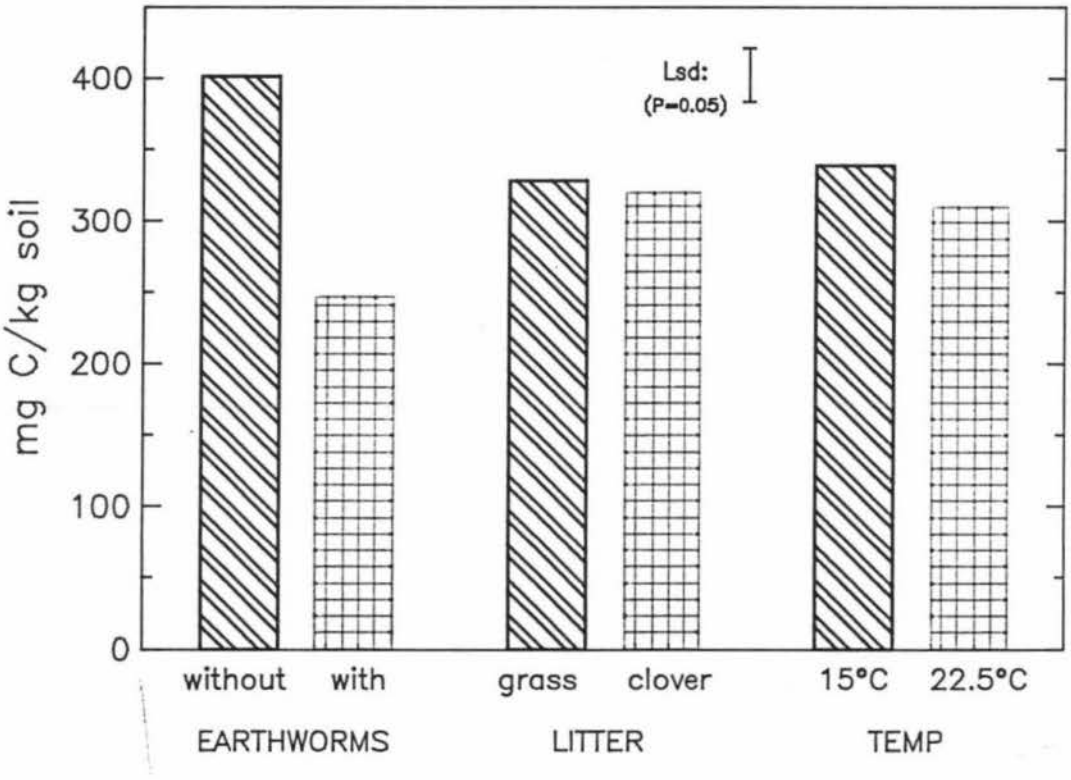

Figure 3.2 Main effects of earthworms, type of litter and temperature on soil microbial biomass

Table 3.6 Soil microbial biomass at the end of the incubation (mg C/kg soil)

Treatments	Microbial biomass (mg C/kg soil)
Grass litter (15 C)	410
Clover litter "	428
Grass + earthworms (15 C)	263
Clover + " "	251
Grass litter (22.5 C)	397
Clover litter "	368
Grass + earthworms (22.5 C)	242
Clover + " "	230
LSD (P=0.05)	73
Check 1 (15 C)	128
Check 2 (22.5)	167

This effect can be appreciated from Plate 3.2, showing fungal colonies in the surface litter, and from Plate 3.3 showing different microbial populations from two destructive samples taken during the incubation period (microbial biomass at week 4 was 523 and 317 mg C/kg of soil with and without earthworms, respectively). This finding is in agreement with evidence produced by Lee (1985). He cited several studies which demonstrated that soil microorganisms (especially fungi and bacteria) are an important component of the diet of earthworms. The mechanism whereby consumption of the microbial biomass by earthworms promotes mineralisation is an attractive, simple explanation for enhanced release of nutrients from microbial tissue.

3.3.1.4 Carbon dioxide evolution and oxygen consumption

Carbon dioxide evolution and oxygen consumption were used to measure the biological activity of the biotic systems assessed in this experiment. Treatments effects on oxygen consumption, carbon dioxide evolution and the respiratory quotient (RQ) are shown in Appendix 1; and the carbon dioxide evolution as a percentage of C added in litter is shown in Appendix 4.

The main effects of treatments (presence of earthworms, two temperatures, and two types of litter) are presented in Figs 3.3 and 3.4 for carbon dioxide evolution and oxygen consumption, respectively. Carbon dioxide evolution increased markedly (26%) in the presence of earthworms and increased moderately (11%) at the higher temperature. The two types of litter showed a lesser effect; carbon dioxide evolution with clover litter being 3.9% higher than that with grass. Moreover, when the addition of litter is compared with the "checks" to which no litter had been added (Fig. 3.5), it is apparent that carbon dioxide evolution was significantly affected; the respiration rate increased by 25 and 35% for grass and clover, respectively.

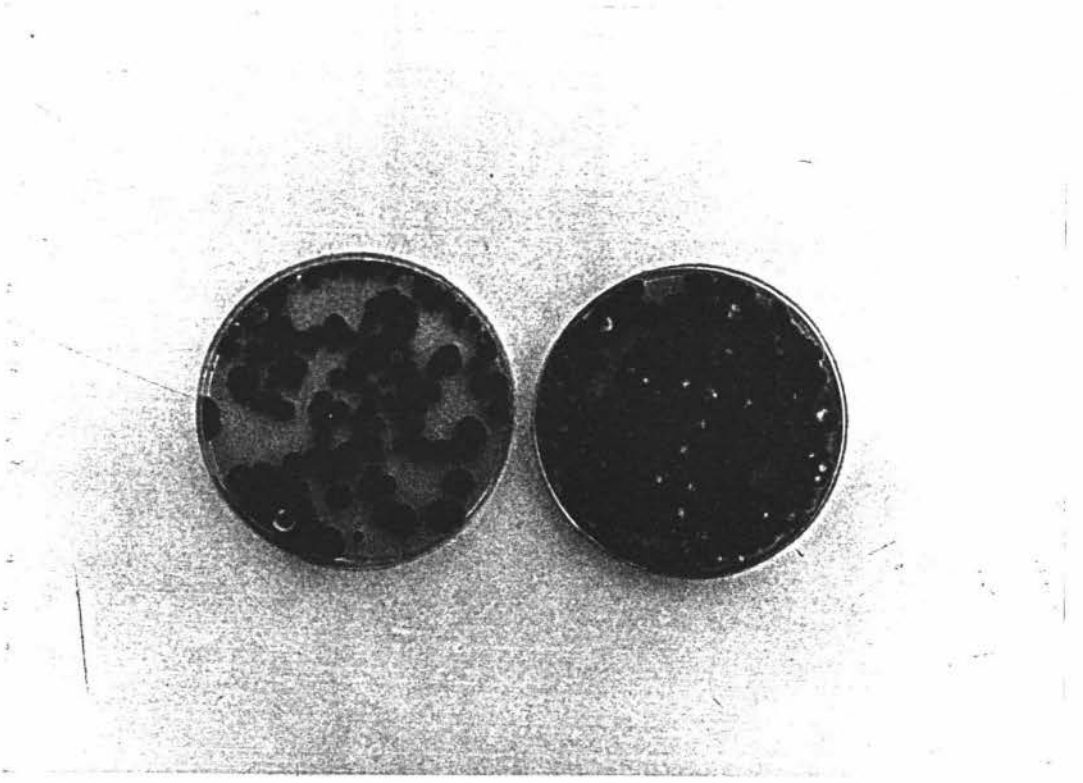

Plate 3.3 Counts of viable mould propagules as determined by a dilution technique

left: with earthworms
right: without earthworms

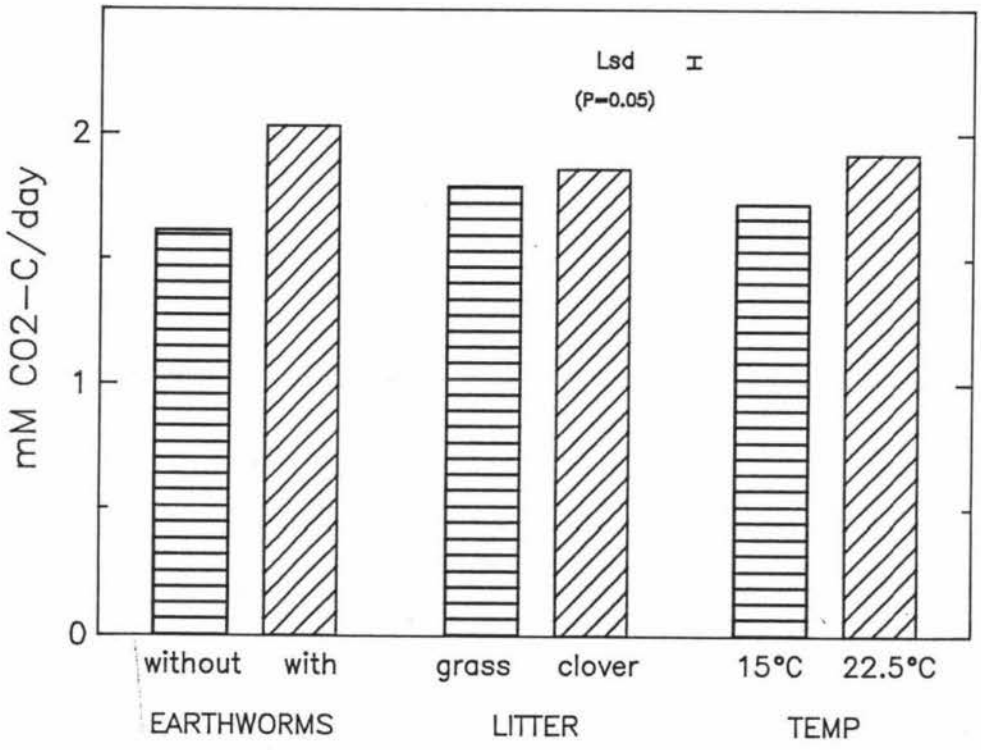

Figure 3.3 Main effects of earthworms, type of litter and temperature on carbon dioxide evolution

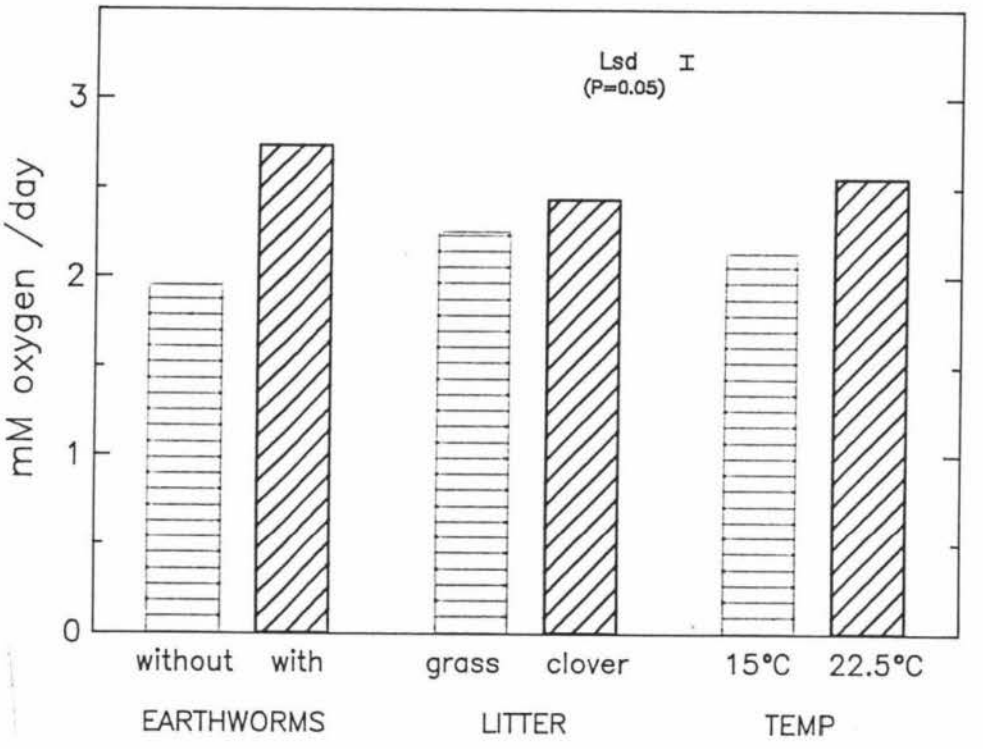

Figure 3.4 Main effects of earthworms, type of litter and temperature on oxygen consumption

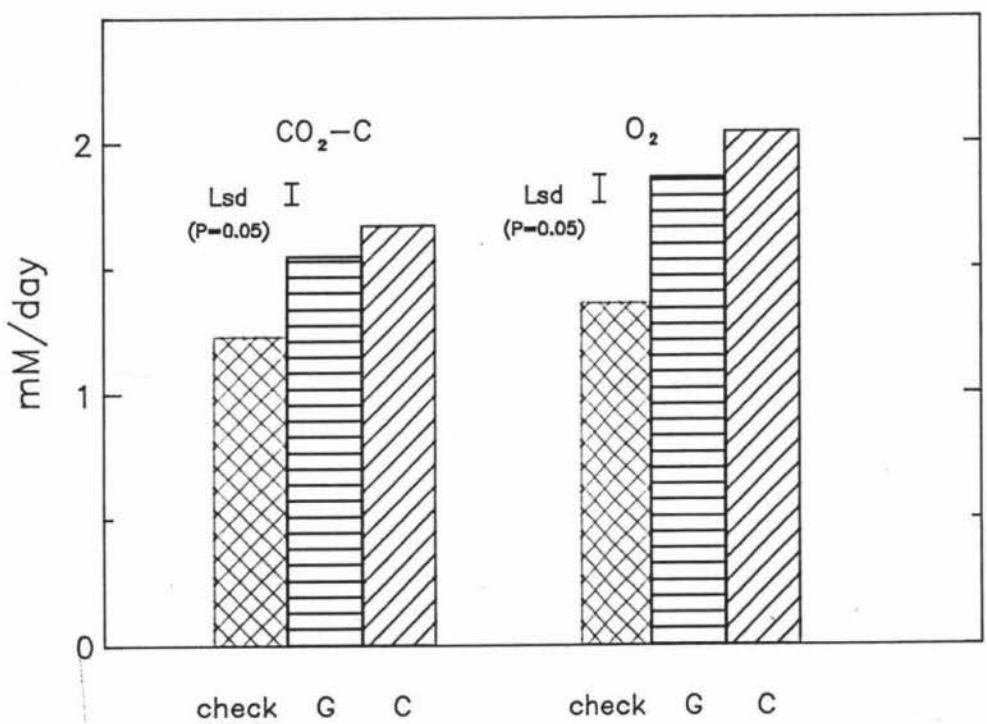

Figure 3.5 Individual effects of grass (G) and clover (C) litter on carbon dioxide evolution and oxygen consumption

Results of oxygen consumption mimicked those for carbon dioxide evolution (Figs 3.3, 3.4 and 3.5). If anything, main treatment differences were more pronounced.

Since the microbial biomass was smaller in the presence of earthworms it is reasonable to conclude that earthworms brought about significant fraction of total soil respiration in this experiment. This result is in contrast to some views cited in the literature (Barley, 1961; Satchell, 1967; Phillipson, 1978). For instance, Phillipson (1978), cited by Stout (1983), estimated that earthworms contribute only about 4 to 5% of the total soil metabolism. However, this discrepancy might result from differences in experimental procedures. Most of the present knowledge about the metabolism of earthworms is based on the respiratory rate of inactive animals. For instance, using such a technique Satchell (1967) estimated that only 8% of total C combustion in a soil was due to direct respiration of a population of L.rubellus. In fact the true consumption of C in an environment more similar to natural conditions is likely to be higher than that previously reported.

The high values for C evolved in this experiment, and attributable in large proportion to the direct effect of earthworms, need not be considered extraordinary or at variance with much of the published literature. Burrowing activity, general motility and feeding at the soil surface (the litter was added weekly) are considered as important avenues for energy consumption, and hence the need for a more active metabolism. If one assumes that carbon dioxide output per unit of microbial biomass was similar in all treatments, indications are that on average earthworms contributed some 50% of total respiration taking place in their presence, during this study. Of course the foregoing assumption cannot be justified without much better definition of the microbial population in individual

treatments. However, this line of thinking serves to illustrate the conjecture that the impact of a numerous, active population of earthworms may have been under-estimated in previous studies.

Soil respiration was more rapid at the beginning of the incubation period. The general trends are illustrated in Figs 3.6 and 3.7 for carbon dioxide evolution and oxygen consumption respectively. In general, during the course of the incubation there was a decline in biological activity, which was significant pronounced in weeks 1 to 5 for the majority of treatments. After that a tendency towards stabilisation could be observed, except perhaps for treatments involving clover at 22.5 C, which declined more consistently over the entire period. This tendency for soil organism activity to decrease might be related to: (i) the effect of previous soil manipulations; (ii) the high rate of litter added at the beginning of the experiment; (iii) changes in the microbial population, or (iv) total earthworm actual biomass with subsequent alteration in recycling of the biomass. Some of these influences have been reported by Jawson and Elliot (1986), to explain the decrease in efficiency of growth of microbial populations with time, especially in those experiments monitoring C evolution over an extended period.

3.3.1.5 Soil organic carbon and soil total nitrogen

The main effects of earthworms, litter and temperature on soil total organic C and N are shown in Table 3.7. No significant difference was found in organic C attributable to the effect of earthworms, type of litter or temperature.

Soil total N was significantly influenced only by temperature. Consequently, the C:N ratio reflected this effect with only temperature causing a significant difference (Table 3.7). While this contrast may not be agronomically very relevant, it must be borne in

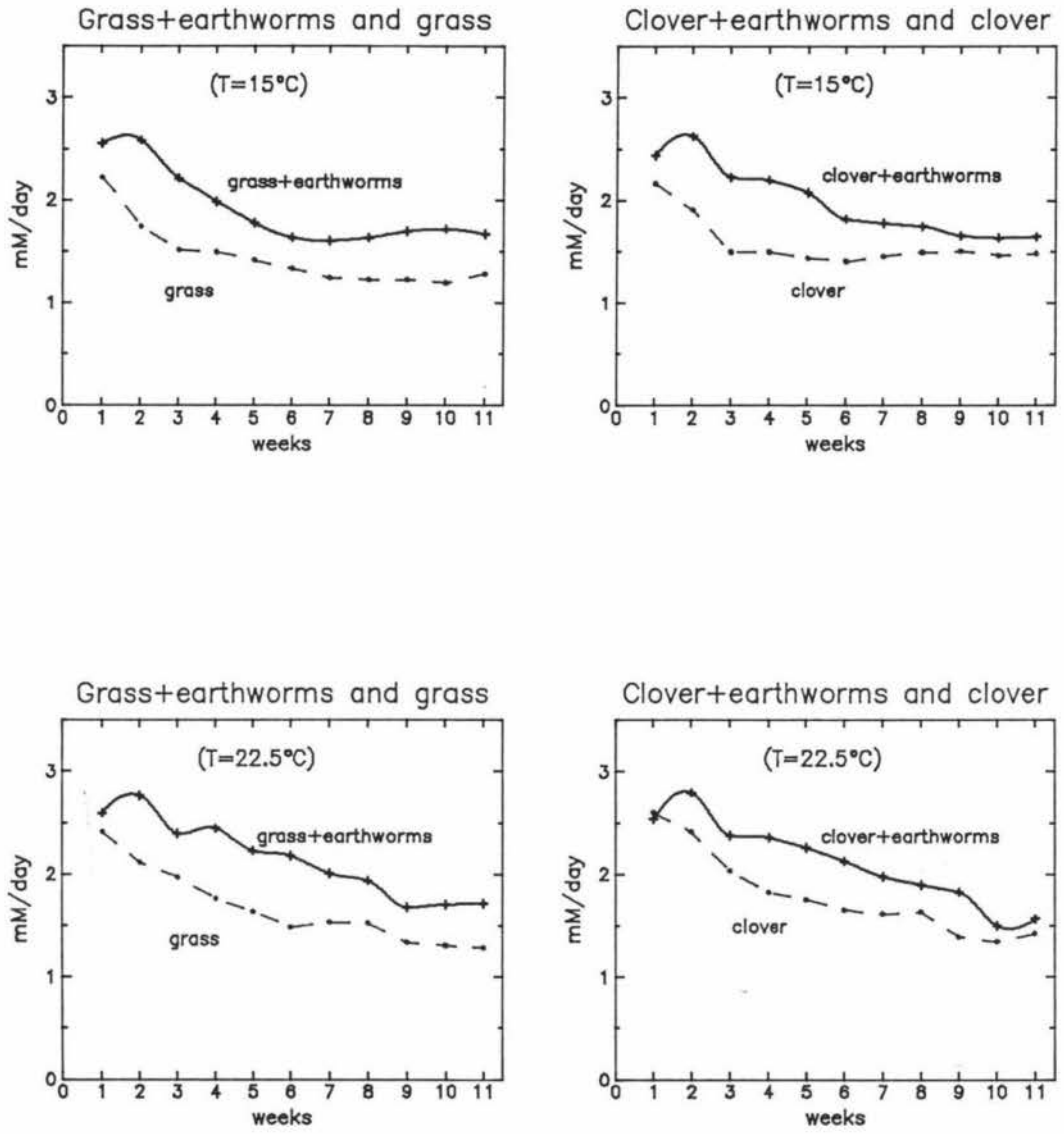

Figure 3.6 Time–course for carbon dioxide evolution during 11 weeks' incubation

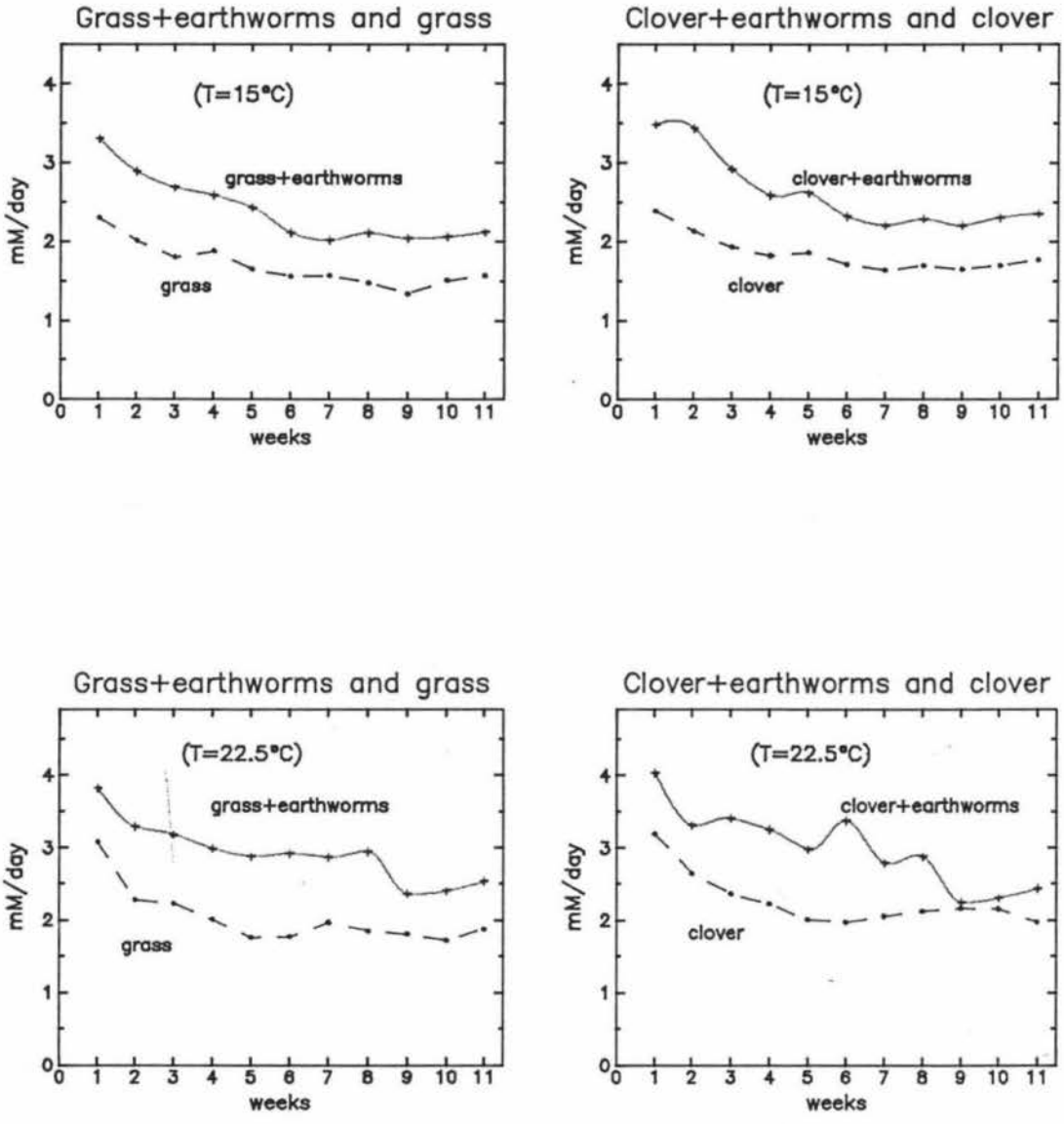

Figure 3.7 Time course for oxygen consumption during 11 weeks' incubation

Table 3.7 Main effects of earthworms, litter and temperature on soil organic carbon and soil total nitrogen at the end of the incubation.

Main effects	% C	% N	C:N
Effect of Earthworms			
with	1.94	0.23	8.43
without	1.93	0.23	8.39
Effect of Litter			
Grass	1.94	0.23	8.43
Clover	1.93	0.23	8.39
Effect of Temp.			
15 C	1.92	0.23	8.35
22.5 C	1.94	0.22	8.81
LSD (P=0.05)	n.s.	0.006	0.21

mind that this significant difference developed within a period of three months, or less than half a growing season.

3.3.2 Effect of earthworms on soil mineral nitrogen

3.3.2.1 Total mineral nitrogen

The main effect of earthworms, temperature and the two types of litter, on total mineral N measured at the end of incubation are shown in Fig. 3.8. Significant differences were found among the main effects, for total mineral N and with earthworms and temperatures for its components (ammonium and nitrate). The individual treatment means are presented in Appendix 2.

i) Main effect of earthworms

It is apparent from the data in Fig. 3.8 that the mineralisation of organic N increased dramatically as a result of earthworm activity. In the soil where earthworms were present, about 50% more mineral N was recorded. The stimulation of mineralisation by earthworm activity has been reported to be related to their own metabolism and/or to the associated stimulation of soil microbial activity (Barley, 1961; Satchell, 1967).

It is difficult to estimate quantitatively the relative contribution to N turnover by the earthworms' own metabolism. Barley (1959) found that 6.4% of organic N ingested by A.caliginosa was excreted in an available form, but interaction with other decomposers must be taken into account. The litter is probably more easily attacked by other organisms after it has been subjected to grinding and to chemical changes in the gut of worms. Moreover, Syers et al. (1979) recorded that 73% of the total content of litter was recovered in casts material, but that the available (inorganic) N in cast was "agronomically insignificant". The same authors suggested that casting activity represents a "pass through" of organic N with some

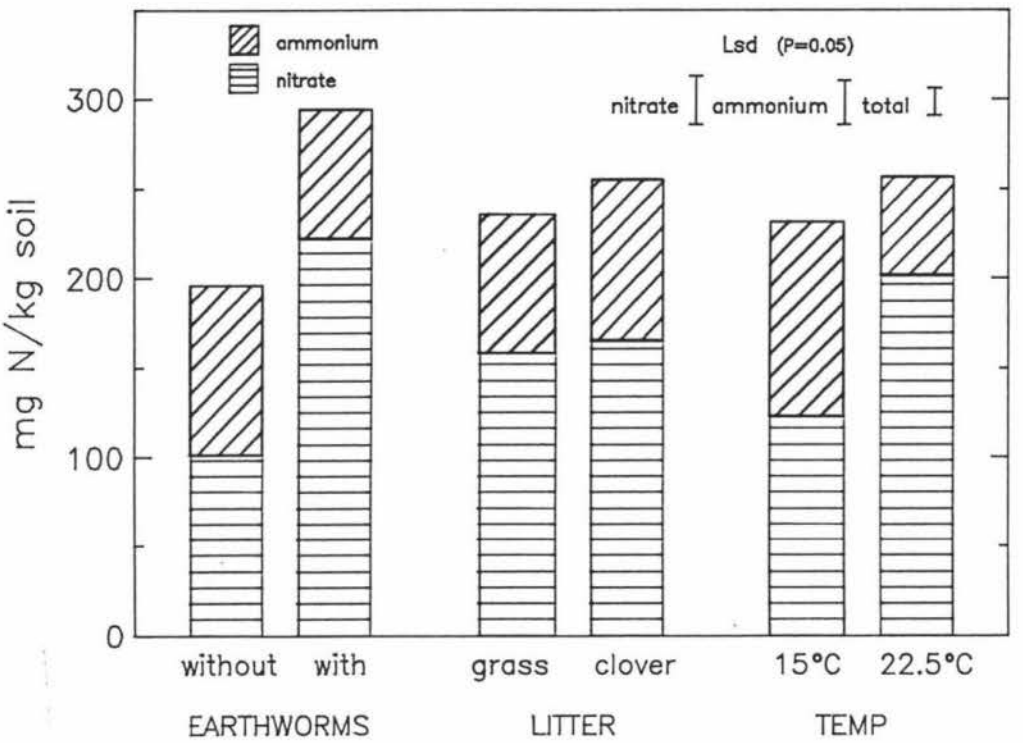

Figure 3.8 Main effects of earthworms, type of litter and temperature on soil mineral nitrogen

increase in mineralisation, perhaps less than that which occurs in litter *in situ*.

It seems clear that some interaction between earthworm activity and other decomposers does occur, and that this relates especially to improvement of the physical properties of the soil and to the mixing of organic residues. However, results from the present experiment suggest that the interactions between earthworms and soil microorganisms are probably more complex than has previously been thought. The earthworms' own metabolic processes could be contributing to a greater extent to nutrient cycles especially when, as in this experiment, a large, active earthworm population is involved and their effect is measured in a soil system. For instance, the total mineral N in soil at the end of incubation greatly exceeded other values reported in the literature. However, the additional inorganic N cannot have been derived from the tissues of earthworms since there was a net immobilisation of N in earthworm biomass when the earthworms increased in weight (Table 3.4). Even though microbial populations can increase because of improvement to the physical properties of a soil (Russell, 1973; Satchell, 1967) and during the passage of residues through the gut (Parle,1963), in this study the soil microbial biomass at the end of the incubation was lower where earthworms were present (Fig. 3.2). Further comminution of added residues appears to have proceeded fairly satisfactorily under the conditions of this experiment, whether or not earthworms were present (see table 3.5). Only with clover addition did the earthworms effect any marked increase in break-down of organic fragments. In all cases, the difference in recovery of uncomminuted fragments in the presence or absence of earthworms constituted less than 10% of total residues added, and so small a difference cannot explain a 50% increase in mineral N formation in soil containing earthworms. Therefore, the additional inorganic N recorded on completion of incubation in this

experiment must have been produced in substantial part by the earthworms' own metabolism and feeding activity.

ii) Main effect of temperature

Mineral N was 9% greater after incubation at 22.5 C than at 15 C. (Fig. 3.8). Also the higher temperature increased the proportion of nitrate, following the same tendency as the overall influence of earthworms. There have been many studies on the effect of temperature on N tranformations to inorganic forms (Anderson et al., 1964; Harmsen et al., 1965; Stanford et al., 1973; Jansson, 1982) and also specifically on nitrification (Frederick, 1956; Scmidt, 1982). The general conclusion is that increasing temperature in the range used in this experiment would increase both mineralisation and nitrification.

iii) Main effect of litter type

The effect of two types of litter on soil mineral N is shown in Fig. 3.8. The C:N ratio of clover litter was substantially lower than that of grass (Table 3.1). This resulted in more mineral N being recovered from clover litter (an extra 18.7 mg N/kg soil). However, this value represents only 53% of the extra N added with clover (70.6 mg N/kg soil) as compared with grass (35.3 mg N/kg soil) litter. Also, from Fig. 3.8 it can be appreciated that clover litter was not significantly different from grass in relation to the form of mineral N (ammonium and nitrate) extracted from the soil.

When the effect of litter was compared with the "check" (without litter) in the absence of earthworms no significant differences in mineral N were observed (Fig. 3.9). In the absence of earthworms, the average mineral N recovery was 196.3 and 193.8 mg N/kg soil for treatments with and without litter respectively. In other words, the substantial quantity of litter added (50 kg DM/ha/day), incorporating

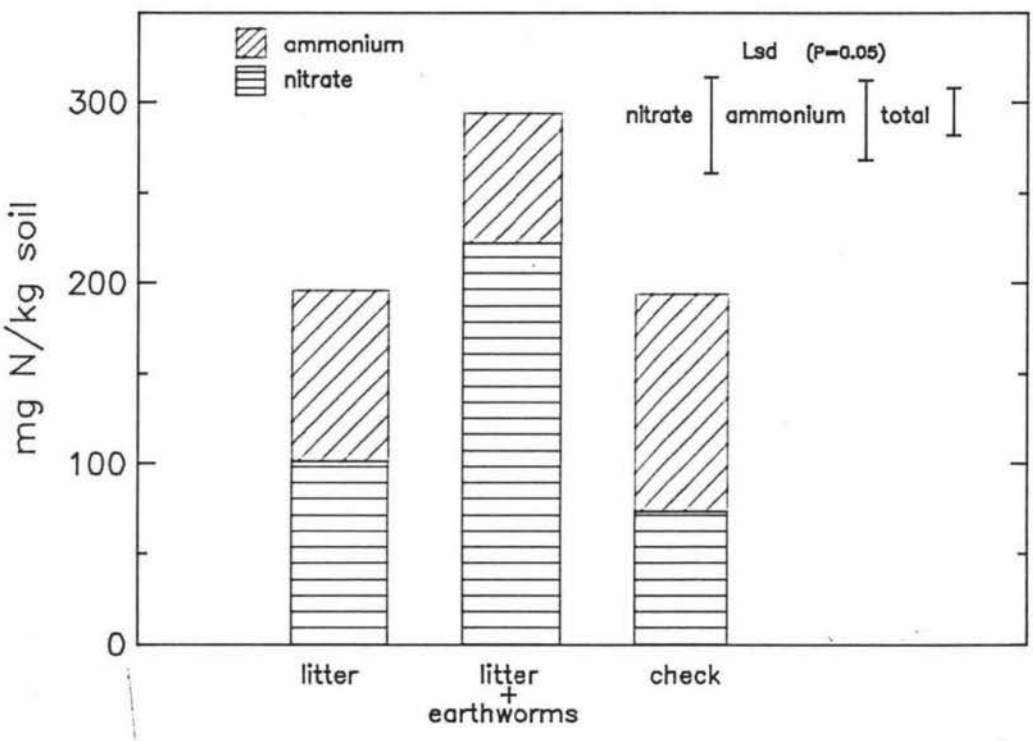

Figure 3.9 Individual effects of litter and litter + earthworms on soil mineral nitrogen

an average total of 52.5 mg N/kg soil in each incubation vessel resulted in no more soil mineral N being recovered in comparison with the "check" treatment, if earthworms were absent.

3.3.2.2 Forms of inorganic nitrogen

The form of inorganic N was dramatically different according to the presence or absence of earthworms. Fig. 3.8 shows that 50% of total mineral N was in the nitrate form when earthworms were excluded, while their presence stimulated nitrification and brought about conversion of about 75% of total mineral N to nitrate. There are several possible explanations for this result. For instance, Syers et al. (1979), working with L.rubellus and A.caliginosa , found that the exchangeable ammonium in cast materials was always greater than nitrate during the casting period. However, nitrification proceeded rapidly in the cast: during the incubation at 16 C about 65% of ammonium was converted to nitrate. Moreover, Parle (1963), cited by Lee (1985), also observed an increase in nitrate and ammonium in the casts of A.longa, relative to the surrounding soil. About 96% was in ammonium form at first, but over a period of 20 days the proportion of nitrate rose.

As ammonia is the end product of biological metabolism of proteins, virtually all organic N breakdown flows through ammonia (Fenchel, 1979). Because this product also predisposes to loss from the system, ammonia volatilisation was monitored over the period to determine the amount of N lost in this way. Appendix 5 shows that an insignificant amount of ammonia was volatilised during the incubation period. Ammonia constitutes the energy substrate for the nitrification process, brought about by obligate autotrophs (Richard, 1974) with carbon dioxide the source of C (Smith, 1982). So it is reasonable to suggest that earthworm activity provides a better environment for nitrifying bacteria than occurs in their absence. In

fact, the mineralisation rate was greater where earthworms were present (Fig. 3.8), and also a higher rate of carbon dioxide evolution from their respiration was recorded (Fig. 3.3). Together both processes guaranteed that energy substrate (ammonia) and cell building material (carbon dioxide) were better supplied in the soil media associated with the earthworms' activities. Thus, nitrification should have proceeded with less limitations.

On the other hand the type of litter did not affect the form of inorganic N, and both grass and clover litter produced a similar pattern of nitrate and ammonium at the end of incubation (Fig. 3.8). However, the temperature significantly affected the proportions. Fig. 3.8 shows that at the higher temperature nitrate was the predominant form of inorganic N. This result might reflect a contrast between temperate and tropical environments in relation to N transformation. The ecological implications of this rapid nitrification in tropical conditions are that loss by leaching of nitrate below the root zone during cropping is likely to be increased as is N loss by denitrification. In this respect, Bartholomew (1977) pointed out, that the unique aspect of shifting cultivation in tropical agriculture is the magnitude of N change. This high potential for leaching losses represents not only a localised pollutant source, but also an inefficient use for N. Therefore, innovative management systems are needed to reduce this loss of N.

CHAPTER 4

CHAPTER 4

EXHAUSTIVE CROPPING EXPERIMENT

4.1 Introduction

Decomposition of litter in the different biotic systems during the incubation experiment (Chapter 3) exerted important effects on soil mineral N. The presence of earthworms increased the level of inorganic N. The type of litter and the temperature during incubation also had significant effects on the amounts and forms of available N, measured at the end of incubation. However, because of the numerous processes which affect N turnover in a soil, the concentration of N dissolved in the soil solution can change considerably over short periods. This applies particularly to nitrate. Several studies have demonstrated that a large difference in available N is not always reflected in plant performance. In microcosm studies, Baath et al. (1978) found that soil fauna enhanced decomposition rate, but no significant increases in plant growth or in nutrient concentration were obtained. In flowing solution culture experiments, with constant N concentrations, a 1000-fold difference in N concentration produced a less than 10 % difference in yield of perennial ryegrass (Lazenby, 1983).

Net mineralisation values from incubations, therefore, may not always be good estimates of plant available N. Mineral N measured by chemical methods presents merely a "snap-shot" of the N present in that pool at that point in time. It can change very rapidly. In particular the readily mineralisable fraction of organic N which may become available very rapidly will not be assessed in a measurement of the mineral N pool.

There is general agreement that of all nutrients supplied to plants, N is the nutrient which has by far the most important impact in increasing plant production. The increase in plant growth, or rather uptake of N by plants (Sahrawat, 1983), provides a more reliable criterion for assessing soil N availability (Chalk et al.,1970; Gasser et al., 1976; Stanford, 1982).

In summary, estimates of net mineralisation or mineral N availability in the absence of living plants may not necessarily reflect the ability of plants to obtain N or the effect of plants in N transformation processes. Carlyle and Malcolm (1986) pointed out that the presence of a plant can have several effects. For instance, estimated net mineralisation gives no indication of the ability of plant roots to compete for N as it is turned over within the microbial pool. Also plant roots, but especially mycorrhizae, may be able to exploit soluble organic sources.

The purpose of this study was to determine whether the enhancement of decomposition rate (measured during incubation), which resulted in an increase in N available (determined by chemical methods after incubation), would be reflected in plant growth, or in nutrient concentration, measured in a glasshouse experiment using ryegrass (Lolium perenne L.) as the test plant.

4.2 Materials and Methods

4.2.1 Glasshouse study

To asses the availability of N to plants a glasshouse experiment was undertaken using soil samples obtained from the incubation study (Chapter 3).

At the end of incubation a representative sample equivalent to 500 g oven dry soil was obtained from each incubation vessel. Each was hand-packed into a plastic pot, with a surface diameter of 110 mm and vertical height of 100 mm. The pots were placed on plastic saucers to retain any leachate, and maintained in a glasshouse with temperature controlled between 12 and 24 C.

Perennial ryegrass (Lolium perenne cv. "Grassland Nui") was used. Approximately 56 seeds were sown and later thinned to 28 plants per pot. Pots were watered to 90 % of field capacity (40% w/w) each day with distilled water. Every two weeks a destructive sampling was carried out to estimate the contribution of plant biomass to the overall weight of pots, thereby allowing adjustment of the watering rate to maintain the same level of soil moisture content throughout. A total of 3 harvests were taken at 8, 12 and 16 weeks. Plants were cut to a height of 10 mm. After the final harvest the roots and stubble ("root") were separated from the soil and washed. The above-ground herbage and the root material were then oven dried at 80 C for 16 h before weighing. The herbage and roots were then finely ground and stored until the end of the experiment for analysis.

4.2.2 Soil and plant analyses

On completion of the experiment, herbage analyses for total N, nitrate, and total P were carried out (for the 3 sample cuts and roots) following the methods described in Chapter 3.2.

Soil samples from all pots were analysed for total N, mineral N, organic C and pH as described in Chapter 3.2. Table 4.1 shows the analytical data for soil at the start and end of this glasshouse experiment.

4.2.3 Experimental design

A total of 30 pots was used, corresponding to 8 treatments and 2 checks (Table 4.1) replicated three times. Earthworms had been removed so, any influence of the earthworm treatments had to reflect some carry-over of activities during the previous incubation. The pots were completely randomised and re-positioned every week to minimise any effect of uneven light and temperature conditions in the glasshouse.

Statistical procedures and limitations were as described in section 3.2.8.

4.3 Results and Discussion

4.3.1 Dry matter yield

The total dry matter yield (TDM) of ryegrass did not exhibit significant differences, regardless of whether the soil used had previously contained earthworms or not. Likewise, no significant differences were found in TDM between soils which had been treated previously with grass litter and those treated with clover litter. Only temperature during incubation had a significant effect (Fig. 4.1), with soils previously incubated at 15 C providing greater TDM than those incubated at 22.5 C. Inspection of the data in Table 4.2 reveals that all this response arose from the root plus stubble fraction.

The distribution of dry matter yield in the different cuts, as well as the yield of the roots plus stubbles at the final harvest, are shown in Fig. 4.1 and in Table 4.2. Both first and second cuts

Table 4.1 Organic carbon, total nitrogen and pH of soil samples at the beginning and at the end of the exhaustive cropping experiment.

Soil	%C		% N		C/N		Min.N		pH	
Sample	start	end	start	end	start	end	start	end	start	end
Grass (15 C)	1.93	1.50	0.24	0.17	8.0	8.8	178.2	7.8	6.1	5.9
Clover "	1.88	1.53	0.23	0.17	7.8	8.8	189.9	9.7	6.2	6.1
G + W "	1.93	1.43	0.23	0.16	8.4	9.4	275.3	9.7	5.3	5.9
C + W "	1.95	1.47	0.24	0.17	8.5	8.8	292.9	8.3	5.2	5.8
grass (22.5)	1.95	1.50	0.22	0.17	8.9	8.8	205.6	7.8	5.2	5.8
Clover "	2.00	1.50	0.23	0.17	9.1	8.8	211.3	7.8	5.1	5.8
G + W "	1.94	1.50	0.23	0.16	8.8	9.4	283.4	7.4	4.9	5.7
C + W "	1.89	1.50	0.22	0.17	8.6	8.8	323.2	7.8	5.3	5.7
lsd(P=0.05)	n.s	n.s.	0.01	n.s.	0.40	n.s.	30.6	1.6	0.31	0.11
Check 1	1.94	1.5	0.23	0.17	8.3	8.8	181.9	7.8	6.2	5.8
Check 2	1.87	1.5	0.22	0.17	8.6	8.8	205.9	8.7	5.3	5.7

(G=grass litter, C=clover litter, W=earthworms)

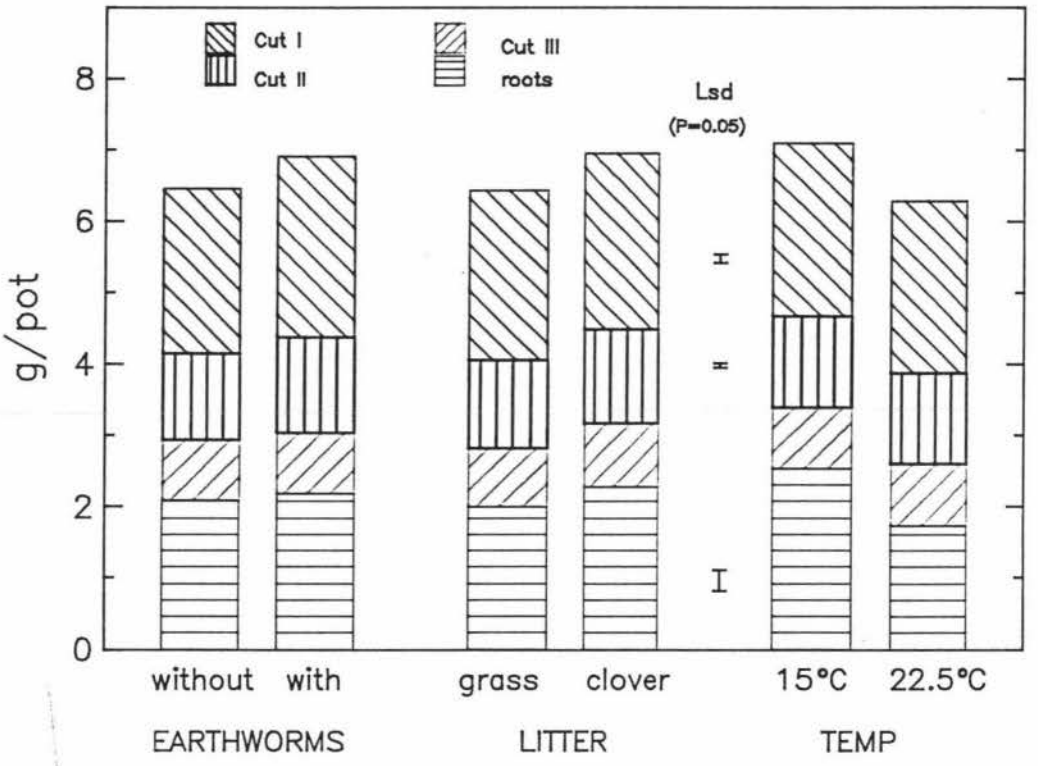

Figure 4.1 Main effects of previous incubation treatments on dry matter yield of ryegrass (see also table 4.2)

showed significant differences in dry matter yield, according to whether the ryegrass was growing in soil samples previously incubated with or without earthworms; earthworms increased DM yield. In addition a large difference was found in root yields in relation to the temperature at which the soil was previously incubated.

Total dry matter yield of ryegrass provided one of the lowest correlation coefficient with the soil mineral N values (smN, measured at the end of the incubation; Table 4.3). However, the dry matter yield of the first cut (DM-I) was resonably closely associated (r=0.78**) with smN. A similar finding was reported by Gasser and Kolembasa (1976), after three cuts of ryegrass in a glasshouse study.

4.3.2 Percentage of nitrogen in the herbage.

The percentage of N in herbage was high in the first cut (Table 4.4). The values ranged between 3.13% and 3.81%, which are in agreement with values cited by Wilman (1965) and Whitehead (1970), for the N content during the early growth stage of ryegrass with an adequate supply of soil mineral N. However, significant differences were found in the percentage of N in herbage at cut 1 according to whether the soil in which the ryegrass was grown had been incubated with or without earthworms. The type of litter applied during the incubation was also reflected in the ryegrass N content at first cut.

The N content declined in the 2nd and 3rd cuts to low values (Table 4.4), which presumably reflected progresive exhaustion of plant available N in the soil medium and this caused a severe restriction to dry matter yield. Differences among treatments in herbage N content in the 2nd and 3rd cuts were not significant, probably as a result of a generalised soil N deficiency after cut 1. This trend is very well

Table 4.2 Dry matter yield (g/pot) of ryegrass

Main effects	Cut 1	Cut 2	Cut 3	Roots	TOTAL
Effect of Earthworms					
with	2.53	1.34	0.85	2.18	6.90
without	2.30	1.21	0.86	2.08	6.45
Effect of Litter					
grass	2.37	1.23	0.83	1.99	6.42
clover	2.46	1.32	0.89	2.27	6.94
Effect of Temp.					
15 C	2.42	1.28	0.86	2.53	7.09
22.5 C	2.41	1.28	0.86	1.73	6.28
Lsd (P=0.05)	0.10	0.07	n.s.	0.51	0.60
Checks	2.16	1.13	0.78	1.83	5.91

Table 4.3 Correlation coefficients (r) between soil mineral N and ryegrass perfomance

	smN	Nitrate (herbage)
total dry matter yield	0.39 *	0.27 ns
dry matter cut 1	0.78 **	0.46 **
N content cut 1	0.86 **	0.75 **
N content cut 2	0.21 ns	0.32 ns
N content cut 3	0.08 ns	-0.13 ns
total N yield (herbage + roots)	0.88 **	0.69 **
N yield cut 1	0.93 **	0.73 **
total herbage N yield	0.91 **	0.72 **
smN	-	0.60 **

smN = soil mineral N at the end of incubation

ns = no significative

* = significative 5% level

** = " 1% "

Table 4.4 Total nitrogen content (%) of ryegrass

Main Effects	Cut 1	Cut 2	Cut 3	Roots
Effect of Earthworms				
with	3.81	1.62	1.33	0.54
without	3.13	1.57	1.33	0.61
Effect of Litter				
grass	3.28	1.58	1.32	0.62
clover	3.67	1.60	1.34	0.63
Effect of Temp.				
15 C	3.33	1.63	1.29	0.61
22.5 C	3.62	1.56	1.37	0.64
LSD (P=0.05)	0.17	0.10	0.09	0.08

illustrated by the highly significant correlation coefficient (r=0.86 **) between the percentage of N in the 1st cut (%N-1) and smN (Table 4.3). By contrast, the correlation coefficients with %N-2 and %N-3 are very low and not significant.

4.3.3 Nitrogen yield in plant (plant N uptake)

The total N yield in ryegrass (TNY) was greater for plants growing in soils that had included earthworms in the previous incubation experiment. The results of the main effects appear in Fig. 4.2 and Table 4.5. Plant N yield was by far largest in the first cut, but it progressively declined in the 2nd and 3rd cuts.

Plant N uptake from soil samples previously incubated with earthworms was 51 mg N/kg dry soil higher than N taken up from soil incubated without earthworms. The type of litter added during the incubation also resulted in significant differences in N yield of ryegrass. This effect represented an increase of about 35 mg N/kg soil, taken up by plants growing in soils that received clover litter previously rather than grass litter. However, it should be remembered that on average vessels receiving clover litter received some 35 mg N/kg dry soil greater input than did vessels receiving grass litter. So this enhanced uptake represented recovery of almost all the extra N added.

Cumulative net mineralisation during the incubation (Chapter 3) was greater for soil containing earthworms, and higher values were also measured with clover litter than grass. Comparing the recoveries of mineral N at the end of the first experiment with the plant N yields in the present study, one can observe (Table 4.3) the high degree of correlation (r=0.88**) between smN and TNY. Absolute values were also very similar in some treatments (Table 4.6). These results

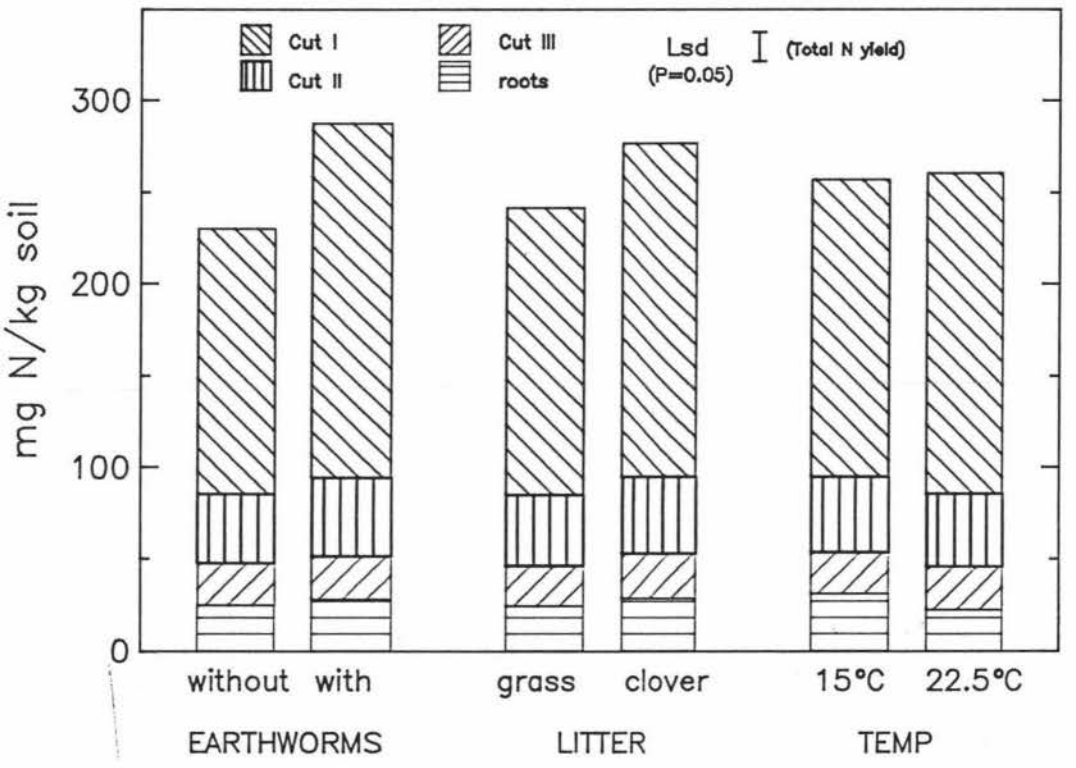

Figure 4.2 Main effects of previous incubation treatments on total nitrogen yield of ryegrass

Table 4.5 Total nitrogen yield (mg N/kg dry soil) of ryegrass

Main Effects	Cut 1	Cut 2	cut 3	Roots	TOTAL
Effect of earthworms					
with	193.1	43.3	22.9	27.9	287.2
without	144.3	37.9	22.8	24.9	229.9
Effect of Litter					
grass	156.1	38.8	21.8	24.3	241.0
clover	181.4	42.4	23.9	28.5	276.2
Effect of Temp.					
15 C	162.1	41.5	22.3	30.8	256.7
22.5 C	175.4	39.6	23.5	22.0	260.6
LSD (P=0.05)	9.3	3.1	1.9	6.6	16.3
Checks	131.6	35.6	20.6	21.9	209.7

Table 4.6 Soil mineral nitrogen at the end of incubation, total nitrogen uptake by ryegrass and soil mineral nitrogen remaining after exhaustive cropping (mg N/kg dry soil)

	Mineral N (at end of incubation)	Total N uptake by ryegrass	Mineral N after (exhaustive cropping)
Grass (15 C)	178.2	209.3	7.8
Clover (")	188.9	246.8	9.7
G + W (")	275.3	274.9	9.7
C + W (")	292.9	294.9	8.3
Grass (22.5 C)	205.6	213.9	7.8
Clover (")	211.3	249.5	7.8
G + W (")	283.4	265.8	7.4
C + W (")	323.2	313.4	7.8
LSD (P=0.05)	30.6	31.7	1.6
Check 1	181.9	203.1	7.8
Check 2	205.9	216.4	8.7

(G = grass litter, C = clover litter, W = earthworms)

agree well with the field and laboratory studies of Carlyle and Malcolm (1985; 1986). They found that N availability estimates derived from both incubation and plant uptake were very similar, and highly correlated, concluding that in their study net mineralisation values from aerobic incubation were good estimators of plant-available N.

The almost 1:1 correspondence between initial mineral N values and plant N yield found in many of the samples (Table 4.6) indicates that the ryegrass was taking up all of the available N. If earthworms were absent during the previous incubation however, initial mineral N recovery was generally rather less than eventual N uptake by ryegrass. While no unequivocal explanation can be provided, collectively these results indicate that much of the added clover N in particular was temporarily immobilised into microbial biomass. It certainly doesn't appear to have persisted in the soil as undecomposed fragments (see section 3.3.1.2), yet it escaped detection during the initial mineral N measurement. It seems likely that this temporarily-immobilised fraction was then progressively released during the course of exhaustive cropping, possibly involving a widening of the biomass C:N ratio in those treatments. On the other hand, where earthworms were present no similar, temporary immobilisation occurred, presumably because of the competition for substrate exerted against the microbial fraction.

Plant N yield was well represented by the values at the first cut. As can be appreciated from Fig. 4.2, about two-thirds of total plant N yield was in the herbage material harvested in the 1st cut, 8 weeks after sowing. Moreover, the quantity of N taken up in the first cut (NY-1) correlated highly with soil mineral N (smN) and also with total N yield (TNY) (Table 4.3). This indicates that the influence of prior incubation on soil N availability was strongly expressed in the

early stages of ryegrass growth. This tendency was also reflected in the virtual absence of nitrate from herbage after the first cut.

4.3.4 Nitrate levels in herbage

Nitrate in herbage (Table 4.7) was only detected in the first cut. The nitrate content was highly variable among samples, and significant differences reflected the effect of earthworms and the type of litter from the preceding incubation experiment.

The internal nitrate concentration has been found to be an excellent measure of the level of N nutrition in grasses (Mackenzie et al., 1963; van Burg, 1966) and in crops (Hylton et al., 1964), although almost all of these studies have been conducted in systems receiving fertiliser N. According to van Burg (1966), to obtain maximum yields the nitrate content of ryegrass ought to be in the region of 100 mM per kg dry matter. He determined that the importance of a certain level of nitrate in herbage is that a low internal nitrate concentration signifies that the grass does not possess any reserve of metabolisable N. Nitrate accumulation, on the other hand, signifies that the grass does possess a certain amount of such a reserve. Other reports from field experiments have indicated that applying fertiliser N consistently increases the nitrate content of the grass. For example, Wilman and Wright (1983) observed values of about 100 ppm of NO_3 (14 mM NO_3 /kg DM) in swards without N fertiliser. However, when 200 or 300 kg N/ha/yr was applied, the nitrate content of herbage was increased to 280 and 600 ppm respectively.

The virtual absence of nitrate in herbage after the first cut confirms the poor level of N nutrition during the 2nd and 3rd regrowth periods and it is presumed that the early stages of growth exploited most of the available soil N.

Table 4.7 Nitrate levels in herbage

(mM nitrate/kg DM)

Main Effects	Nitrate concentrations (mM /kg DM)
Effect of earthworms	
with	341.5
without	28.8
Effect of Litter	
grass	72.5
clover	297.6
Effect of Temp.	
15 C	132.5
22.5 C	237.8
LSD (P= 0.05)	210.5
Checks	191.7

In Table 4.7 it can be seen that the nitrate content in herbage was very much higher in treatments from soils incubated with earthworms. This result might be expected, simply because earthworms resulted in the production of more nitrate (Fig. 3.8). A significant difference was also observed according to the type of litter added during the incubation; clover resulted in a four fold increase in nitrate level over ryegrass litter. This result is somewhat surprising, as there was very little difference between these two treatments in soil nitrate content after incubation (see Fig. 3.8). So, it seems likely that more adequate substrate availability quite simply enhanced nitrification in those soil samples which had received clover residues.

The correlation coefficients between herbage nitrate and other parameters measured in this experiment appear in Table 4.3. The strongest association was with percentage N and N yield in the first cut, and with total herbage N yield (THNY).

4.3.5 Soil mineral N at the end of cropping

A small amount of soil mineral N was recorded on completion of the pot experiment (Table 4.6), indicating that practically all mineral N available at the end of the incubation was taken up by the test plant. This was presumeably the main source for N nutrition, because a N budget indicates (Appendix 6) that very limited net mineralisation occurred during the growing period. This is confirmed by the significant reduction in plant N levels recorded in cuts 2 and 3 for most treatments. In only one case (clover residues relative to grass) did a significant increase in N yield by ryegrass occur through cuts 1, 2 and 3 (Table 4.5), indicating that mineralisation of the clover N continued to make some contribution, albeit small, to plant nutrition right through the cropping sequence. In contrast, the

residual influence of earthworms was exerted only through cuts 1 and 2, while that of temperature did not extended beyond cut 1 (Table 4.5)

4.3.6 Conclusions

In general, the effects that the different treatments generated on soil mineral N during the incubation phase were reflected in the exhaustive cropping of these soil samples with ryegrass. It is clear that the measurements obtained from the first cut provided the best indices for evaluating the availability of soil N as indicated by soil total mineral N content at sowing. The largest part of mineral N from the previous incubation was taken up during the first regrowth period. However, the total dry matter yield showed a weak correlation with available N. Therefore, in this experiment TDM provided a poor criterion for evaluating soil N availability. It was found that N uptake in the 1st cut (NY-1), N uptake in total herbage yield (THNY), total plant N uptake (TNY) and dry matter yield in the 1st cut (DM-1) were more useful plant characteristics for evaluating the availability of soil N. Chalk and Waring (1970), suggested that N uptake is a better index of available N than DM yield. Similarly, from a glasshouse study with 15 British soils having a range in texture, organic C and total N contents, Gasser and Kolembasa (1976) reported that N uptake by ryegrass from soil without the application of fertiliser N correlated better with N availability than did dry matter yield. In contrast it was found that with added fertiliser, dry matter yields of ryegrass at the first cut correlated better with available soil N than did N uptake.

The relationships between soil mineral N and ryegrass performance in the present experiment are in agreement with most of the studies reported for wheat, ryegrass and rice, summarised by Sahrawat (1983). The common conclusion from those experiments is that N uptake by

plants is a better criterion for soil N availability than dry matter yield, because plant growth is not governed only by N availability but also by numerous other factors, and uptake of N by the plant does not necessarily result in increased dry matter production. However, it is interesting to note that despite good agreement between the pattern of results from the present study and those cited in the relevant literature, there was a large difference in the approach to provide variation in soil available N to be tested against plant uptake and growth. While most of those studies cited, N was supplied as fertiliser, or utilised very contrasting soil types to vary soil N avilability, in the present experiment all the variation in soil mineral N was induced by manipulation of important biological components within a common soil medium.

The additional mineralisation that occurred because of the activities of earthworms in the previous incubation was significant for increasing plant growth. This result agrees well with Ingham et al. (1985), who concluded that while the advantages of increased N mineralisation by macrobes may be short-term, they may occur in many ecosystems in those short periods of ideal conditions when plant growth can occur. Certainly, surface mixing earthworms are very active in NZ pastures during the important growth periods of winter and spring. Therefore macrobes may perform important regulatory functions at critical times in the growth of plants.

CHAPTER 5

CHAPTER 5

GENERAL DISCUSSION

As a succession proceeds towards a more mature system, either grassland (Clark, 1977) or forest (Cole et al., 1977), a greater proportion of the total system N becomes tied up in biomass, and the internal cycling of N assumes major significance. Under such conditions, the ecological importance of macrobes (especially earthworms in temperate habitats) is a key feature, not only in influencing the rate of litter disappearance, but also the potential for mineralisation of contained nutrients. The availability of these nutrients for plant uptake depends on the amount of organic C available for microbial growth, and hence nutrient immobilisation, in competition with plant uptake.

The results obtained under the conditions of this study confirmed that earthworms have a low ecological efficiency (i.e. substantial ingestion of substrate but limited production; Reichle, 1977). Almost all the C was excreted; a large proportion was oxidised to CO_2 through respiration and removed from the system. (Appendix 4). Consequently a good deal of the N (doubtless, P and S also) bound to this C was released in mineral form (Fig. 3.9). This aspect (low ecological efficiency) highlights an important consideration which has encouraged investigation of any direct relationships betweeen decomposition rate and soil fertility.

Accumulation of mineral N in soil may be considered to result from a supply of N in excess of the needs of soil microorganisms (Stojanovic et al., 1956). Thus, if energy materials are present in excess, immobilisation of inorganic N takes place and significant quantities are not mineralised until the imbalance is in favour of N

release. During the preliminary incubation in this study, a similar quantity of mineral N accumulated in soils whether they were receiving herbage residues or not (earthworms were absent in both cases; see Fig. 3.9). An explanation may lie with the dynamics of the microbial population in the systems compared here. Importantly, the soil microbial biomass of the "check" samples was lower than those in soils receiving grass or clover residues (Table 3.6). We must conclude that most of the additional N provided in residues was simply incorporated into additional microbial biomass, or immobilised. All other nutrients were adequately supplied.

Figure 3.9 also illustrates the dramatic impact of earthworms in mineralisation. The additional N mineralised by the activities of earthworms (97 mg N/kg soil) is equivalent to almost twice the N provided on average to the system by litter (53 mg N/kg soil). The activities of earthworms may have induced some sort of "priming effect" (Smith, 1982); but, whatever the precise explanation, they certainly stimulated a release of mineral N well in excess of that achieved by the microbial fraction alone.

The presence of earthworms reduced microbial biomass (Table 3.6). This change relative to those treatments receiving only litter may have been due to competition for C as a result of the substantial consumption and respiration by the earthworms. Hence, energy was limiting both the soil microbes' growth and their capacity to immobilise N. This point illustrates the intimate linkage between energy (C) and nutrient flow in an ecosystem (Macfadyen, 1978), and suggests interesting possibilities for manipulating the different components of the soil ecosystem through manipulating organic substrates. Importantly, in spite of metabolic activities being considered as a key feature to interpreting the rate of nutrient flow, the relationship between substrate assimilation and biomass production

is not uniform and varies among different soil organisms within the soil biota (Reichle, 1977).

The high respiration rate of earthworms (in relation to their low production efficiency), when compared with microbes, probably stems from their substantial energy expenditures for burrowing, locomotion and feeding activities (Lee, 1985). However, there is another important difference in the manner in which microbes and earthworms satisfy their demands for energy and nutrients. While microbes (bacteria and fungi) absorb organic subtances from solution, earthworms ingest mainly solid particles (Richard, 1974). Other phagotrophes do likewise. This difference is fundamental, because fungi and bacteria may or may not utilise the same substance both for energy and as a source of nutrients. Thus, microbes can establish a better equilibrium between energy and nutrient requirements, because they may discriminate as to substrate utilisation. In contrast to this, when consuming organic materials for energy (C), earthworms are coincidentally ingesting other nutrients which may greatly exceed their nutritional requirements. Therefore, excess nutrients are excreted in both mineral forms and as more elaborated, organic materials. The latter was suggested by Bouche (1982), to indicate pathways for energy flow other than the respiration of C to carbon dioxide or the differences in energy between food and faeces.

The significance of examining carbon metabolism is that C (or energy) provides a common metabolic denominator by which it is possible to compare the intensity and significance of decomposer activities. Moreover, cycling of C and N within soil organic matter has been shown to be closely linked (McGill et al., 1981). Mineralised N, S and P are released from soil organisms as waste products during their search for energy, which is obtained through oxidation of C to carbon dioxide. The N cycle is intimately dependent

upon the activities of decomposers and, accordingly, available C can be a modifying factor.

Results of this study, even though obtained under very different conditions, confirm the recent finding of Reinertsen et al. (1984) about the primary importance of available C (rather than the simple C:N ratio) in decomposition processes and nutrient release. For instance, Table 4.1 shows that the C:N ratio at the end of incubation was very similar to the C:N ratio at the end of the exhaustive cropping experiment, with values being low enough to promote N mineralisation. However, only during the continuous addition of herbage residues in the incubation experiment was significant net mineralisation of N observed (Table 4.6). After that, during the cropping experiment, net mineralisation was minor. Consequently, N content and hence dry matter yield of ryegrass was adequate only at the early stages of plant growth. Perfomance at later harvests was poor (Tables 4.2 and 4.5). Therefore, despite the low C:N ratio in soil during the cropping phase the absence of fresh residues to provide a continuing source of available C and N appears to have been an important factor in limiting subsequent organic matter decomposition and, hence, a more appropriate N supply to the test plant.

BIBLIOGRAPHY

BIBLIOGRAPHY

Alexander, M. 1977. Introduction to Soil Microbiology. Wiley and Sons. New york. 476 p.

Anderson, D.E., and F.C. Boswell. 1964. The influence of low temperature and various concentration of ammonium nitrate on nitrification in acid soils. Soil Science Society of America Proceedings 28: 525-529.

Anderson, J.P.E. 1982. Soil respiration. In Methods of Soils Analysis Part II Chemical and Microbiological Properties. C.A. Black (ed.), pp. 831-872, Agronomy Monograph No. 9, American Society of Agronomy, Madison, Wisconsin.

Anderson, R.V., D.C. Coleman, and C.V. Cole. 1981. Effect of saprotrophic grazing on net mineralisation. In Terrestrial Nitrogen Cycles. F.E. Clark and T. Rosswall (eds.), pp. 201-216, Ecological Bulletin No. 33, Stockolm.

Ausmus B.S., N.T. Edward, and M. Witkamp. 1976. Microbial immobilisation of carbon, nitrogen, phosphorus and potassium: Implication for forest ecosystem processes. The 17th Symposium of the British Ecological Society. April, 1975, London, England, pp.397-416.

Ayanaba, A., S.B. Tuckwell, and D.S. Jenkinson. 1976. The effect of clearing and cropping on the organic reserves biomass of tropical forest soils. Soil Bilology and Biochemestry 8: 519-525.

Baath, E., U. Lohm, B. Lundgreen, T. Soderstrom, and B. Wiren. 1978. The efffect of N and C supply on the development ot soil organisms population and pine seedling. A microcosm experiment. Oikos 31: 153-163.

Ball, P.R. 1979. Nitrogen relationships in grazed and cut grass clover system. PhD. Thesis, Massey University, 217 pp.

Ball, P.R. 1982. Nitrogen balances in intensively managed pastures. Proceedings of a Workshop on Nitrogen Balances in Terrestrail Ecosystems in New Zealand. P.W. Gandar (ed.) pp. 47-66, Department of Scientific and Industrial Research, New Zealand.

Ball, P.R., and T.R.O. Field. 1982 . Responses to nitrogen as affected by pasture characteristics, season and grazing management. In Nitrogen Fertilisers in New Zealand Agriculture. P.B. Lynch (ed.), pp. 45-64, Ray Richard, Auckland.

Ball, P.R., and D.R. Keeney. 1983. Nitrogen losses from urine-affected areas of a New Zealand pasture, under contrasting seasonal conditions. Proceedings of the XIV International Grassland Congress, Lexington, pp. 342-344.

Ball, P.R., and J. Ryden. 1984. Nitrogen relationships in intensively managed temperate grasslands. Plant and Soil 76: 23-33.

Ball, P.R., and T.R.O. Field. 1985. Productivity and economics of legumes based pastures and grassland receiving fertiliser nitrogen. Procceding of the Trilateral Workshop on the Forage Legumes for Energy-Efficient Animal Production. R.F Barnes, P.R. Ball, R.W. Brougham, G.C. Martin, and D.J. Minson (eds.), pp. 47-55, Palmerston North.

Ball, P.R., and J.R. Crush. 1986. Prospect for incresing symbiotic nitrogen fixation in temperate grasslands. Proceedings of the XV International Grassland Congress, Kyoto, pp. 56-62.

Ball, P.R., and T.R.O. Field. 1986. Nitrogen cycling in intensively-managed grassland: A New Zealand view-point. Proceedings of the Symposium on the Nitrogen Cycling in Agricultural Systems ot Temperate Australia. P.E. Bacon, and J. Evans (eds.), in press, Australian Society of Soil Science, Wagga Wagga, Australia.

Barkoff, E. 1954. Contribution to the colourimetric determination of humus in soil. The Journal of the Scientific Agricultureal Society ot Finland 26: 198-210.

Barley, K.P. 1961. The abundance of earthworms in agriculture land and their possible significance in agriculture. Advances in Agronomy 13: 249-268.

Barley, K.P., and A.C. Jenning. 1959. Earthworms and soil fertility. III Influence of earthworms on the availabilitty of nitrogen. Australian Journal of Agricultural Research 10: 364-370.

Barley, K.P., and C.R. Kleining. 1964. The ocupation of newly irrigated land by earthworms. Australian Journal of Science 26: 290-291.

Bouche, M.B. 1982. Ecosysteme prairial. 4.3 Un example d'activite animale: Le role des lombriciens. Acta Oecologica, Oecologia Generalis 3 (1): 127-154.

Bremmeer, J.M. 1965. Total nitrogen. In Methods of Soil Analysis Part II Chemical and Microbiological properties. C.A. Black (ed.), pp. 1149-1176, Agronomy Monograph No. 9, American Society of Agronomy, Madison, Wisconsin.

Burden, R.J. 1982. Nitrate contamination of New Zealand aquifers: A review. New Zealand Journal of Science 25: 205-220

Campbell, C.A., and W. Souster. 1982. Loss of organic matter and potentially mineralisable nitrogen from Saskatchewan soils due to cropping. Canadian Journal of Soil Science 62: 651-656.

Campbell, C.A., E.A. Paul, and W.B. McGill. 1976. Effect of cultivation and cropping on the amount and forms of soil N. Proceeding of the Western Canada Nitrogen Symposium, Calgary, Alta, pp. 7- 101.

Carlyle, J.C., and D.C. Malcolm. 1985. Nitrogen availability beneath pure spurce and mixed larch + spurce stand growing on a deep peat. I. Net N mineralisation as measured by field and laboratory incubation. Plant and Soil 93: 95-113.

Carlyle, J.C., and D.C. Malcolm. 1986. Nitrogen availability beneath pure spurce and mixed larch + spurce stand growing on a deep peat. II. A comparison ot N availability as measured by plant uptake and long-term laboratory incubation. Plant and Soil 93: 115-122.

Carran, R.A. 1983 Changes in soil nitrogen during psture-crop sequences-A review. Proceedings Agronomy Society of New Zealand 13: 29-32

Chalk, P.M., and S.A. Waring. 1970. Evaluation ot a rapid test for assesing nitrogen availability in wheat soils. I. Correlation with plant indices ot availability obtained in pot culture. Australian Journal of Experimental Agriculture and Animal Husbandry 10: 298-305.

Chauhan, B.S., J.W. Stewart, and E.A. Paul. 1979. Effect of carbon addition on soil labile inorganic, organic and microbially held phosphate. Canadian Journal of Soil Science 59: 387-396.

Cheng, H.H., and J.M. Bremmer. 1965. Gaseous torm ot nitrogen. In Methods of Soil Analysis Part II Chemical and Microbiological properties. C.A. Black (ed.), pp. 1287-1323 Agronomy Monograph No. 9, American Society of Agronomy, Madison, Wisconsin.

Clarholm, M. 1981. Protozoan grazing of bacteria in soil impact and importance. Microbial Ecology 7: 343-350.

Clark, F.E. 1977. Internal Cycling ot N in shortgrass prairie. Ecology 58: 1322-1333.

Clement, C.R., M.J. Hopper, and L.H.P. Jones. 1978. The uptake of nitrate by Lolium perenne from flowing nutrient solutions. Journal of Experimental Botany 29: 453-464.

Cole, C.V., G.S. Innis, and J.W.B. Stewart. 1977. Simulation of phosphorus cycling in semiarid grassland. Ecology 58: 1-15.

Coleman, D.C., C.V. Cole, H.W. Hunt, and D.A. Klein. 1978. Trophic interaction in soils as they affect energy and nutrient dynamics. I. Introduction. Microbial Ecology 4: 345-349.

Coleman, D.C., C.P.P. Reid, and C.V Cole. 1983. Biological strategies of nutrient cycling in soil systems. Advances in Ecological Research 13: 1-53.

Crooke, W.M., and W.F. Simpson. 1971. Determination of ammonium in Kjeldahl digest of crop by an automated procedure. Journal of the Science of Food and Agriculture 22:9-10.

Darwin, C. 1904. The Formation of Vegetable Mould through the Action of Worms, with Observations on their Habits. Murray, London, 298 pp.

Douglas, L.A., and J.M. Bremmer. 1970. Extraction and colourimetric determination of urea in soils. Soil Science Society of America Proceedings 34: 859-862.

Edward, C.A., and G.W. Heath. 1963. The role of soil animals in breakdown of leaf materials. In Soil Organisms. J. Doeksen, and I. der Drift (eds.), pp. 76-80, North-Holland Publ. Co., Amsterdam.

Elliot, E.T., D.C. Coleman, R.E. Ingham, and J.A. Trofymow. 1984. Terrestrial ecosystems. Proceedings of the Third International Symposium on Microbial Ecology. M.J. Klug, and C.A. Reddy (eds.), pp. 424-433, American Society of Microbiology, Michigan, United States.

Fenchel, T., and T.H. Blackburn. 1979. Bacteria and Mineral Cycling. Acadamic Press, London, 225 pp.

Field, T.R.O., and P.R. Ball. 1978. Tactical use of fertiliser nitrogen. Proceedings Agronomy Society of New Zealand 8: 129-133

Forbes, R.S. 1974. Decomposition of agricultural crop debris. In Biology of Plant Litter Decomposition. C.H. Dickinson, and G.J.F. Pugh (eds.), pp. 723-742, Academic Press, London.

Frederick, L.R. 1956. The formation of nitrate from ammonium nitrogen in soil. I. Effect of temperature. Soil Science Society of America Proceedings 20: 496-500.

Freeney, J.R., and C.H. Williams. 1983. The sulphur cycle in soil. In The Global Biogeochemical Sulphur Cycle. M.V. Ivanov, and J.R. Freeney (eds), pp. 129-201, John Wiley and Sons, Inc. New York.

Gardner, W.H. 1965. Water content. In Methods of Soils Analysis Part I Physical and Mineralogical Properties, Including Statistics of Measurements and Sampling. C.A. Black (ed.), pp. 82-129, Agronomy Monograph No. 9, American Society of Agronomy, Madison, Wisconsin.

Gasser, J.K.R., and S.J. Kolembasa. !976. Soil nitrogen. IX. The effects of leys and organic manures on the available N in clay and sandy soils. Journal of Soil Science 27: 237-249.

Gillingham, A.G., J.K Syers, and P.E.H. Gregg. 1984. Nutrient cycling and management in grazed pastures. New Zealand Agricultural Science 18 (3) 115-119.

Goh, K.M., and S. Heng. 1985. Effect of forest conversion on organic matter accumulation and soil nutrient status in Golden Downs State Forest, Nelson. Proceedings of the Soil Dynamics and Land Use Seminar. I.B. Campbell (ed.), pp. 294-312, New Zealand Society of Soil Science and New Zealand Soil Conservation Association, Blenheim, New Zealand.

Golebiouska, J., and L. Ryszkowski. 1977. Energy and carbon fluxes in soil compartments of ecosystems. Proceedings of the VI International Soil Zoology Colloquium of the International Society of Soil Science. U. Lohm, and T. Persson (eds.), 274-283, Uppsala, Sweden.

Gray, T.R.G., and S.T. Williams. 1971. Soil Microorganisms. Oliver and Boyd, Edinburgh, 116 pp.

Hargrave, B.T. 1976. The central role of invertebrate faeces in sediment decomposition . The 17th Symposium of the British Ecological Society. April, 1975, London, England, pp. 301-322.

Harmsen, G.W., and G.J. Kolenbrander. 1965. Soil inorganic nitrogen. In Soil Nitrogen. W.V. Bartholomew, and F.E. Clark (eds.), pp. 43-92, Agronomy Monograph No. 10, American Society of Agronomy, Madison, Wisconsin.

Harmsen, G.W., and D.A. van Schrevon. 1955. Mineralisation of organic nitrogen in soil. Advances in Agronomy 8: 300-398.

Hausenbuiller, R.J. 1980. Soil Science. Principles and Practices. 2nd Edition, Publisher, Iowa, 611 pp.

Heal, O.W., and P. Ineson. 1984. Carbon and energy flow in Terrestrial Ecosystems: Relevance of microflora. Proceedings of the Third International Symposium on Microbial Ecology. M.J. Klug, and C.A. Reddy (eds.), pp. 394-403, Michigan, United States.

Henzell, E.F., and P.J. Ross. 1973. The nitrogen cycle of pasture ecosystems. In Chemistry and Biochemistry of Herbage. G.W. Butler,and P.W. Bailey (eds.), pp. 227-246 Academic Press, New York.

Henzell, E.F., I. Vallis, and J.E Lindquist. 1968. Automatic colourimetric methods for the determination of nitrogen in digest and extracts of soils. CSIRO, Paper No. 53, Canberra.

Hoglund, J.H. 1985. Grazing intensity and soil nitrogen accumulation. Proceedings of the New Zealand Grasland Association 46: 65-69.

Holmes, W. 1980. Grass, its Production and Utilisation. Blackwell Scientific Publications, Oxford, 295 pp.

Hunt, W.F. 1971. Leaf death and decomposition during pasture regrowth. New Zealand Journal of Agricultural Research 14: 208-218.

Hylton, L.O., D.F. Williams, A. Ulrich, and D.R. Cornelius. 1964. Critical nitrate levels for growth of italian ryegrass. Crop Science 4: 16-19.

Jackman, R.H. 1964. Accumulation of organic matter in some New Zealand soils under permanent pasture. New Zealand Journal of Agricultural Research 7: 445-471.

Jansson, S.L., and J. Persson. 1982. Mineralisation and immobilisation of soil nitrogen. In Nitrogen in Agricultural Soils. F.J. Stevenson (ed.), pp. 229-248, Agronomy Monograph No. 22, Madison, Wisconsin.

Jawson, M.D., and L.F. Elliot. 1986. Carbon and nitrogen transformations during wheat straw and root decomposition. Soil Biology and Biochemistry 18: 15-22.

Jenkinson, D. S., and D.S. Powlson. 1976. The effects of biocidal tratments on metabolism in soil. V. A method for measuring soil biomasss. Soil Biology and Biochemistry 8: 209-213.

Kaplan, D.L., R., Hartenstein, E.F. Neuhauser, and M.R. Malecki. 1980. Physicochemical requirements in the environment ot the earthworms Eisenia Foetida. Soil Biology and Biochemistry 12: 347-352.

Keogh, R.G. 1979. Lumbricid earthworm activities and nutrient cycling in pasture ecosystem. Proceedeings of the 2nd Australasian Conference Grassland Invertebrates Ecology. T.K. Crosby, and R.P. Pottinger (eds.), pp. 49-51, Government Printer, Wellington, New Zealand.

Keeney, D.R. 1985. Mineralisation of nitrogen from legume residues. Proceedings ot the Trilateral Workshop on the Forage Legumes for Energy-Efficient Animal Production. R.F Barnes, P.R. Ball, R.W. Brougham, G.C. Martin, and D.J. Minson (eds.), pp. 177-182 Palmerston North.

Laird, J.M., and M. Kroger. 1981. Earthworms. Critical review in environmental control 11: 189-188.

Lazenby, A. 1983. Nitrogen relationships in grassland ecosystems. Proceedings of the XIV International Grassland Congress, Lexington, pp. 56-63.

Lee, K.E. 1985. Earthworms. Their Ecology and Relationships with soils and land use. Academic Press, Sydney, 399 pp.

Lee, K.E., and J.N. Ladd. 1984. Some recent advances in soil biology and biochemistry. Proceedings ot the National Soil Conference, Brisbane, pp. 83-103.

Levy, E.B. 1970. Grassland of New Zealand. Government Printer, Wellington, 344 pp.

Lofty, J.R. 1974. Oligochaetes. In Biology of Plant Litter Decomposition. C.H. Dickinson, and G.J.F.Pugh (eds.), pp. 467-488, Academic Press, London

Mackay, A.D., and E.J. Kladivko. 1985. Earthworms and rate of breakdown of soybean and maize residues in soil. Soil Biology and Biochemistry 17: 851-857.

Madsen, B.L. 1972. Detritus on stones in small streams. Memorias dell'Istituto Italiano di Idrobiologia 29: 385-404

Maldague, M.E. 1979. Role des animaux edaphiques dans la fertilite des sols forestiers. Publications de l'Institut National pour l'Etude Agronomique du Congo. Serie Scientifique No. 112.

Malone, C.R., and D.E. Reichle. 1973. Chemical manipulation of biota in a fescue meadow. Soil Biology and Biochemistry 5: 629-639.

Marshall, K.C., and M. Alexander. 1960. Competition between soil bacteria and fusarium. Plant and Soil 12: 143-153.

Mengel, K., and E.A. Kirkby. 1982. Principles of Plant Nutrition. Third Edition, International Potash Institute, Bern, Switzerland, 655 pp.

MacFadyen, A. 1978. The role of the fauna in decomposition processes in grasslands. Scientific Proceedings, Royal Dublin Society Serie A 6: 197-206.

MacKenzie, A.J., W.F. Spencer, K.R. Stockinger, and B.A. Krantz. 1963. Seasonal nitrate-nitrogen content of cotton petioles as affected by nitrogen application and its relationships to yield. Agronomy Journal 55: 55-59.

McGill, W.B., C.V. Cole. 1981. Comparative aspect of cycling of organic C, N, S, and P through soil organic matter. Geoderma 26: 267-286.

O'Connor, M.B, and B.W. Parker. 1984. Applying nutrients. Making the best decisions. New Zealand Agricultural Science 18: 119-122.

Parkinson, D., T.R. Gray, and S.T. Williams. 1971. Methods for studying the ecology of soil microorganisms. Blackwell Scientific Publications, London, 116 pp.

Parle, J.N. 1963. A microbiological study of earthworm casts. Journal of General Microbiology 31: 13-22.

Parr, J.F., and R.I. Papendick. 1978. Factors affecting the decomposition of crop residues by microorganisms. In Crop Residue Management Systems. W.R. Oschwald (ed.), pp. 109-209, Special Publication No. 31, American Society of Agronomy, Madison, Wisconsin.

Paul, E.A., and R.P. Voroney. 1980. Nutrient and energy flows through soil microbial biomass. In Contemporary Microbial Ecology. D.C. Elwood (ed.), pp.215-237, Academic Press, London.

Paul, E.A., and R.P. Voroney. 1984. Field interpretation of microbial biomass activity measurements. Proceedings of the Third International Symposium on Microbial Ecology. M.J. Klug, and C.A. Reddy (eds.), pp. 509-514, American Society of Microbiology, Michigan, United States.

Petersen, H., and M. Luxton. 1982. A comparative analysis of fauna population and their role in decomposition processes. Oikos 39: 288-388.

Phillipson, J., R. Abel, J. Steel, and S.R.J. Woodell. 1978. Earthworms Numbers, biomass and respiratory metabolism in a beech woodland. Oecologia 33: 291-309.

Powlson, O.S., and D.S. Jenkinson. 1976. The effect of biocidal treatment on metabolism in soil. II. Gamma irradiation, autoclaving, air drying and fumigation with chloroform on methyl bromide. Soil Biology and Biochemestry 8: 179-188.

Raw, F. 1962. Studies of earthworms populations in orchard. I. Leaf burial in apple orchards. Annals of Applied Biology 50: 389-404.

Reichle, D.E. 1977. The role of soil invertebrates in nutrient cycling. Proceedings of the VI International Soil Zoology Colloquium of the International Society ot Soil Science. U. Lohm, and T. Persson (eds.), pp. 145-156, Uppsala, Sweden.

Reinertsen, S.A., L.F. Elliot, V.L. Cochran, and G.S. Campbell. 1984. Role of available carbon and nitrogen in determinig the rate ot wheat straw decomposition. Soil Biology and Biochemistry 16: 459-464.

Richard, B.N. 1974. Introduction to the soil ecosystem. Longman, New York 260 pp.

Russell, E.W. 1973. Soil Conditions and Plant Growth. 10th Edition, Longman, London, 849 pp.

Saggar, S., J.R. Bettany, and J.W. Stewart. 1981. Sulphur transformation in relation to carbon and nitrogen in incubated soils. Soil Biology and Biochemistry 13: 499-511.

Sahrawat, K.L. 1983. Correlation between index of soil nitrogen avilability percent in plant, nitrogen uptake, and dry matter yield of rice grown in greenhouse. Plant and Soil 74: 223-228.

Satchell, J.E. 1967. Lumbricidae. In Soil BIology. A. Burges, and F. Raw (eds.), pp. 259-322, Academic Press, London.

Satchell, J.E., and D.G. Lowe. 1967. Selection ot leat litter in Lumbricus terrestris. In Progress in Soil Biology. O. Graft, and J.E. Satchell (eds.), pp.102-119 North-Holland Pub. Company, Amsterdam.

Satchell, J.E. 1974. Introduction. In Biology of Plant Litter Decomposition. C.H. Dickinson, and G.J.F. Pugh (eds.), Vol. I, pp. i- xxxvi, Academic Press, London.

Schmidt, E.L. 1982. Nitrification in soils. In Nitrogen in Agricultural Soils. F.J. Stevenson (ed.), pp. 253-283, Agronomy Monograph No. 22, Madison, Wisconsin.

Scott, D., J.M. Keoghan, G.G. Gossens, L.A. Manssell, M.J.S. Floate, B.J. Wills, and G. Douglas. 1985. Limitations to pasture production and choice of species. Proceedings of the Conference Using Herbage Cultivars. R.E. Burges, and J.L. Brock (eds.), pp. 9-16, New Zealand Grassland Association, Lincoln College, New Zealand.

Sears, P.D. 1962. Exploitation of high production pastures in New Zealand. Proceedings of the New Zealand Ecological Society 9: 57-63.

Sears, P.D., and L.T. Evans. 1953. Pasture growth and soil fertility. III. The influence of red and white clovers, superephosphate, lime, and dung and urine on soil composition and on earthworms and grass-grub population. New Zealand Journal of Science and Technology 35 A: 42-52.

Sears, P.D., V.C. Goodall, R.H. Jackman, R.H. Robinson. 1965a. Pasture growth and soil fertility. VIII. The influence of grasses, white clover, fertiliers and the return of herbage clippings on pasture production of an impoverished soil. New Zealand Journal of Agricultural Reserch 8: 270-283.

Sears, P.D., V.C. Goodall, R.H. Jacknman. 1965b. Pasture growth and soil fertility. IX. Repeated cropping of a soil previously under permanent pasture. New Zealand Journal of Agricultutral Research 8: 497-510

Sharpley, A.N., and J.K. Syers. 1976. Potential role of earthworms cast for phosphorus enrichment of runoff waters. Soil Biology and Biochemistry 8: 341-346.

Shen, S.M., G. Pruden, and D.S. Jenkinson. 1984. Mineralisation and immobilisation of nitrogen in fumigated soil and the measurement of microbial biomass nitrogen. Soil Biology and Biochemistry 16: 427-444.

Sinclair, A.G., and I.S. Cornforth. 1984. A modification of the "superchoice" phosphate maintenance model. New Zealand Journal of Experimental Agriculture 12: 141-144.

Smith, O.L. 1982. Soil microbiology: A model of decomposition and nutrient cycling. CRC Press Inc., Florida, 273 pp.

Soil Survey Staff. 1975. Soil Taxonomy. A basic system of soil classification for making and interpreting soil surveys. U.S. Department of Agriculture, Agriculture Handbook No. 36.

Spedding, C.R.W., and E.C. Diekmahan. 1972. Grasses and Legumes in British Agriculture. Commenwealth Agricultural Bureaux, Bulletin No. 49, 511 pp.

Stanford, G. M.H. Frere, D.H. Schwaninger. 1973. Temperature coefficient of soil mineralisation. Soil Science 115: 321-323.

Stanford G. 1982. Assesment of soil nitrogen availability. In Nitrogen in Agricultural Soils. F.J. Stevenson (ed.), pp. 229-248, Agronomy Monograph No. 22, Madison, Wisconsin.

Steele, K.W. 1982. Nitrogen in grassland soils. In Nitrogen Fertilisers in New Zealand Agriculture. P.B. Lynch (ed.), pp. 29-44, Ray Richard, Auckland.

Stockdill, S.M.J. 1959. Earthworm improve pasture growth. New Zealand Journal of Agriculture 98: 227-233

Stockdill, S.M.J. 1966. The effect of earthworms on pastures. Proceedings of the New Zealand Ecological Society 13: 68-74.

Stockdill, S.M.J. 1982. Effects of introduced earthworms on the productivity of New Zealand pastures. Pedobiologia 24: 29-35.

Stojanovic, B.J. and F.E. Broadbent. 1956. Immobilisation and mineralisation of nitrogen during decomposition of plant residues in soil. Soil Science Society of American Proceedings 20: 213-218.

Stout, J.D., K.R. Tate, and L.F. Molloy. 1976. Decomposition processes in New Zealand soils with particular respect to rates and pathways of plant degradation. The 17th Symposium of the British Ecological Society. April, 1975, London, pp. 97-144.

Stout, J.D. 1980. The role of protozoa in nutrient cycling and energy flow. Advances in Microbial Ecology 4: 1-50.

Stout, J.D. 1983. Organic matter turnover by earthworms. In Earthworm Ecology. From Darwin to Vermiculture. J.E. Satchell (ed.), pp. 35-48, Chapman and Hall, London.

Syers, J.K., A.N. Sharpley, and D.R. Keeney. 1979. Cycling of nitrogen by surface-casting earthworms in a pasture ecosystem. Soil Biology and Biochemistry 11: 181-185.

Syers, J.K., and J.A. Springett. 1983. Earthworm ecology in grassland soils. In Earthworm Ecology. From Darwin to Vermiculture. J.E. Satchell (ed.), pp. 67-83, Chapman and Hall, London.

Syers, J.K., and J.A. Springett. 1984. Earthworms and soil fertility. Plant and Soil 76: 93-104.

Taylor, N.H., and I.J. Pohlen. 1968. Classification of New Zealand soils. New Zealand Soil Bureau, bulletin No. 26, 142 pp.

Trudinger, P.A. and D.J. Swine. 1979. Biogeochemical cycling of mineral-forming elemnts. Elsevier Scientific Publishing Co., Amsterdam, 648 .pp.

Twine, J.R., and C.H. Williams. 1971. The determination of phosphorus in Kjeldahl digest of plant material by automatic analysis. Communications in Soil Science and Plant Analysis 2: 485-489.

van Burg, P.F.J. 1966. Nitrate as an indicator of the nitrogen-nutrition status of grass. Proceedings of the X International Grassland Congress, Finland, pp. 267-272.

van Veen, J.A., and E.A. Paul. 1981. Organic carbon dynamics in grassland soils. 1. Background information and computer simulation. Canadian Journal of Soil Science 61: 185-201.

van Veen, J.A., J.N. Ladd, and M. Amato. 1985. Turnover of carbon and nitrogen through the microbial biomass in a sandy loam and clay soil incubated with glucose and ammonium sulphate under different moisture regimes. Soil Biology and Biochemestry 17: 747-756.

Vimmerstedt, J.P., and J.H. Finney. 1973. Impact of earthworms introduction on litter burial and nutrient distribution in Ohio strip mine spoil banks. Soil Science Society of American Proceedings 37: 388-391.

Voroney, R.P., J.A. van Veen, and E.A. Paul. 1981. Organic carbon dynamics in grassland soils. 2. Model validation and simulation of the long-term effects of cultivation and rainfall erosion. Canadian Journal of Soil Science 61: 211-224.

Wallwork, J.A. 1976. The Distribution and Diversity of Soil Fauna. Academic Press, London, 335 pp.

Waters, R.A.S. 1955. Numbers and weights of earthworms under a highly productive pasture. New Zealand Journal of Science and Technology 36A: 516-525.

Whitehead, D.C. 1970. The Role of Nitrogen in Grassland Productivity. Commenwealth Agricultural Bureaux, Bulletin No. 48, London, 202 pp.

Wilkinson, S.R., and R.W. Lowrey. 1973. Cycling mineral nutrients in pasture ecosystems. In Chemistry and Biochemistry of Herbage. G.W. Butler, and P.W. Bailey (eds.), pp. 247-315 Academic Press, New York.

Wilman, D. 1965. The effect of nitrogenous fertilizer on the rate of growth of italian ryegrass. Journal of British Grassland Society 20: 248-254.

Wilman, D., and P.T. Wright. 1983. Some effects of applied nitrogen on the growth and chemical composition of temperate grasses. Herbage Abstracts 53 (8) 387-393.

Witkamp, M., and B.S. Ausmus. 1976. Processes of decomposition and nutrient transfer in forest systems. The 17th Symposium of the British Ecological Society. April, 1975, London, England, pp. 375-396.

Yeates, G.W. 1979. Soil nematodes in terrestrial ecosystems. Journal of Nematology 11: 213-229.

APPENDICES

Appendix 1 Soil respiration as oxygen consumption and carbon dioxide evolution (mM/day)

Treatments	nM/day		
	O2	CO2	R.Q.
Grass litter (15 C)	1.69	1.44	0.85
Clover litter "	1.83	1.57	0.86
Grass + W "	2.38	1.91	0.80
Clover + W "	2.62	1.97	0.75
Grass litter (22.5 C)	2.02	1.66	0.82
Clover litter "	2.26	1.78	0.79
Grass + W "	2.92	2.14	0.73
Clover + W "	2.99	2.10	0.70
LSD (=0.05)	0.12	0.09	0.05
Check 1 (15 C)	1.32	1.10	0.83
Check 2 (22.5 C)	1.39	1.37	0.98

(W = earthworms)

Appendix 2 Soil mineral N at the end of incubation

(mg N/kg soil)

	NH4	NO3	TOTAL
Grass (15)	123.4	54.9	178.2
Clover (15)	131.0	58.9	189.9
Grass + W (15)	92.6	182.7	275.3
Clover + W (15)	99.7	193.2	292.9
Grass (22.5)	58.0	147.6	205.6
Clover (22.5)	66.7	144.6	211.3
Grass + W (22.5)	34.6	248.8	283.4
Clover + W (22.5)	59.4	263.8	323.2
LSD (P=0.05)	49.8	53.5	30.6
check 1	120.0	61.9	181.9
check 2	121.2	84.7	205.9

(W = earthworms)

Appendix 3 Total dry matter of yield ryegrass

Treatments	g/pot
Grass (15 C)	6.73
Clover (15 C)	7.29
Grass + W (15 C)	7.08
Clover + W (15 C)	7.24
Grass (22.5 C)	5.42
Clover (22.5 C)	6.35
Grass + W (22.5 C)	6.42
Clover + W (22.5 C)	
LSD	1.18
check 1	6.01
check 2	5.81

(W = earthworms)

Appendix 4 Carbon dioxide evolution, and CO2-C as a % of C Added in Litter (1)

Treatments	CO2-C /11 weeks (mM CO2-C)	Net % of C added (2)
Grass litter (15 C)	110.9	28.3
Clover litter "	120.9	39.2
Grass + W "	147.1	67.5
Clover + W "	151.7	75.2
Grass litter (22.5 C)	127.8	24.1
Clover litter "	137.1	34.2
Grass + W "	164.8	64.0
Clover + W "	161.7	60.8
Check 1 (15 C)	84.7	-
Check 2 (22.5 C)	105.5	-

(W = earthworms)

(1) 92.3 mM C added in litter

(2) Corrected for activity in "check" samples

Appendix 5 Ammonia volatilisation during incubation

Main effects	mg N/kg soil/11 weeks
Earthworms	
with	0.02
without	0.07
Litter	
grass	0.01
clover	0.08
Temperature	
15 C	0.04
22.5 C	0.05
Lsd (P=0.05)	0.01
Check 1	0.02
Check 2	0.02

Appendix 6 Partial mass balances for N added (1)

mg N/kg dry soil

	Grass	Clover	Grass+Worms	Clover+Worms
INPUTS	35.3	70.6	35.3	70.6
APPARENT RECOVERY				
Microbial biomass (2)	36.6	35.8	15.0	13.3
Earthworms biomass	-	-	3.4	4.1
Min. N (end incub.)	-1.8	6.7	84.5	114.1
Ammonia volat.	0.0	0.1	0.0	0.0
Plant uptake	1.9	38.4	60.7	94.5

a) At the end of incubation

Systems	Inputs	App. recovery	Difference
Grass	35.3	34.8	0.50
Grass+earthworms	35.3	102.9	-67.60
Clover	70.6	42.6	28.00
Clover+earthworms	70.6	131.5	-60.90

(cont. Appendix 6)

b) At the end of exhaustive cropping

Systems	Inputs	App. recovery	Difference
Grass	35.3	38.5	-3.20
Grass+earthworms	35.3	79.1	-43.80
Clover	70.6	74.3	-3.70
Clover+earthworms	70.6	111.9	-41.3

(1) All values corrected by checks,

(2) N in microbial biomass was estimated from the values of C biomass according to the relation propossed by Ayanaba et al. (1976)

Appendix 7 Analysis of variance

(Levels of significance: 0.05= 4.60; 0.01= 8.86)

a) Carbon dioxide evolution

SOURCE OF VARIATION	DF	SS	SS%	MS	VR
reps STRATUM	2	0.003908	0.28	0.001954	
reps.*UNITS* STRATUM					
temperat	1	0.246038	17.56	0.246038	93.200
litter	1	0.028704	2.05	0.028704	10.873
worms	1	1.054204	75.23	1.054204	399.338
temperat.litter	1	0.006338	0.45	0.006338	2.401
temperat.worms	1	0.003037	0.22	0.003037	1.151
litter.worms	1	0.018704	1.33	0.018704	7.085
temperat.litter.worms	1	0.003504	0.25	0.003504	1.327
RESIDUAL	14	0.036958	2.64	0.002640	
TOTAL	21	1.397488	99.72	0.066547	
GRAND TOTAL	23	1.401396	100.00		
GRAND MEAN	1.822				
TOTAL NUMBER OF OBSERVATIONS		24			

STRATUM STANDARD ERRORS AND COEFFICIENTS OF VARIATION

STRATUM	DF	SE	CV%
reps	2	0.0156	0.9
reps.*UNITS*	14	0.0514	2.8

(cont. appendix 7)

b) Oxygen consumption

SOURCE OF VARIATION	DF	SS	SS%	MS	VR
reps STRATUM	2	0.008958	0.18	0.004479	
reps.*UNITS* STRATUM					
temperat	1	1.037504	21.00	1.037504	201.248
litter	1	0.175104	3.54	0.175104	33.965
worms	1	3.611505	73.08	3.611505	700.534
temperat.litter	1	0.002204	0.04	0.002204	0.428
temperat.worms	1	0.009204	0.19	0.009204	1.785
litter.worms	1	0.001504	0.03	0.001504	0.292
temperat.litter.worms	1	0.023438	0.47	0.023438	4.546
RESIDUAL	14	0.072175	1.46	0.005155	
TOTAL	21	4.932638	99.82	0.234888	
GRAND TOTAL	23	4.941597	100.00		
GRAND MEAN	2.338				
TOTAL NUMBER OF OBSERVATIONS	24				

STRATUM STANDARD ERRORS AND COEFFICIENTS OF VARIATION

STRATUM	DF	SE	CV%
reps	2	0.0237	1.0
reps.*UNITS*	14	0.0718	3.1

(cont. Appendix 7)

c) Soil nitrate at the end of the incubation.

SOURCE OF VARIATION	DF	SS	SS%	MS	VR
reps STRATUM	2	6706.1	4.61	3353.0	
reps.*UNITS* STRATUM					
temperat	1	37193.6	25.58	37193.6	40.191
litter	1	264.0	0.18	264.0	0.285
worms	1	87338.5	60.08	87338.5	94.377
temperat.litter	1	2.3	0.00	2.3	0.002
temperat.worms	1	649.0	0.45	649.0	0.701
litter.worms	1	223.3	0.15	223.3	0.241
temperat.litter.worms	1	48.7	0.03	48.7	0.053
RESIDUAL	14	12955.9	8.91	925.4	
TOTAL	21	138675.3	95.39	6603.6	
GRAND TOTAL	23	145381.4	100.00		
GRAND MEAN		161.8			
TOTAL NUMBER OF OBSERVATIONS		24			

STRATUM STANDARD ERRORS AND COEFFICIENTS OF VARIATION

STRATUM	DF	SE	CV%
reps	2	20.47	12.7
reps.*UNITS*	14	30.42	18.8

(Cont. Appendix 7)

d) Soil ammonium at the end of the incubation

SOURCE OF VARIATION	DF	SS	SS%	MS	VR
reps STRATUM	2	2634.3	6.90	1317.1	
reps.*UNITS* STRATU					
temperat	1	19425.7	50.90	19425.7	24.029
litter	1	876.0	2.30	876.0	1.084
worms	1	3210.9	8.41	3210.9	3.972
temperat.litter	1	132.5	0.35	132.5	0.164
temperat.worms	1	366.6	0.96	366.6	0.453
litter.worms	1	91.3	0.24	91.3	0.113
temperat.litter.worms	1	106.7	0.28	106.7	0.132
RESIDUAL	14	11317.9	29.66	808.4	
TOTAL	21	35527.6	93.10	1691.8	
GRAND TOTAL	23	38161.8	100.00		
GRAND MEAN		83.2			
TOTAL NUMBER OF OBSERVATIONS		24			

STRATUM STANDARD ERRORS AND COEFFICIENTS OF VARIATION

STRATUM	DF	SE	CV%
reps	2	12.83	15.4
reps.*UNITS*	14	28.43	34.2

(cont. Appendix 7)

e) Total soil mineral N at the end of the incubation

SOURCE OF VARIATION	DF	SS	SS%	MS	VR
reps STRATUM	2	1825.4	2.64	912.7	
reps.*UNITS* STRATUM					
temperat	1	2853.6	4.13	2853.6	9.348
litter	1	2096.3	3.03	2096.3	6.867
worms	1	57027.8	82.52	57027.8	186.814
temperat.litter	1	98.8	0.14	98.8	0.324
temperat.worms	1	40.8	0.06	40.8	0.134
litter.worms	1	597.0	0.86	597.0	1.956
temperat.litter.worms	1	297.5	0.43	297.5	0.975
RESIDUAL	14	4273.7	6.18	305.3	
TOTAL	21	67285.5	97.36	3204.1	
GRAND TOTAL	23	69110.9	100.00		
GRAND MEAN		245.0			

TOTAL NUMBER OF OBSERVATIONS 24

STRATUM STANDARD ERROR AND COEFFICIENTS OF VARIATION

STRATUM	DF	SE	CV%
reps	2	10.68	4.4
reps.*UNITS*	14	17.47	7.1

(cont.Appendix 7)

f) Soil microbial biomass

SOURCE OF VARIATION	DF	SS	SS%	MS	VR
reps STRATUM	2	2709	1.51	1355	
reps.*UNITS* STRATUM					
temperat	1	5017	2.79	5017	2.707
litter	1	477	0.27	477	0.257
worms	1	143685	79.84	143685	77.534
temperat.litter	1	852	0.47	852	0.460
temperat.worms	1	376	0.21	376	0.203
litter.worms	1	57	0.03	57	0.031
temperat.litter.worms	1	852	0.47	852	0.460
RESIDUAL	14	25945	14.42	1853	
TOTAL	21	177261	98.49	8441	
GRAND TOTAL	23	179971	100.00		

GRAND MEAN 323.9

TOTAL NUMBER OF OBSERVATIONS 24

STRATUM STANDARD ERRORS AND COEFFICIENTS OF VARIATION

STRATUM	DF	SE	CV%
reps	2	13.01	4.0
reps.*UNITS*	14	43.05	13.3

(cont. Appendix 7)

g) Total N yield ot ryegrass

SOURCE OF VARIATION	DF	SS	SS%	MS	VR
reps STRATUM	2	179.4	0.55	89.7	
reps.*UNITS* STRATUM					
temperat	1	104.2	0.32	104.2	0.299
litter	1	7413.1	22.53	7413.1	21.267
worms	1	19745.6	60.01	19745.6	56.647
temperat.litter	1	245.8	0.75	245.8	0.705
temperat.worms	1	1.6	0.00	1.6	0.005
litter.worms	1	10.7	0.03	10.7	0.031
temperat.litter.worms	1	324.1	0.99	324.1	0.930
RESIDUAL	14	4880.0	14.83	348.6	
TOTAL	21	32725.1	99.45	1558.3	
GRAND TOTAL	23	32904.5	100.00		

GRAND MEAN 258.6

TOTAL NUMBER OF OBSERVATIONS 24

STRATUM STANDARD ERRORS AND COEFFICIENTS OF VARIATION

STRATUM	DF	SE	CV%
reps	2	3.35	1.3
reps.*UNITS*	14	18.67	7.2

(cont.Appendix 7)

h) Total dry matter yield ot ryegrass

SOURCE OF VARIATION	DF	SS	SS%	MS	VR
reps STRATUM	2	0.3348	2.21	0.1674	
reps.*UNITS* STRATUM					
temperat	1	3.8962	25.74	3.8962	8.026
litter	1	1.7442	11.52	1.7442	3.593
worms	1	1.3301	8.79	1.3301	2.740
temperat.litter	1	0.1926	1.27	0.1926	0.397
temperat.worms	1	0.5922	3.91	0.5922	1.220
litter.worms	1	0.2501	1.65	0.2501	0.515
temperat.litter.worms	1	0.0000	0.00	0.0000	0.000
RESIDUAL	14	6.7960	44.90	0.4854	
TOTAL	21	14.8014	97.79	0.7048	
GRAND TOTAL	23	15.1362	100.00		
GRAND MEAN	6.68				
TOTAL NUMBER OF OBSERVATIONS	24				

STRATUM STANDARD ERRORS AND COEFFICIENTS OF VARIATION

STRATUM	DF	SE	CV%
reps	2	0.145	2.2
reps.*UNITS*	14	0.697	10.4

(cont. Appendix 7)

i) Nitrate content of ryegrass herbage.

SOURCE OF VARIATION	DF	SS	SS%	MS	VR
reps STRATUM	2	5437033	4.53	2718517	
reps.*UNITS* STRATUM					
temperat	1	3390017	2.82	3390017	1.082
litter	1	15488267	12.89	15488267	4.942
worms	1	29882018	24.87	29882018	9.534
temperat.litter	1	3666017	3.05	3666017	1.170
temperat.worms	1	3285600	2.73	3285600	1.048
litter.worms	1	11788017	9.81	11788017	3.761
temperat.litter.worms	1	3315267	2.76	3315267	1.058
RESIDUAL	14	43879896	36.53	3134278	
TOTAL	21	114695096	95.47	5461671	
GRAND TOTAL	23	120132128	100.00		
GRAND MEAN		1322			
TOTAL NUMBER OF OBSERVATIONS		24			

STRATUM STANDARD ERRORS AND COEFFICIENTS OF VARIATION

STRATUM	DF	SE	CV%
reps	2	582.9	44.1
reps.*UNITS*	14	1770.4	134.0

(cont. Appendix 7)

j) Soil mineral N at the end of exhaustive cropping ot ryegrass.

SOURCE OF VARIATION	DF	SS	SS%	MS	VR
reps STRATUM	2	5.8800	16.67	2.9400	
reps.*UNITS* STRATUM					
temperat	1	8.1667	23.15	8.1667	9.211
litter	1	0.3267	0.93	0.3267	0.368
worms	1	0.0000	0.00	0.0000	0.000
temperat.litter	1	0.0000	0.00	0.0000	0.000
temperat.worms	1	0.3267	0.93	0.3267	0.368
litter.worms	1	2.9400	8.33	2.9400	3.316
temperat.litter.worms	1	5.2267	14.81	5.2267	5.895
RESIDUAL	14	12.4133	35.19	0.8867	
TOTAL	21	29.4000	83.33	1.4000	
GRAND TOTAL	23	35.2800	100.00		

GRAND MEAN 8.30

TOTAL NUMBER OF OBSERVATIONS 24

STRATUM STANDARD ERRORS AND COEFFICIENTS OF VARIATION

STRATUM	DF	SE	CV%
reps	2	0.606	7.3
reps.*UNITS*	14	0.942	11.3