PARTLY HUMIFIED ORGANIC MATTER IN SOILS:

ITS CONTRIBUTION TO MINERALIZABLE NITROGEN

A Thesis submitted

by

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SUMMARY

The principal aim of this investigation was the assessment of the contribution made to the reserves of mineralizable N in soils by non- or only partly-humified organic matter. To examine the hypothesis that this material is a labile, readily mineralized fraction, a method was developed for its separation from the soil. The most efficient and reproducible separation of 'free' organic matter from the clay-organic complex was achieved by ultrasonic dispersion of finely ground soil in a heavy (SG 2.0) organic liquid containing small amounts (c. 0.1% w/v) of a surfactant ('Aerosol OT'). The "light fraction" (LF) so obtained was shown to consist essentially of finely comminuted and partly humified plant fragments, together with phytoliths and trace amounts of soil minerals.

Chemical studies showed that although some 'humic' materials were present, the gross composition of the LF more nearly approximated that of fresh plant material than of 'humic' extracts of soils.

The total content of hydrolysable neutral carbohydrates in the LF was intermediate between that of undecomposed plant material and humic acid. Hexoses (notably glucose) accounted for the largest proportion (c. 75%) of the total neutral sugars in the LF, pentoses for c. 25% and 6-deoxy-hexoses for 1-2%. Some 35-80% of the N contained in the LF was released as amino acids on acid

hydrolysis. Generally the ratio of nitrogen to carbohydrate in the LF was sufficiently high for its decomposition to be accompanied by a net release of mineral N. Incubation studies indicated that the LF was a potentially available substrate for soil organisms, and that it decomposed some 4-40 times more rapidly than did the remainder of the soil N. During decomposition of the LF there was little change in the amounts of the individual amino acids present, although as the relative proportions of rhamnose and fucose increased it was suggested that the LF separated after incubation contained newly synthesised material.

The amounts of LF separated from a range of soils varied with soil type and agronomic history, and accounted for an appreciable proportion of the total organic N. For a loam (Red brown earth), a sand (Solonised brown soil) and a clay (Grey soil of heavy texture) under fallow-wheat (F-W) and pasture-fallow-wheat (P-P-F-W) rotations, the LF-N represented some 20%, 15-29%, and 3-7% of their total N respectively. Plots under a P-P-F-W rotation contained more LF-N than plots under a F-W rotation, and plots under pasture significantly more than similar plots under wheat. The pools of 'inert' N in these soils were estimated to represent some 30%, 40-50%, and 50-70% of the mean soil N in the loam, sand and clay respectively.

Seasonal changes in LF-N in the sand and clay were relatively small, and not obviously related to the patterns of

seasonal change in mineralizable N. During incubation at 35°C for 4 weeks at c. pF 2, plots under the P-P-F-W rotation released some 7% of their total N. However changes in the amount of LF-N were proportionally much greater, for while the LF only represented 3-30% of the total N, the loss of LF-N during incubation accounted for 25-60% of the mineral N released.

Thus the original hypothesis that the LF is a more labile part of the soil N was confirmed. Whilst the determination of the amount of LF-N provided a better estimate of the available N accumulated under pasture than did total soil N, it did not represent the only important source of mineralizable N in the soils examined.

As chemical fractionation procedures have generally proved unsatisfactory for the estimation of available N, the fractionation technique developed during this study will be of value in future investigations designed to locate and identify the labile N in soil.

PREFACE

Some of the preliminary results discussed in this thesis are based on material presented by the author to the University of Adelaide in partial fulfilment of the requirements for the degree of Bachelor of Agricultural Science with Honours.

This thesis contains no other material which has been accepted for the award of any other degree or diploma in any University, and to the best of the author's knowledge and belief, the thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

G. W. Ford
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I INTRODUCTION

The close relationship between the organic content of soils and their fertility has long been recognized, and has generally been attributed to the effects of the organic fraction on the physical and chemical properties of soils.

Organic matter is added to the soil by the activities of plants, and is subsequently modified and assimilated by the soil fauna and flora, the end product of this process being a fairly stable amorphous dark-coloured material generally termed 'humus'. Soil organic matter is thus a heterogeneous complex of plant and animal residues, microbial tissue, and 'humus'. Little is known of the relative importance of these 3 fractions in the biological cycling of N in arable soils, although the turnover of N in this complex "mineralization-immobilization cycle" has been intensively studied by many authors e.g. Jansson (1958). Humified materials commonly represent a large proportion of the soil N, but as they are apparently relatively resistant to decomposition they provide little of the N required by crops. Microbial tissue comprises only a small proportion of the total N, but whilst it is a highly active fraction its N is not normally released unless the soil is subjected to partial sterilization treatments (Jenkinson 1966a). Plant residues account for most of the remaining soil N, but although fresh plant material is generally rapidly decomposed in soil little is known of the dynamics of partly humified plant material.

Its possible significance in arable soils is indicated by observations of the rapid loss of plant residues from soil following the cultivation of old pastures (Saunder and Grant (1962)), and of a concomitant rapid mineralization of the soil N (Sears 1953; Kulakov 1960; Nye and Greenland 1964). It may therefore be postulated that this labile N consists of non-humified or only partly-humified plant material recently added to the soil.

In Australia most wheat is grown in rotation with grass-legume pastures, the yield and protein content of the crop being largely dependent upon the accretion of readily mineralizable N during the pasture phase of the rotation. The dynamics of partly humified plant material in some soils under pasture-wheat rotations has therefore been examined and related to changes in the mineralizable N in the soil. In order to do this it was first necessary to develop a method for the quantitative separation of this material from soil. As other authors had achieved partial separations using densimetric techniques (Khan 1959; Monnier, Turc and Jeanson-Luusinang 1962), the initial phase of the present study involved the critical examination and modification of these procedures. The "light fraction" (LF) so obtained was then examined to determine whether it was in fact composed of plant residues. Changes in the amounts of LF in some soils under pasture-wheat rotations were then related to the amount of mineralizable N present, with a view to evaluating the contribution made by the labile N in the LF to the total pool of labile N in the soil.

II REVIEW OF LITERATURE ON DYNAMICS OF ORGANIC MATTER WITH SPECIAL REFERENCE TO NITROGEN IN ARABLE SOILS

(A) Turnover of Organic Matter in Soil

(i) Concepts

Under a given land system the organic matter content of a soil will ultimately attain an equilibrium level where the rate of accretion to the soil from plant and animal sources balances the rate of mineralization. This process of simultaneous loss and gain is described as turnover, and may be defined as "the flux of organic matter through the organic matter in a given sample of soil" (Jenkinson 1963a).

The time required to attain equilibrium, and the actual level which is ultimately reached, will depend upon the interaction of many factors, the most important - for a given soil type and rotation - being:

(a) the quantity and quality of the organic matter present initially, e.g. forest (McGarity 1959; Nye and Greenland 1964) and natural and improved pastures (O'Connor, Robinson and Jackman 1962; Saunder and Grant 1962; Jackman 1964a, 1964b) all react differently to clearing, cultivation and cropping;

(b) the availability of nutrient elements required for plant growth, such as N, P, S or trace elements (C.H. Williams 1962), or whether lime is applied (Walker 1957);

(c) the system of land management practised, e.g. for pastures: the species, yield and management (e.g. whether grazed or cut

for fodder conservation; Melville and Sears 1953; Walker 1956a, 1956b; Clement and Williams 1964; Kleinig 1966; but cf. Watson and Lapins (1964) who found no effect from these treatments), or whether the land is periodically fallowed prior to cropping. Decomposition is depressed under actively growing plants, possibly due to competition for moisture (Jenkinson 1964a), and is stimulated by bare fallowing (e.g. Penman 1949).

However this equilibrium will be upset following a change in management practice such as cultivation or drainage, or a change in the type of vegetation. Thus the greater return or organic matter to the soil from grass or leguminous leys than from cereal crops leads to the net accumulation of organic matter in arable soils placed under such leys (e.g. Richardson 1938; Low 1955; Walker 1956c; Hood 1960; C.H. Williams 1962; Clement and Williams 1964; Heard 1965). Conversely, cultivation and cropping of such pasture land leads to a net loss of soil organic matter, the rate of loss apparently being greatest immediately following cultivation (e.g. Clarke and Marshall 1947; Sears 1953; McGarity 1959; Saunder and Grant 1962; Greenland 1962).

Such changes in soil organic matter content, whether considered on an annual basis or as a result of seasonal or other periodic changes (e.g. Russell 1964a) have obvious importance in relation to the choice of the optimal system of land utilization for any given environment. Consequently there is considerable interest

in describing the process of turnover of soil organic matter under different situations. Various approaches to this problem are discussed below.

(ii) Mathematical models and their validity

Russell (1962) has presented a comprehensive equation relating changes in soil organic matter (or nitrogen) with time to the processes of addition and decomposition of organic matter, which are themselves functions of time, viz:

$$\frac{dx}{dt} = - Px + Q \qquad (1)$$

where x is the organic matter content of the soil expressed as C or N, t is time, and P and Q time-dependent decomposition and addition parameters respectively. The general solution to equation (1) is

$$x \exp (\int P\, dt) = \int Q \exp (\int P\, dt)dt + C \qquad (2)$$

Such an equation may be used to describe both seasonal changes in soil organic matter during the year and also annual changes from year to year although its evaluation is rather complex (Russell 1964a).

Generally P and Q have been considered as constants (e.g. Jenny 1941), equation (1) then being expressed in the form

$$\frac{dx}{dt} = - K_1 x + K_2 \qquad (3)$$

where K_1 and K_2 are decomposition and addition constants respectively. The solution to this equation is

$$x_t = x_E - (x_E - x_0)\exp(-K_1 t) \qquad (4)$$

where x_0, x_t and x_E represent the soil organic C or N content initially, at time t and at equilibrium respectively.

At equilibrium,

$$\frac{dx}{dt} = 0 \quad \text{and} \quad x_E = \frac{K_2}{K_1} \qquad (5)$$

Derived terms often used in studies of organic matter kinetics are (after Russell 1962; and Jenkinson 1963a):

(a) the half-life ($t_{\frac{1}{2}}$), defined as the time taken for half the change from the original organic matter level to the new equilibrium level to occur, i.e.

$$t_{\frac{1}{2}} = \frac{K_1}{\log_e 2} = \frac{0.693}{K_1} \qquad (6)$$

and

(b) the turnover time (t_t), or average age of the organic matter (Bartholomew and Kirkham 1960), defined as the time required for the mineralization of an amount of organic matter equal to the amount present in the soil, i.e.

$$t_t = \frac{x}{K_1 x} = \frac{1}{K_1} \qquad (7)$$

It is noteworthy that both these parameters depend only on K_1, the fraction of organic matter decomposed annually.

Typical half-life values for soils in temperate regions are in excess of 25 years, so that detailed records of soil organic matter changes over a long period are required for their satisfactory evaluation.

A number of equations similar to (3) have been proposed, these varying somewhat in the definition of terms used and in the expression of the addition term K_2. Usually K_2 is defined as the increment to the native soil organic matter resulting from the humification of added crop residues or organic manures (Nikiforoff 1937; Jenny 1941; Dawson 1950), thus implying, although not specifying, another decomposition parameter which relates the actual addition of fresh organic matter to the resultant increment in soil organic matter. This latter has been considered to be identical to K_1 (e.g. Richardson 1938) or to differ for various fractions of the soil organic matter (Tyurin 1937, quoted by Kononova 1961; Hénin and Dupuis 1945; Woodruff 1949; Hénin, Monnier and Turc 1959; Greenland and Nye 1959; Nye and Greenland 1960). Before discussing these equations further it is necessary to consider some of the assumptions involved.

Validity of Mathematical Model

The major assumptions made in applying equation (3) to soils are:

1. that losses of soil organic matter, C and N are proportional to one another at all stages of decomposition. This will only be valid where both the C and N in the decomposing substrates are equally available to the soil microorganisms.

2. that the annual addition of organic matter to the soil (K_2) is a continuous process, and occurs at a constant rate independent of the level of soil organic matter. This is unlikely to hold under conditions of declining soil fertility as the organic matter return will also decline (e.g. Jenkinson 1963a), or where the soil is under a rotation including a pasture phase, but it may be a reasonable approximation where a steady state level has been attained.

A useful means of dealing with the case of land under a rotation in which the accretion and loss processes may markedly differ in magnitude during successive phases of the rotation was proposed by Nye and Greenland (1960), and may be written in the form (Greenland 1962)

$$x_E = \frac{(K_2)_p t_p + (K_2)_c t_c}{(K_1)_p t_p + (K_1)_c t_c} \qquad (8)$$

where x_E, K_1, K_2 and t have the same meaning as in equation (3), except that the subscripts indicate that appropriate values for either the pasture or cropping phases of the rotation are referred to.

3. that the rate of decay of organic matter (K_1) is independent of the amount added. Although it is known that additions of readily decomposable organic matter affect the decomposition rate

of native soil humus, the significance of this "priming effect" in field studies where decomposition occurs over relatively long periods has not been established (e.g. Jenkinson 1963a, 1963b; Jansson 1963a; Harmsen 1964). It has been suggested that the value of K_1 may also vary with time, and whilst it seems inherently probable that this may hold for soils recently ploughed from the virgin state (Clarke and Marshall 1947; Greenland 1962), or for soils subjected to alternate wetting and drying cycles or other partial sterilization treatments (Jenkinson 1966a), the experimental evidence for the importance of this phenomenon under field conditions is slight.

4. that all parts of the organic matter are equally decomposable. Many authors have recognized the heterogeneous nature of the soil organic matter, and have attempted to allow for this by modifying equations (3) and (4) in various ways.

e.g. (a) by assuming that the different forms of organic matter decompose independently, Woodruff (1949) proposed the use of pairs of addition and decomposition parameters for each form (i) considered, i.e.

$$x_t = \sum_{i=1}^{n} x_{iE} - (x_{iE}-x_{i0})\exp(-K_i t) \qquad (9)$$

(b) by assuming that the relatively rapid decomposition of fresh plant residues produced humified material which subsequently decomposed more slowly, Hénin and Dupuis (1945) proposed an equation of the form

$$\frac{dx}{dt} = KA - \beta x' \qquad (10)$$

where x' represents the "humified" organic matter, and K the "isohumic coefficient" i.e. the fraction of the total plant material (A) returned in humified form to the soil each year. The fraction (β) of the soil humus decomposing each year was assumed to be the same for all parts of the humus carbon.

A more sophisticated model was proposed by Hénin, Monnier and Turc (1959), in which two distinct forms of organic matter were recognized. These were (i) fraction A; labile organic matter, "light fraction" with a high C:N ratio, which was the precursor of (ii) fraction B; stable organic matter ("humus"), with a C:N ratio close to 10.

The equations proposed were complex, and of the form

$$A_t = A_E - (A_E - A_0)\exp(-\alpha t) \qquad (11)$$

and

$$B_t = B_E - (B_E - B_0)\exp(-\beta t) - K(A_E - A_0)\frac{\alpha}{\alpha-\beta}\left[\exp(-\beta t) - \exp(-\alpha t)\right] \qquad (12)$$

where α and β are decomposition parameters, and K (the "isohumic coefficient") the fraction of A changed to B each year.

However, as discussed on p. 19 , even attempts such as these to split the decomposition process into two parts are probably inadequate, as many fractions of differing biological stability are probably involved at different stages of the

decomposition process (e.g. Jenkinson 1963a, 1965a, 1966a).

(iii) Studies of Turnover

1. Based on trends in soil N

Most attempts to evaluate the magnitudes of the turnover either of added organic matter or of native soil "humus" have been based on the application of equation (3) to changes in soil N status. In temperate regions, in soils under crop or pasture, only 1-3% of the total soil N (N_t) mineralizes each year i.e. K_1 = 0.01-0.03 (e.g. Nikiforoff 1937; Richardson 1928; Walker, Orchiston and Adams 1954; Stevenson 1965a). This is in marked contrast to the rapid decomposition of fresh plant or animal residues added to soils, and implies that the bulk of the soil humus is relatively resistant to decomposition, although the mechanisms involved are not clear (e.g. E.W. Russell 1961; Greenland 1965b; Bremner 1965a).

Some typical values obtained by European and American authors are given in Table 1. Corresponding data from Australian studies is discussed later (p.23).

The values of K_1 were either calculated by fitting equation (3) to data referring to long-term changes under a mono-culture or continuous rotation (Richardson 1938; Jenny 1941; Bartholomew and Kirkham 1960; Jenkinson 1963a; Muller 1966), or estimated using various equations from soil and plant analyses (Nikiforoff 1937; Hénin and Dupuis 1945; Woodruff 1949; Monnier 1965).

TABLE 1. TYPICAL VALUES FOR CONSTANTS IN TURNOVER EQUATIONS

Locality	Rotation or Amendment	Period (years)	Constant			Reference
			Equation	Symbol	Value	
U.K.	Permanent grassland	200	(3)	K_1	0.028	(7)
"	Continuous barley	61	"	"	0.025	(3)
N.America	Continuous maize	33	"	"	0.061	(4)
"	Continuous maize	45	"	"	0.022	(1)
"	Continuous corn-oats-clover	45	"	"	0.036	(1)
France	Continuous vegetable crops					
"	a) + mineral N fertilizer	12	"	"	<0.006	(6)
"	b) + straw	12	"	"	0.008	(6)
"	c) + sheep manure	12	"	"	0.032	(6)
N.America	Continuous maize	33	(9)	mean K_1	0.020	(8)
"	Continuous wheat	33	"	"	0.010	(8)
France	Legume residues	-	(10)	K	0.10	(2)
"	Cereal residues	-	"	K	0.15	(2)
"	Typical value	-	"	β	0.020	(2)
France	Cultivated soil	-	(11,12)	α	0.5-1.0	(5)
"	Rendzinas, poorly drained soils	-	"	α	0.02-0.1	(5)
"	Raw humus, heath soil	-	"	α	c.0.03	(5)
"	Fresh green manure	-	"	K	0	(5)
"	Cereal residues	-	"	K	0.08-0.20	(5)
"	Pasture residues	-	"	K	0.15-0.30	(5)
"	Peat	-	"	K	1.0	(5)
"	Typical values	-	"	β	0.008-0.012	(5)

- = not stated

References:

(1) Bartholomew and Kirkham (1960)
(2) Hénin and Dupuis (1945)
(3) Jenkinson (1963a)
(4) Jenny (1941)
(5) Monnier (1965)
(6) Muller (1966)
(7) Richardson (1938)
(8) Woodruff (1949)

It is generally agreed that K_1 is usually small, corresponding to turnover times in the range 20-100 years. It is interesting to examine the results of the French authors more closely, as they relate to the likely magnitude of the differences between various soils and management practices.

Hénin and Dupuis (1945) suggest that only 10-15% of the added plant residues are converted to humus, this then mineralizing at a relatively slow rate (c. 2% p.a.). Monnier and Turc (1964) have suggested that decomposition of the free organic matter accumulated under pastures may account for most of the initially rapid rate at which the soil organic matter is decomposed following cultivation. Monnier (1965), elaborating on the earlier results of Hénin, Monnier and Turc (1959) has also quoted data - but not its derivation - illustrating the effect of soil texture and frequency of cultivation on the decomposition rate of plant residues (α). The contribution of plant residues to the final equilibrium value of "free organic matter" (A_E) may be estimated from the values of α given in Table 1. Thus for a cultivated silt this contribution was approximately equal to the annual addition of fresh organic matter, whereas for a rendzina and a peat it represented some ten and thirty times the annual addition respectively. The decomposition rate of the soil humus (β) was also stated to vary (in an unspecified manner) with soil type; and in addition B_E depended upon the system of land management, as the proportion of fresh organic matter ending up as humus at

equilibrium depended on the ratio of the "isohumic coefficient" (K) to β. The fact that the proportion (K) of added fresh organic material varied so markedly with the nature of the addition (i.e. with its relative ease of decomposition) was in accordance with the general experience of other investigators, green manure contributing very little to soil humus reserves, and cereal residues proportionally more than pasture residues.

However, the validity of these conclusions, and of the values of K, α and β proposed by Monnier (1965) is doubtful, as not only was virtually no supporting evidence presented by these authors, but the fractionation procedure used only achieved at best a partial separation of the "free" material, and moreover was markedly influenced by both the soil type and the dispersion technique used (see pp. 46-50 and Chapter III).

There is no other data available relating to the effect of soil type rotation and management on dynamics of the "free" and "bound" fractions, probably largely because a suitable and quantitative densimetric fractionation procedure has not been developed.

Other information as to the relative sizes and activities of the pools involved in the turnover of soil C and N is available from studies using isotopic (^{14}C and ^{15}N) labelling, but only the results of major significance to considerations of the dynamics of soil organic matter are considered here.

2. Studies of Turnover based on ^{15}N and ^{14}C Studies

(a) ^{15}N: The complexity of the processes involved in the biological turnover of N in the soil have been indicated by laboratory incubation studies using ^{15}N, notably by Jansson (1958). Few comparable field studies, apart from experiments aimed at determining the recovery of tagged fertilizer N by crops, have been conducted, as the interpretation of the results in an unambiguous manner has been recognised to pose formidable problems (Jansson 1963a). Perhaps the most significant conclusions from such studies, apart from confirming the complexity of the interrelated processes involved in the turnover of soil N (the "mineralization-immobilization cycle"), were

(i) that the dominant factor influencing the rate and net result of these processes was the availability of an energy source for the soil heterotrophic microorganisms, and that the associated "priming effect" was real but of limited importance (Jansson 1963a), and

(ii) that the soil organic N consisted of an "active" phase, representing a small proportion (10-15%) of the total N and involved in its turnover, and a much larger "passive" phase, which was relatively inert as far as turnover was concerned. The active phase was considered to consist essentially of microbial tissue (the "biomass"), and the passive phase to represent the more humified organic matter.

Studies of the decomposition of plant residues (Kuo and

Bartholomew 1963; Bartholomew 1965) indicate that most of the plant protein is readily decomposed and replaced by newly synthesised microbial protein. Measurements of the net loss of plant N added to a soil and then partly decomposed probably underestimate the actual turnover of the plant N, especially as it may be subjected to several relatively rapid cycles of microbial degradation and resynthesis. The net mineralization of immobilized N, either from additions of plant-N or fertilizer-N, appears to proceed at a relatively slow rate, comparable to that of the native soil humus (Jansson 1963b). However the chemical nature of mineralizable-N is obscure (Keeney and Bremner 1964; Keeney 1965; Simpson and Freney 1967).

There are at least three fractions involved in the turnover of soil organic matter; relatively undecomposed plant and animal residues, microbial tissue, and soil humus. Some further information relating to the relative sizes and turnover rates of these pools is available from studies using radioactive C (^{14}C or ^{13}C), and in some cases from cross-tagging experiments involving the use of both carbon and nitrogen isotopes.

(b) ^{14}C: Information on turnover rates of soil organic matter or of added plant material has come both from incubation studies and from radiocarbon dating of various fractions of the organic matter.

(1) Incubation studies

Estimates of turnover are readily obtained by measuring

the CO_2 flux of soil amended with a ^{14}C-labelled substrate, the relative decomposition rates of substrate and native soil organic matter being estimated from the $^{14}C:^{12}C$ ratio in the evolved CO_2.

Laboratory studies of the changes occurring during incubation of soil amended with labelled sugars, plant or microbial tissue, or various constituents isolated from the plant tissue (e.g. cellulose, hemicellulose or protein) have been conducted by a number of investigators (e.g. see review papers of Jenkinson 1963b; Simonart 1964; Greenland and Oades 1968; and also Mutatker and Wagner 1967).

These studies have generally confirmed the rapid decomposition rate of the added substrate, and stimulation of the mineralization rate of the native humus. Although Jansson (1960), using ^{14}C-glucose, found evidence of active and passive pools analagous to those found during his previous ^{15}N investigations, relatively little detailed knowledge of the humification process has been obtained from applying the classical fractionation procedures to the labelled soil humus, as in general the label was found to be rapidly distributed between humic acids, fulvic acids and humin. This indicates both that a dynamic equilibrium must exist between part at least of these components of soil organic matter and the added substrates, and also that the biological significance of these fractions is probably rather doubtful. Jenkinson (1962, 1963d) has suggested the use of cold 0.1 N $Ba(OH)_2$ as an extractant

for the estimation of biomass material, but Keeney (1965) found that this did not provide a good estimate of the amount of readily mineralizable-N. The use of other extractants to estimate the pool of labile organic matter in soil is discussed further below (p. 36).

Some studies of the decomposition of labelled plant residues under field conditions have also been reported, notably by Jenkinson (1963c; 1964a, 1964b; 1965a, 1965b; 1966a). The main conclusions from these papers may be summarised as follows.

1. The decomposition rate of uniformly labelled ryegrass in the field was little affected by differences in soil reaction, texture or organic matter content, but was slightly greater under bare fallow than under grass. Under bare fallow, seasonal fluctuations due to soil temperature variations were detected. However the decomposition rate was not influenced by the level of the ryegrass addition, nor (after the first year) by the origin of the plant tissue (i.e. tops cf. roots). Under bare fallow the decomposition rate was very rapid during the first 6 months, but thereafter the ryegrass-C decomposed at a rate appreciably faster than that of the native soil organic matter, the corresponding half-lives being 4 and 25 years respectively. Evidently all the ryegrass-C was not equally decomposable, for in a calcareous soil although the median half-life of the plant-C was only 95 days, one tenth of the carbon had a half-life of greater than 5.8 years, implying that the

proportion of the residual plant-C decomposing annually may vary with time i.e. that K_1 for the plant-C is not necessarily constant, especially immediately following its incorporation into the soil (cf. Clarke and Marshall 1947; Greenland 1962).

2. It was possible to estimate the size and relative activity of the biomass by applying partial sterilization treatments to soil containing labelled organic matter. The biomass was estimated to comprise a small proportion (c. 2-3%) of the native soil organic matter, and to represent a variable proportion of the added plant-C (e.g. 10-12% after 12 months decomposition of the ryegrass, decreasing to 4% after 4 years). The biomass was postulated to consist of two broad fractions, namely a zymogenic part, derived from recently added plant residues and having a half-life of c. 2 years, and an autochthonous part which exhibited little change in size over the 4 year period studied, despite recent additions of plant material.

It is clear from these results that, as suggested by Jansson (1958), the organic matter in soils (apart from undecomposed plant material) may be broadly split into two groups i.e. an active phase, including the biomass and representing some 2-15% of the total organic matter, and a much larger passive phase. However, each of these will evidently contain a number of components of variable size and activity, reflecting the quantity and quality of any recent organic matter additions, as well as the time elapsed

since such materials were incorporated into the soil. Thus the use of the equations discussed above to predict the loss of organic matter from soils will be complicated, especially for soils to which organic matter is periodically added (e.g. during a pasture phase).

(2) Radiocarbon dating of organic matter

Estimates of the mean decomposition rate of the native soil humus (c. 2-3% per year) correspond to turnover times in the surface of arable soils of at the most 100 years. Rather greater average ages have been obtained from the radiocarbon dating of various fractions of the organic matter from a range of soils. This is largely due to the inaccuracy of the radiocarbon method for samples < 200-300 years old (Tamm and Östlund 1960; Talibudeen 1964). Typical results obtained from various fractions from a range of soil types are presented in Table 2.

The limited data which is available suggests that there is a profound effect of soil type and vegetation on the turnover of soil organic matter, and that the more humified fractions are relatively inert (e.g. with turnover times of >1000 years). Material in deeper horizons of the surface soil was usually older than that nearer the surface, although these differences were reduced if undecomposed plant residues were removed (Paul, Campbell, Rennig & McCallum 1964). These authors also reported that on a chestnut soil the inclusion of a clover phase in a 3 year cropping

TABLE 2. APPARENT AVERAGE AGES OF ORGANIC MATTER IN SOME SOILS AS INDICATED BY RADIOCARBON DATING

Country	Soil	History	Horizon and Depth (cm)	Fraction of Soil Organic Matter	Mean Age (years)	Reference
U.S.A.	silt loam	grassland	A_1: 0-15	total	410 ± 100	(1)
"	"	"	A_2: 20-30	"	840 ± 200	
"	brown acid soil	woodland	A-B: 0-57	coarse organic material	<50	
"	"	"	A: 0-30	NaOH-soluble	50-200	(3)
"	"	"		" -residue	c.2000	
Nether-lands	podzol	-	B: 15-20	total	940 ± 20	(1)
Sweden	"	forest	B: -	"	370 ± 100	(2)
Canada	"	(forest)	A: -	total (*)	360 ± 60	
"	black chernozem	cultivated	A: 0-15	total (*)	990 ± 60	
"	"	"	"	fulvic acids	630 ± 60	
"	"	"	"	humic acids	1308 ± 64	(4)
"	"	"	"	humin	1240 ± 60	
"	"	"	A: 15-25	total (*)	1130 ± 70	

- = not stated. * undecomposed plant material removed by floatation and hand-picking.

References: (1) Broecker, Kulp and Tucek (1956); Simonson (1959)
(2) Tamm and Östlund (1960)
(3) Broecker and Olson (1960)
(4) Paul et al. (1964)

rotation maintained the level of soil organic matter at a higher level than in an adjacent continuously cropped (WWF) plot, even though the average age of the organic matter was considerably lower (1680 ± 80 cf. 2250 ± 90 years).

The data discussed above indicates that the turnover of organic matter in soil involves a number of simultaneous accretion and loss processes, all of which may vary in rate with time, soil type, vegetation and management (e.g. frequency of cultivation). At least three major components have been recognized:

(a) undecomposed plant residues, which are usually relatively rapidly decomposed (depending on their composition), to form

(b) more humified material, of which only an average of 1-3% mineralizes annually, although very resistant materials are also present within this fraction, and

(c) biomass material, representing probably <10% of the total organic matter, and turning over relatively rapidly, and subject to the influence of soil temperature, moisture and aeration conditions, and to the frequency, quantity and quality of additions of fresh plant material.

It is the nature of the dynamic equilibria between these fractions, and the manner in which they are influenced by soil type and agronomic management, which largely determines the fertility of a soil in a given environment. Data relating to the fertility of Australian agricultural soils under pasture-wheat

rotations is presented below, and discussed with reference to the possible role of partly humified plant material in the dynamics of nitrogen in such soils.

(iv) Importance in Australian Wheat Soils

1. Fertility trends

In southern Australia the importance of the level of organic matter in arable soils has been emphasised by the widespread observation that whilst cultivation leads to a deterioration of soil structure and to an accelerated rate of loss of organic matter - often of the order of 2-5% p.a. (e.g. Clarke and Marshall 1947; Hallsworth, Gibbons and Lemerle 1954; Martin and Cox 1956; Drover 1956; Colwell 1958; Williams and Lipsett 1961; Greenland 1962), the use of ley farming practices offsets these effects (e.g. Cook 1939; Penman 1949; Purchase, Vincent and Ward 1949; Sims and Jardine 1949; Woodroffe 1949; Bath 1951; Davies 1952; Sims 1953; Donald and Williams 1954; Williams and Donald 1957; Hingston 1959; Russell 1960; Bath 1962). However, even long term pastures may not restore the soil N status to its original level (Bath 1949; Donald and Williams 1954; Russell 1960; Greenland 1962).

2. Estimates of turnover parameters

Most studies of the dynamics of organic nitrogen in Australian soils have been limited to investigations of the net

accretion rates under improved grass-legume pastures or of the rate of depletion of soil nitrogen reserves under relatively intensive cropping regimes. There is little data on the accretion and decomposition parameters for the cropping and pasture phases of soils under pasture-wheat rotations, so that information on the details of the process of organic matter turnover under such conditions is rather meagre.

Under pasture-cropping regimes the equilibrium level of soil N is only approached very slowly, and is largely governed by a comparatively small fraction (<3%) of the organic N decomposing each year. The nature and dynamics of this fraction has not been studied in detail, although it has been proposed to include plant residues derived mainly from the concentration of roots in the surface layer of soil (Troughton 1957; Russell 1960) together with plant stem butts, nodules and burrs (seeds) either naturally buried in the soil (Watson and Lapins 1964) or incorporated by faunal (e.g. earthworm) activity (Barley 1961; Barley and Kleinig 1964). The contribution of dung and urine from grazing animals is not known, as most data refers to grazed pastures. As excreted N is readily mineralized (Barrow 1961) such material may well stimulate turnover rather than increase the net accretion of soil N (Watson and Lapins 1964), despite its action in enhancing the growth of grasses in mixed swards (e.g. Davies 1952; Walker 1956a).

Typical values for estimates of these parameters for a range of soil types under relatively permanent pasture or cropping rotations are given in Table 3. Most of the data refers to changes following the establishment of improved annual grass-legume pastures on land of low initial fertility, although the nature of the equilibrium process involved appears also to be similar for land of relatively high initial N content (Russell and Harvey 1959). These estimates are most reliable where data derived from long-term experiments has been fitted to suitable mathematical equations of the types previously discussed (e.g. Russell and Harvey 1959; Greenland 1962). In many cases (e.g. McGarity 1959; Watson and Lapins 1964), the estimated values of mean N accretion rates under pastures are derived from herbage analyses making assumptions similar to those of Walker, Orchiston and Adams (1954), and Walker (1956b), or from observed changes in soil nitrogen content together with estimated bulk density values (e.g. Cook 1939; Penman 1949; Parker 1957). However, despite these limitations the data presented probably reflects reasonably satisfactorily the approximate magnitudes of the several processes involved.

It is clear that accretion occurs mainly in the surface few inches of soil, and that the rates under improved legume-based pastures exceed by up to twofold those under non-legume or volunteer legume swards. Although the reported values for accretion rates are obviously influenced by differences in climate, soil type and management, in general values of the order of 35-80 kg N/ha/year are

TABLE 3. ESTIMATES OF TURNOVER PARAMETERS FOR SOME AUSTRALIAN SOILS

Land Use	Pasture Type	Soil Group and Location	Sample Depth (cm)	Mean Net Annual Change in Soil N: Gains K_2 (kg N/ha/year)	Mean Net Annual Change in Soil N: Losses K_1	Half-life of Equilibration $t_{½}$ (years)	Source
A. Relatively long-term annual pastures, grazed	G-M	Solonised brown; Walpeup, Vic.	0-15	34			A.P.Mann (1967) (pers.comm.)
	G-NL	" " ; " , "	0-15	21			
	G-C	Podzolic soils; Crookwell, NSW	0-10	47			Donald and Williams (1954)
	G-VL	Solonetzic soils; Kybybolite, SA	0-15	73			Cook (1939)
	G-C	" " ; " , "	0-15	52			
	G-C	" " ; " , "	0-5	58	0.029	24	Russell (1960)
	G-C	Lateritic sand; Kojonup, WA	0-10	81(81)			Watson and Lapins (1964)
	G-C	Transitional podsol; Rutherglen, Vic.	0-15	56 (range 34-220)			Penman (1949)
	G-C	" " ; " , "	not stated	107			Bath (1962)
	G-VL	Red-brown earth; Gunnedah, NSW	not stated	24			Purchase, Vincent and Ward (1949)
	G-C+	" " ; Deniliquin, NSW	0-7.5	68			Kleinig (1966)
	G-C	" " ; Waite Institute, SA	0-23	39			Woodroffe (1949)
	G-NL	" " ; Truro, SA	12.7	32			
	G-NL	" " ; " , "	"	59			Clarke (1963)
	Clover	" " ; " , "	"	155			
	G-C	Grey and brown calcareous solonised soils; Merredin, WA	0-25	91			
	G-NL		0-25	64			Parker (1957)
	G-C+	Alluvial clay; Mypolonga, SA	0-23	300	0.022	31	Russell and Harvey (1959)
	G-C+	Grey and brown soils of heavy texture; Wakool, NSW	0-10	68			
	G-M	" " ; Longerenong, Vic.	0-15	80			Kleinig (1966)
	G-NL	" " , "	0-15	49			D.R.Rooney and C.L.Tuohey (1965, pers.comm.)
	G-C	Krasnozem; Lismore, NSW	0-15	45[a] (73)	(0.0025)	(280)	
	G-C	" ; " , "	0-15	67[b] (111)	(0.0043)	(160)	McGarity (1959)
				a=wet season b=dry season			
B. Continuous cropping or short-term annual pasture-wheat rotations	G-C in 3- or 4-course rotation. Cont.W	Red-brown earth; Waite Institute, SA	0-20	67	0.01	69	Greenland (1962)
		" " ; " " , "	0-20	50	0.03	23	
	G-M in 4-course rotation. Cont. FW	Grey soil of heavy texture; Longerenong, Vic.	0-15	67	0.013	56	R.H. Laby (1963, pers.comm.)
			0-15	28	0.027	28	

NB: (i) Sward Composition: G-C=grass (e.g. Lolium rigidum) + clover (Trifolium sp.)
G-M= " (" " ") + medic (Medicago sp.)
G-VL= grass + volunteer legumes
G-NL= grass + no legumes

(ii) Parameters K_1, K_2, $t_{½}$ as defined in text. Values in parenthesis refer to ungrazed pastures.

+ = irrigated.

not uncommon. Under favourable circumstances accretion rates of the order of 200 or more kg N/ha/year may be attained (Penman 1949; Russell and Harvey 1959). The accretion rates of 20-55 kg N/ha/year reported under non-leguminous pastures or crops (Parker 1957; Greenland 1962; Clarke 1963; personal communications from Laby 1963, Rooney and Tuohey 1965, and Mann 1967) largely reflect non-symbiotic fixation, and are similar to those reported by overseas authors (E.W. Russell 1961).

For a given soil type, decomposition (mineralization) rates of soil N under pastures are approximately half those under intensive cropping rotations. Commonly under both short and long-term pastures, as well as under crops, K_1 values of the order of 1-3% per year, corresponding to turnover times of the order of 30-100 years have been reported, although much lower K_1 values (<0.5%) have been estimated for some krasnozems under sod-seeded pastures (McGarity 1959).

Wetselaar (1967) has used a novel method for estimating the amount of soil N mineralized during as short a period as a single growing season. Where both N losses from the soil (from leaching or denitrification) and N gains to the soil (from rainwater or non-symbiotic fixation) were negligible, the decomposition rate was indicated by the "mineralization coefficient" (M.C.). This was defined as the amount of NO_3-N formed in a single season *in situ* by bare fallowing the soil, expressed as a percentage of the

organic N in the topsoil at the onset of the growing season. Although its potential application to the cereal growing areas of southern Australia would probably be limited by losses during the winter and spring months (e.g. Storrier 1965c), Wetselaar has obtained some interesting results for two tropical soils recently cleared from the virgin condition. In a clay loam (a lateritic red earth) the M.C. remained fairly constant at c. 5% during 3 years of bare fallow, whereas in a sand it rapidly decreased from an initially high rate of 12.5% in the first year to 5% after 4 years of bare fallow. This was interpreted as indicating an initial rapid breakdown of labile organic material in the sand, followed by a slower rate of mineralization from more stable material. A similarly rapid mineralization (c. 7%) was observed in the clay loam following the ploughing of a 7 year old leguminous pasture. Saunder and Grant (1962) have also observed that mineralization rates under bare fallow were greater in coarse textured than in clay soils, and that following cultivation of pastures on the sandy soils a rapid comminution of plant residues not associated with the clay fraction occurred.

3. Seasonal Variations in Mineralizable-N

The extensive literature on the seasonal fluctuations in mineral-N levels under crops, pastures or bare fallow conditions testifies to the importance attached to the estimation of the quantity of plant-available nitrogen in arable soils. Such studies

only permit inferences to be drawn regarding actual mineralization rates under field conditions, as appreciable losses (e.g. due to leaching, denitrification, microbial assimilation, or plant uptake) are usually also involved. Nevertheless it has been established that mineralization is dependent not only on the reserves of readily mineralizable N in the soil, but also on the moisture content and temperature of the soil.

The results of overseas studies have been reviewed by Harmsen and van Schreven (1955) and by Harmsen and Kolenbrander (1965). In temperate areas the maximum levels of mineralizable N occur during spring and the minimum during summer, although Richardson (1938) reported an opposite pattern under grassland at Rothamsted. In areas subjected to hot, dry summers the maximum levels are generally found at the end of the summer period, and much lower levels during winter (Saunder, Ellis and Hall 1957; Greenland 1958). These variations have been ascribed to changes in the amount and nature of the reserves of readily mineralizable N, associated either with changes in the quantity and composition of the plant root exudates and residues added to the soil at various stages of growth, or to partial sterilization effects resulting either from winter frosts or summer desiccation.

Although the organic N accumulated under relatively long-term grass legume pastures may mineralize rapidly following their cultivation (Penman 1949; Bath 1949, 1951) no comprehensive

studies of the effects of soil type and rotation on the seasonal variations in readily available N have been reported for Australian soils. Jensen (1940) found that the mineralizable N in 46 wheat soils was closely related to their total N content, and Waring (1963) found a close correlation between mineralizable N and the N uptake by wheat in pot culture.

Some correlations between field N data (e.g. total soil N, or NO_3-N at seeding) and the yield and N content of wheat grown under field conditions have been reported (Sims 1953, 1956, 1961, 1964; Sims and Mullaly 1962; Storrier 1962, 1965a, 1965b, 1965c; Russell 1964b) but other authors have been unable to demonstrate such relationships (Waring and Teakle 1960; Williams and Lipsett 1961; Storrier 1962). Evidently factors other than the supply of mineral N to the growing crop are involved, and these will include the marked influence of both wheat variety (Russell 1964b) and the growing season rainfall (Martin and Cox 1956; Sims 1961; Rooney, Sims and Tuohey 1966).

(B) Methods for the Estimation of "Labile" Organic Matter

Soil organic matter has traditionally been analysed in terms of total C, N or oxidizable substances (e.g. Broadbent 1965). Such non-isolative chemical methods have not permitted any distinction to be made even between unhumified matter and humus. A wider range of methods has been proposed for the isolation of

various fractions of the soil organic matter (e.g. Mortensen 1965; Stevenson 1965b), but most suffer from a marked lack of selectivity, especially in differentiating between labile and passive fractions.

Direct estimation of the size of the labile pool is best achieved by determination of the CO_2 flux during decomposition (see p. 16), as the net N mineralization may be a poor index, especially if the labile material has a wide available C:N ratio. Despite the often dominant influence of changes in the nature and form of both plant residues and biomass material associated with the various pretreatments to which the sample may be subjected, some indications of the types of organic materials which might be involved in the labile pool have been obtained from soil test procedures where the net N mineralization during decomposition of readily mineralizable organic matter is determined (e.g. Harmsen and van Schreven 1955; Bremner 1965c; Scarsbrook 1965).

(i) Indirect methods

These are aimed at estimating the size and lability of the total pool of mineralizable organic matter in a soil, without necessarily attempting to measure the amounts or determine the physical and chemical nature of the fractions involved. Such estimates are usually correlated with crop response to various levels of fertilizer additions, in either pot culture or field experiments (see Bremner 1965c). Both biological and chemical approaches to the problem of estimating the availability of soil N have been used.

1. Biological methods

Bioassays in which the growth of microorganisms or higher plants is measured over relatively short periods have not been found as useful as methods where the growth response is largely dependent on the mineralization of organic N during the test. The best correlations have been obtained between crop growth and/or yield response in pot culture and the mineral N released during incubation (Harmsen and van Schreven 1955; Gasser 1961; Bremner 1965c).

Whilst the net N mineralization during an arbitary incubation period is not a good index of the absolute amount of potentially mineralizable N in a sample, it has been widely used in empirical soil test procedures aimed at estimating the potential availability of N to crops. The need for rigorous standardization of such tests reflects the effects of the various treatments to which the soil is subjected (sampling, drying, grinding and sieving, storage and incubation) on the decomposability of biomass and plant tissue material in the soil. The lethal effect of desiccation on portion of the biomass (Paul and Tu 1965; Jenkinson 1966a) undoubtedly accounts for a large part of the observed effects of time of sampling, drying pretreatment and time of storage on the amount of N subsequently released on incubation. A similar lethal effect due to freezing and thawing would explain the failure of deep-freeze storage of field-moist soils to prevent changes similar to those observed for corresponding soils stored air dry (Harding and

Ross 1964). The increase in mineralizable N following fine grinding and sieving of the soil to <80 mesh was attributed by Waring and Bremner (1964b) to the increased accessibility of organic matter normally protected within aggregates, possibly in micropores as suggested by Rovira and Greacen (1957). However mechanical comminution of plant fragments may also be involved, as fine grinding increases the decomposition rate of fresh plant tops (van Schreven 1964) and of wood sawdust (Neal, Bollen and Nu 1965), probably by increasing the physical accessibility of plant carbohydrate materials to enzymatic attack.

The rate and extent of N mineralization is markedly affected by the incubation procedure, this probably reflecting the composition and activity of the microbial population developed under the particular conditions used. Anaerobic procedures (e.g. Waring and Bremner 1964a) may produce erratic results, particularly in samples containing appreciable amounts of undecomposed organic material (Keeney and Bremner 1966a) or of NO^3-N (Robinson 1967). Aerobic procedures have generally been preferred, and although satisfactory correlations between the net release of mineral N and crop response have been reported by many authors (Gasser 1961; Gasser and Williams 1963; Keeney and Bremner 1966a, 1967) other investigators have only obtained useful correlations when the total mineral N present after incubation was used (Smith 1966). In general, such incubation studies have yielded little information

regarding the relative contributions of plant residues, biomass or "humus" materials to the soil reserves of mineralizable N.

2. Chemical methods

In an attempt to determine the chemical nature of the mineralizable N in soils, extensive studies have been made of the changes in the amounts and distribution of these different forms of organic N after a large proportion of it has been mineralized. Keeney and Bremner (1964) examined a range of 10 virgin soils and their cultivated analogues, and Keeney (1965) and Keeney and Bremner (1966b) examined a wide variety of soils before and after aerobic incubation for 10-12 months. Although all the forms of N other than non-exchangeable NH_4^+-N decreased in total amount as a result of mineralization, on average there was comparatively little change in the relative proportions of hydrolyzable and non-hydrolyzable forms of N. There was no consistent pattern discernible between soil type or its agronomic history and the percentage contributions of these forms of organic N to the readily mineralizable soil N. This was interpreted as indicating that the various forms of organic N determined by these authors were equally susceptible to mineralization. Keeney and Bremner (1966b) therefore concluded that "any chemical method of assessing the availability of soil N based solely on determination of a particular form of soil N will prove unsatisfactory".

This conclusion is consistent with the concept of the

different relative contributions of plant (and animal) residues, biomass material and "humus" to the mineralizable N in soils. The differences between these contributions reflect the range of chemical complexity of these materials, particularly with regard to their "available energy:available nutrient" status. Most constituents of fresh plant tissue, with the possible exception of lignin, are relatively rapidly degraded by the soil microflora. Although their relative rates of decomposition are markedly influenced by the distribution of lignified tissues (see Kononova 1961) a major proportion of the plant N is normally readily mineralized. The biomass-N, although turned over rapidly, probably does not contribute appreciably to soil reserves of mineralizable N unless portion is killed e.g. by freezing or desiccation. The soil "humus"-N is generally regarded as normally turning over relatively slowly, and is known to be chemically extremely complex. The inertness of the "humus"-N has been variously attributed to its stabilization (i.e. enhanced resistance to microbial degradation) by reaction with other organic soil constituents (e.g. lignin, carbohydrates, tannins, phenols or quinones) or by adsorption onto clay minerals, especially expanding-lattice types, and also to its relatively low content of energy rich materials suitable for the maintenance of an active microbial population. The relative importance of these various factors in a given soil, and the amounts of plant residues, biomass and "humus" material present, would be sufficiently variable to obscure any trends in the overall changes

in composition associated with mineralization. It follows that a fractionation procedure which takes cognizance of these factors will be more likely to yield meaningful results. Such an approach would be one aimed at quantitatively separating plant residues from the soil humus. Techniques for both the chemical and physical separation of these fractions have been proposed, and although the distribution of the biomass between these fractions has not been determined, it is probably intimately associated with organic material in all stages of decomposition.

(ii) Direct methods

Separation of relatively undecomposed plant and animal residues from soil humic materials has been hindered by the intimate association of these materials with one another and with the soil minerals (Greenland 1965a, 1965b). A large number of procedures have been proposed, including both selective destruction or chemical extraction of either fraction and their physical separation by floatation.

1. Selective destruction or extraction of either fraction

Methods based on selective destruction or dissolution have met with limited success, as they commonly lack adequate specificity. Thus estimation of the relative amounts of undecomposed and humified materials based on weight losses after combustion at arbitary temperatures (Bouyoucos 1934), or determination of the "readily

oxidizable" fraction (e.g. with dilute H_2O_2, NaOCl, NaOI, or chromic acid) have generally proved unsatisfactory (e.g. Jackson 1958).

A number of selective dissolution procedures have been applied to soil samples and have recently been reviewed by Mortensen (1965). Aqueous extractants used include strong or weak alkalis, dilute acids, and chelating agents. Humified materials have also been extracted with various organic reagents, e.g. dimethylformamide (Whitehead and Tinsley 1964) and acetylacetone (Halstead, Anderson and Scott 1966). The most widely used extractants have been dilute acids and alkalis, and Kononova (1961) has reviewed the extensive Russian literature dealing with the composition and dynamics of the various classes of humified materials, isolated by Tyurin's procedure, as influenced by soil type and system of land management. Despite uncertainties regarding possible chemical changes during extraction, the properties of the extracted humic colloids have been demonstrated to be characteristic of the soil type in which they were formed, and to reflect the stabilizing influence of the exchangeable calcium, sesquioxide and clay mineral content of the soil. However these studies, whilst demonstrating the net loss of humic colloids associated with the cultivation of various soils, have provided little direct evidence as to the relative contribution of plant residues and humic colloids to the reserves of mineralizable soil N.

Acetyl bromide has also been used by a number of authors,

as it is claimed to dissolve non-humified plant materials (Scheffer and Ulrich 1960); however lack of knowledge of its precise mode of action and its unpleasant chemical characteristics have probably prevented its general acceptance and use.

Hoyt (1967) has proposed that the amount of undecomposed plant material in arable soils may be estimated by determining the amount of chlorophyll-type compounds extracted from soils with aqueous acetone. Decreases in the amounts extracted following the ploughing of a ley were obtained, and in addition there was evidence that the amounts of these materials were closely correlated with the N uptake by ryegrass in pot culture. This technique warrants further examination as it has the attraction of being both rational and simple.

2. Densimetric fractionation of "free" organic matter

a) Principle of method

As the density of soil minerals (c. 2.6-2.7 g/cc) is much greater than that of plant tissue constituents e.g. cellulose (1.5-1.6 g/cc; Hermans 1949) and lignin (1.30-1.45 g/cc; Brauns 1952), or soil organic matter (e.g. 1.6 g/cc; Smith 1943), it should be possible to separate soil minerals from organic matter by suspending the soil in a liquid of suitable intermediate density. Organic matter bound to soil minerals will have an average density considerably higher than that of non-bound material, so that a separation of "free organic matter" from the "clay organic complex"

should also be possible.

However, whilst this concept is simple, considerable experimental difficulties have been encountered in devising a rapid, efficient and reproducible separation procedure. The major problems have been associated with the difficulty of obtaining complete dispersion of the soil in the suspending liquid, and quantitative recovery of the "free" organic material floated out of the soil.

b) Fractionation procedures using aqueous solutions

Fresh plant residues contain entrapped air, so that their SG is only c. 1.0 (Williams and Baker 1957). In addition, their generally cylindrical shape results in a sedimentation velocity appreciably slower than that of soil mineral grains, so that separation of plant root material by floatation in water is feasible. The procedures devised permit large samples to be processed, but are usually inefficient and may be rather tedious.

A complex wet sieving and floatation technique was proposed by McCalla, Duley and Goodding (1943). Barley (1953) investigated a simpler technique in which the sample was suspended in a NaCl solution of SG 1.2, centrifuged and the separate decanted, and obtained recoveries of 80-100%. The use of 'Calgon' as a dispersing agent, together with mechanical dispersion of the soil and wet sieving of the suspension was also examined (Barley 1955), but the separate obtained was appreciably contaminated with soil and so had to be refractionated. A similar problem of variable soil contamina-

tion of the separates was reported by Williams and Baker (1957). Their procedure was also tedious, involving washing of soil cores through a 60 mesh sieve and then repeated decantation and resieving of the separate. Kulakov (1960) obtained a better separation by washing the cores through a bank of sieves (2 mm, 0.25 mm, 0.1 mm), and recovering very fine particles by flocculating the washings with $AlCl_3$ and passing them through a 0.05 mm sieve. A similar multiple sieving technique was used by Roulet, Dubach, Mehta, Müller-Vonmoos and Deuel (1963), but these authors further improved the quality of the separation by shaking the soil with water for 4 hours and then completing the disruption of soil aggregates in a conical apparatus fitted with a central water jet. No plant fragments were visible in thin sections of the <0.06 mm soil residue, and recoveries of 85-100% of the soil organic C were obtained. This technique would appear to be suitable for studies involving the fractionation of relatively large samples of soil as up to 8 kg soil/day may be processed.

Khan (1959) succeeded in further fractionating and partly characterising the organic matter of several Russian soils by first separating <1μ material by decantation, then successively fractionating the residue by centrifuging in Toulet (aqueous K_2HgI_4) solutions of decreasing SG (2.45, 2.30 and 1.80). Each separate was then centrifuged and decanted four times in order to obtain a homogeneous fraction. Typical results are given in Table 4.

TABLE 4. COMPOSITION OF FRACTIONS SEPARATED FROM SOME RUSSIAN SOILS

(after Khan 1959)

Soil	SG of Fraction		Fraction g/100 g Soil	% C in Fraction	% of Soil C in Fraction	% Ash in Fraction
Chernozem	<1 μ separate		43.00	8.50	77.94	-
	>1 μ residue	<1.80	1.60	43.60	14.78	10.50
		1.80-2.30	3.40	7.50	5.35	-
		2.30-2.45	3.00	1.04	0.64	-
		>2.45	49.00	0.13	1.28	-
Podzol	<1 μ separate		17.10	5.64	58.89	-
	>1 μ residue	<1.80	0.50	34.40	10.43	16.20
		1.80-2.30	3.11	12.27	23.31	-
		2.30-2.45	6.10	1.20	4.30	
		>2.45	73.18	0.07	3.00	-

- = not stated.

Khan recognized that he had achieved a clear separation of the "free" and "combined" fractions, as a large proportion (60-80%) of the soil C was associated with the clay (<1μ) fraction, and a smaller proportion (c. 20%) with the fractions of SG <2.3. The least dense fraction (SG <1.8) consisted of particles of organic material with a C:N ratio of c. 25-30, and an 'ash' content of 9-16%,

due to mechanical admixture of the soil minerals with the separate. Part of this fraction was soluble in dilute (0.1 N) alkali, suggesting that it was partly decomposed. The organic material of SG 1.8-2.3 was associated with biogenic amorphous silica (phytoliths), and was also partly soluble in dilute alkali. Denser fractions contained little organic matter.

This study confirmed the feasibility of physically separating "free" organic matter from soil, and demonstrated that the use of dense liquids had improved the separation, and so permitted an estimate of the amounts of such partly decomposed material in a range of soil types. However the procedure suffered from two main disadvantages, in that it employed a highly toxic and corrosive liquid, and the separation of "free" organic matter from mineral contaminants was incomplete, even after four successive fractionations.

c) Fractionation procedures using heavy organic liquids

A number of authors have proposed densimetric fractionation procedures involving the use of mixtures of bromoform and a less dense solvent (e.g. benzene, carbon tetrachloride or ethanol). As the nature of the sample pretreatment, the solution density, the method of dispersing the soil, the suspension concentration, and the method of sedimentation all materially influence the efficiency and reproducibility of the fractionation, the following discussion emphasises the relevant details of the procedures used

and the results obtained.

Attempts at characterising the separates have usually been limited to some microscopic examinations, together with some determinations of the 'ash', C or C:N contents of the fractions. Generally the fractionation procedures used have not been critically examined, and little attempt has been made to evaluate the agronomic significance of the fractions obtained other than to describe differences between various soil types. No detailed studies of the chemical composition or the dynamics of these fractions have been attempted.

Lein (1940) employed a heavy organic liquid (a mixture of bromoform and 'pseudocumene' (1,2,4-trimethyl benzene)) as this was found to be more satisfactory than an aqueous Toulet solution. He suspended a 5.0 g sample in 100 ml of the liquid (of SG 1.7, 2.0, 2.25 or 2.57) by agitating with a glass rod, then centrifuged the suspension at 10,000 rpm in a centrifuge designed to enable continuous separation of the suspension. The fractions were collected on filters and washed free of the solvent with benzene. Typical results for two soils of contrasting texture are given in Table 5. The contents of inorganic materials have been calculated by difference, based on the organic matter contents of each fraction.

Although Lein established that plant fragments were concentrated in the lightest fractions (especially in the separate

TABLE 5. COMPOSITION OF FRACTIONS SEPARATED FROM SOME RUSSIAN SOILS

(after Lein 1940)

Soil	SG of Fraction	Fraction g/100 g Soil	% C in Fraction	% of Soil C in Fraction	Approx. % Inorganic Material in Fraction
Chernozem	<1.7	1.24	39.96	8.17	27
	1.7 - 2.0	6.49	22.87	24.44	55
	2.0 - 2.25	27.35	11.13	50.12	76
	2.25 - 2.57	28.20	2.59	12.02	94
	2.57 - 2.76	34.87	0.66	3.77	98
Podzol	<1.7	1.14	39.00	12.24	28
	1.7 - 2.0	4.90	19.08	25.72	62
	2.0 - 2.25	13.15	9.02	32.63	80
	2.25 - 2.57	29.48	2.67	21.65	93
	2.57 - 2.76	48.56	0.51	6.85	98

of SG <1.7), and the clay-organic complex in the denser fractions (particularly the 2.0 - 2.25 fraction), the less dense separates were heavily contaminated with inorganic materials. There was no 'critical' density at which the best separation of "free" from clay-combined organic matter was obtained, so that the choice of the solution SG for a particular soil would be an arbitrary one.

Hénin and Turc (1949) attempted to improve this by disrupting the aggregates prior to fractionation by boiling the soil in 0.1% $CaCl_2$ and drying in the presence of ethyl alcohol to prevent reaggregation. The dried powder was then suspended in bromoform-benzene solutions of SG 1.5 - 2.5, and the suspension allowed to sediment under gravity.

Satisfactory recoveries of ground plant material were obtained from synthetic peat-clay mixtures using this technique, and the ability of the method to differentiate between plant and humic acid materials when mixed with soil clays was demonstrated, the plant material being recovered in solutions of SG 1.75 - 2, and the humic acid only in denser solutions. Application of this procedure to a number of soils yielded the results shown in Table 6.

The separations obtained using solutions of "critical densities" (SG 1.75 - 2) were variable, and depended markedly on the soil type. Hénin and Turc attributed this to the increase in both the degree of comminution and the extent of contamination with soil minerals of the material floated out of the soil as the density of the separating solution was increased. Whilst large differences in the amounts of material obtained using different solution densities were found for the soil examined, the C:N ratios of the light fractions (SG <2) were generally higher (c. 20-25) than those of the denser fractions (SG >2) from the same soil.

TABLE 6. COMPOSITION OF FRACTIONS SEPARATED FROM SOME FRENCH SOILS

(after Hénin and Turc 1949)

a) Amounts

Soil	% C in Soil	g Fraction/100 g Soil with SG of				
		<1.5	1.5-1.75	1.75-2.0	2.0-2.25	2.25-2.5
Heath Soil	5.8	7	3.5	2.5	7	6
Pine Humus	25.6	49	23	2	3.5	
Cultivated Silt	1.6	0.3	0.7		0.3	9.7

b) Composition

Soil	C:N of Fraction with SG of	
	<2	>2
Heath Soil	19	17.9
Pine Humus	25.8	16.1
Cultivated Silt	19.1	7.9

Similar results were subsequently reported for a rendzina, (Turc 1949; Hénin and Turc 1950) and in general the best separations (as assessed by the C:N ratio of the separate) were obtained using solutions of SG 1.75 - 2.

Jeanson-Luusinang (1960) recognised that destruction of soil aggregates with water was tedious, and reported that fine

grinding and sieving (<0.5 mm) was as efficient and more convenient. A bromoform-ethanol solution of SG 2.0 proved generally satisfactory, although she reported that a range of fractions of varying physical appearance were obtained by using a series of solutions of SG 1.75 - 2.25. The least dense fraction (SG <1.75) consisted of coarse plant fragments, finer particles being obtained in the separate of SG 1.75 - 2.0. The clay-organic complex was concentrated in the fraction of SG 2.0 - 2.25. She concluded that sedimentation under gravity (in beakers or special separating funnels) was unsatisfactory both for fine textured soils or for samples containing appreciable amounts of "free" organic material, even though the light and heavy separates were repeatedly refractionated.

Monnier, Turc and Jeanson-Luusinang (1962) subsequently improved this technique by centrifuging the soil suspension (cf. Lein 1940). A suspension of 5-10 g air dry, <0.5 mm soil in 100 ml of bromoform-ethanol solution of SG 2.0 was centrifuged for 5 min at 1,000xg, and the supernatant decanted on to a filter. Refractionation of the soil pellet was found to be necessary in order to recover mechanically entrained material. With samples containing large amounts of "free" material (SG <2.0), refractionation of the separate was sometimes also required. The separates were oven dried at 105°C, or washed with ethanol and dried at a lower temperature. The bromoform-ethanol solution was reused after filtration and readjustment of its SG,

but periodically had to be redistilled to remove dissolved organic materials.

A comparison of the amounts and composition of the light fractions obtained from several soils using either sedimentation in special separating funnels or centrifuging to separate the two fractions is given in Table 7.

The modified procedure resulted in greater (30-60%) yields of light fraction having a similar or higher carbon content to that obtained by sedimentation. The reproducibility of the fractionation, especially for finer textured soils, was stated to be improved. The lower C:N ratio of the light fraction was considered to reflect a more complete separation, the sedimentation method only recovering coarser particles of relatively higher C:N ratio. Refractionation of the heavy fraction from soil previously fractionated by the sedimentation method yielded an additional quantity of fine light fraction particles which accounted for the observed difference in yield between the two methods. Both procedures recovered similar amounts of coarse (2.0 - 0.2 mm) straw particles from synthetic subsoil-straw mixtures, but the centrifuging procedure recovered a greater proportion of fine (<0.2 mm) particles. However incomplete recoveries from some soils were obtained even with the modified procedure.

Monnier (1965) has commented further on the procedure of Monnier et al. (1962). He noted that refractionation of the soil pellet was necessary only where the sample contained large

TABLE 7. COMPOSITION OF FRACTIONS SEPARATED FROM SOME FRENCH SOILS BY SEDIMENTATION UNDER GRAVITY OR BY CENTRIFUGING

(after Monnier et al. 1962)

Soil	Vegetation	Mean Composition of LF (SG <2) obtained by					
		Sedimentation			Centrifuging		
		LF % of Soil*	C % of LF	C:N of LF	LF % of Soil*	C % of LF	C:N of LF
Fine Sand	young pasture	1.29	19.9	18.1	2.07	20.1	17.1
Silt	old pasture	2.93	18.1	22.3	3.36	24.0	20.4
White Rendzina	not stated	1.33	23.9	27.7	1.93	22.8	26.2

* 10g sample of air dry, <0.5 mm soil fractionated either by technique of Jeanson-Luusinang (1960) or by technique developed by Monnier et al. (1962).

amounts of light fraction. For calcareous samples, particularly those containing relatively large amounts of clay-combined organic matter, a solution SG of 1.75 was stated to be preferable to the SG of 2.0 generally recommended.

Lefebvre-Drouet (1963) has further examined the effect of varying the degree of grinding of the soil prior to fractionation by the method of Monnier et al. (1962). For the two rendzinas studied, the results (Table 8) showed that grinding the soil <0.2 mm (rather than <0.5 mm as recommended by Monnier et al. 1962) resulted in the release of an additional amount of light fraction equivalent to 25-30% of that obtained from a <0.5 mm sample. However, as the C:N ratio of the material obtained by either grinding treatment was similar, she nevertheless concluded that there was no advantage to be gained by fine grinding of the soil for future studies.

Lefebvre-Drouet and Meriaux (1966) reported that a greater proportion of the total N was mineralized during incubation for four months at 28°C of a cultivated grey rendzina than of a brown-red rendzina under grass. This was interpreted as reflecting the influence of the relative amounts and compositions of the light fractions (SG <2) in these soils, as in the cultivated soil this separate represented 10% of the soil N and had a C:N ratio of 16, whereas in the grassed soil the corresponding values were 3% and 29 respectively. However no attempt was made either to measure the

TABLE 8. EFFECT OF GRINDING ON SUBSEQUENT DENSIMETRIC FRACTIONATION OF SOME FRENCH SOILS

(after Lefebvre-Drouet 1963)

Soil	History	Mean Composition of LF (SG <2) obtained from Soil					
		<0.5 mm			<0.2 mm		
		LF % of Soil	C:N of LF	% of Soil C in LF	LF % of Soil	C:N of LF	% of Soil C in LF
Grey Rendzina	Cultivated	1.32	15.3	10.9	1.65	15.6	12.4
Brown-red Rendzina	Grassed	2.72	26.4	10.2	3.52	23.0	10.5

NB: (i) LF separated by method of Monnier et al. (1962)
(ii) Composition of LF calculated from other data in cited paper
(iii) Site history from Lefebvre-Drouet and Meriaux (1966).

loss of light fraction during incubation or to make allowance for the probable effects of other differences between the samples (e.g. time of sampling or sample preparation) on the net N mineralization.

3. Conclusions

The range of results obtained by various authors using different densimetric fractionation procedures is indicated by the results in Table 9. Few of these procedures have been critically examined, the separates have usually been characterised only on the basis of their physical appearance and C:N ratio, and little attempt has been made to justify the hypothesis that the "free" organic matter is a more labile fraction than is the clay-organic complex. Nevertheless it is apparent that for a wide variety of soil types at least partial separation of these fractions has been achieved, and that the "free" organic fraction can account for an appreciable proportion of the soil C, and generally has a C:N ratio similar to or greater than that of the soil from which it was separated. Khan (1959) found that the light fraction was intimately associated with phytoliths, and most authors have obtained separates which were appreciably contaminated with soil mineral matter.

The failure to obtain satisfactory separations was partly a result of inadequate disruption of soil aggregates and dispersion of the sample in the heavy liquid. Although grinding of the soil prior to fractionation was found more convenient than aqueous destruction methods (Monnier et al. 1962), the results obtained

TABLE 9. AMOUNTS AND COMPOSITION OF "FREE" ORGANIC MATTER OBTAINED BY DENSIMETRIC FRACTIONATION OF VARIOUS SOILS

References	Fractionation Procedure				Soil	Vegetation or History	Depth (cm)
	Soil Pretreatment	Solution Composition	Method of Dispersion	Method of Sedimentation			
(1)	use >1μ separate	aqueous (Toulet)	-	centrifuge	Chernozem Podzol	- -	- -
(2)	-	organic bromoform + trimethyl benzene	-	centrifuge	Chernozem Podzol	steppe reserve forest	0-10 (A_1) 2-12
(3)	boil in water, dry with EtOH	bromoform + benzene	(Manual agitation)	gravity	Pine Humus Silt	(forest) cultivated	- -
(4)					Rendzina	-	-
(5)	air dry, and grind <0.05 mm	bromoform + ethanol	manual agitation	centrifuge	Fine Sand Silt Rendzina	young pasture old pasture cultivated	- - -
(6)							
(7)	"	"	"	"	Rendzina Rendzina	grassed cultivated	0-15 0-15
(8)	"	"	"	"	Brown Podzolic	deciduous forest	[(A_{11}) 0-2 (A_{12}) 2-20
					Podzol	pine forest	[(A_{11}) 0-10 (A_{12}) 10-25

References: (1) Khan (1959)
(2) Lein (1940)
(3) Hénin and Turc (1949)
(4) Hénin and Turc (1950)
(5) Monnier et al. (1962)
(6) Monnier (1965)
(7) Lefebvre-Drouet and Meriaux (1966)
(8) Duthion and Chretien (1966)

- = not given.

TABLE 9 (continued)

References	Soil	pH (water)	g/100 g Soil				SG	"Free" Organic Matter			
			<2μ	C	N	C:N		% of Soil	Ash Content (g/100 g)	C:N	% of Soil C contained in Fraction
(1)	Chernozem	-	-	4.7+	-	-	<1.8	1.6	10.5	32.4+	14.8
	Podzol	-	-	1.6+	-	-		0.5	16.2	-	10.4
(2)	Chernozem	6.7	19.0	6.1	0.5	12.1	<1.7	1.2	27+	-	8.2
							<2.0	7.7	49+	-	32.6
	Podzol	5.0	24.8	3.5	0.3	13.0	<1.7	1.1	28+	-	12.2
							<2.0	6.0	56+	-	37.9
(3)	Pine Humus	-	-	25.6	-	-	<2	74	-	25.8	-
	Silt	calc.	-	1.6	-	-		1.0	-	19.1	-
(4)	Rendzina	-	-	10.8+	-	-	<1.75	5.5	-	18.5*	17
							<2	16.5	-	10.3	17+
(5)	Fine sand	-	-	-	-	-	<2	2.1	-	17.1	-
(6)	Silt	-	-	-	-	-		3.4	-	20.4	-
	Rendzina	-	-	-	-	-		1.9	-	26.2	-
(7)	Rendzina	(1.4% $CaCO_3$)	54	5.0	0.34	14.8	<1.75	1.2	-	35.5*	5.6+
							<2	2.2	-	32.3*	9.0+
	Rendzina	(49.5% $CaCO_3$)	41.5	2.6	0.20	13.0	<1.75	0.3	-	21.0	4.1+
							<2	1.6	-	17.0*	13.9+
(8)	Brown	4.4	22.5	8.0	0.44	18.5	<1.75	8.5	-	27.0	34.7+
	Podzolic	4.9	19.5	4.1	0.27	15.0		1.2	-	25.6	9.9
	Podzol	4.0	21.5	12.3	0.70	17.4		21.8	-	22.9	56.2
		4.6	21	4.7	0.27	17.5		2.3	-	25.2	16.4

References:
(1) Khan (1959)
(2) Lein (1940)
(3) Hénin and Turc (1949)
(4) Hénin and Turc (1950)
(5) Monnier et al. (1962)
(6) Monnier (1965)
(7) Lefebvre-Drouet and Meriaux (1966)
(8) Duthion and Chretien (1966)

+ = calculated from data in paper;
* = weighted mean of data in paper;
- = not given;
calc. = calcareous.

Suite.

depended on the degree of grinding, a <0.2 mm sample yielding more "free" separate than a <0.5 mm one (Lefebvre-Drouet 1963). Manual agitation, which was normally used to suspend the soil in the heavy liquid, was not entirely satisfactory since the relatively high suspension concentrations used (e.g. 5-10 g soil/100 ml) led to mechanical entanglement of the fractions during sedimentation. Consequently repeated refractionation of the separates was often required (Khan 1959; Monnier et al. 1962).

Separation in heavy organic liquids appears to be the most promising method for the separation of "free" organic matter from the clay-organic complex. Critical examination and refinement of this technique is required if a quantitative and reproducible separation is to be achieved. Further characterization of the separates and studies of their dynamics are also needed in order to evaluate the importance of "free" organic matter in arable soils.

III DENSIMETRIC FRACTIONATION OF SOIL ORGANIC MATTER

If the relative contributions of non-humified and humified organic matter to the N turnover in arable soils is to be examined, it is essential that if possible a quantitative and reproducible separation of these two fractions be obtained.

(A) Preliminary Studies

(i) Initial investigations

The fractionation procedure used initially was developed as a result of an examination of some of the major factors affecting the separation achieved using the procedure of Monnier et al. (1962). The principal modifications to their technique involved a reduction in the soil:solution ratio, and the use of ultrasonic vibration to improve the efficiency of dispersion of the soil in the bromoform solution. The various factors affecting the efficiency and reproducibility of the separation are discussed in more detail in section A(ii) below.

This technique was evaluated by applying it to samples from plots under various pasture-wheat rotations.

1. Soils

The soils used were samples from the surface horizon of plots from the permanent rotation trial (C1) at the Waite Institute, Adelaide. The soil, Urrbrae fine sandy loam, is a red-brown earth (Piper 1938). Samples from the (0-10)cm layer were

obtained by subsampling from composites of 6 cores (Veihmeyer tube) from each plot (c. 0.05 ha), several samplings being made during the 1963 growing season. The soils were crushed <2 mm, and subsamples dried at 105°C before being ground to pass a 0.25 mm sieve.

2. Methods

5.0 g samples of soil were oven dried at 105°C, ground <0.25 mm and suspended in c. 150 ml bromoform-petroleum spirit (b.r. 80°-100°C) solution of SG 2.0. Dispersion was achieved by ultrasonic vibration (Mullard E 7680/3 Ultrasonic drill, 60 watts, 20 ± 4 Kc/sec) for 3 minutes. The suspension was centrifuged at c. 2,000 R.C.F.* for 30 minutes (M.S.E. 'Medium" centrifuge, swing-out head), and the supernatant and material floating on it (the "light fraction", LF) decanted onto a filter paper (Whatman No. 50) under suction. The LF was washed with petroleum spirit, dried at 70°C, and weighed.

3. Results and discussion

The amounts of light fraction (LF) from various samples of plots under different rotations are presented in Table 10. There are evidently gross differences in the amounts of LF accumulated in plots under the various rotations. Thus after 38 years continuous wheat (C1:17) there was some 0.6% LF present in the soil at the end of summer, this representing 13% of the total

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* R.C.F. = relative centrifugal force = $(1.12\ RN^2)10^{-5}$
where R = radius (cm) from centre of centrifuge spindle to outside lip of tube, and
N = speed (rpm) of centrifuge spindle.

TABLE 10. EFFECT OF ROTATION AND TIME OF SAMPLING ON LF LEVELS IN URRBRAE LOAM (C1 TRIAL, 0-10 cm)

Plot No.	Rotation and Phase	g LF/100 g OD Soil*		
		May'63	July'63	Oct.'63
2	FW : W	0.24	0.16	0.26
1	" : F	0.29	0.26	0.17
17	Continuous W	0.58	ND	0.41
32	PPFW : P_2	0.77	0.90	0.71
30	" : W	0.52	0.59	0.40
13	PPPPWW: P_1	0.85	0.64	0.56
12	" : P_3	1.01	0.74	0.89
11	" : P_4	1.56	0.99	0.81
14	" : W_1	1.05	0.98	0.66
29	Continuous P	1.71	1.23	1.45

* means of quadruplicates

ND = not determined

P = pasture (P_1= first year pasture, P_2= second year pasture, etc.)

F = fallow

W = wheat (W_1= first year wheat).

soil carbon (as determined by dry combustion). Corresponding figures for a plot which had been under continuous grass-legume pasture for 14 years (C1:29) were 1.7% LF, and 21% of the soil carbon. Intermediate amounts of LF were present in plots at

various stages of 4-course or 6-course rotations. The amount of LF generally increased with time under pasture, and with the relative duration of the pasture and cropping phases of the rotation. Conversely, the LF level tended to fall during the cropping phase. These trends were similar to those reported by Greenland (1962) for soil N in these rotations, suggesting that the LF might be of use as an index of the fertility status of plots under such rotations.

There was also some indication of seasonal trends within some of the plots sampled (Table 10, but these could in part be due to seasonal variations in bulk density. Whilst the results indicate that the fractionation technique used yielded amounts of LF which reflected the agronomic history of the plot sampled, some problems were encountered with regard to the reproducibility of the separation. Further studies were therefore undertaken of the factors affecting the fractionation.

(ii) Detailed Studies of the Densimetric Fractionation Procedure

1. Soils and methods

Details of the soils examined are given in Table 14. The analytical procedures used are described in Chapter IV (A(i)-(iii)) and in Appendix B.

2. Choice of densimetric liquid

a) Composition

Previous authors have reported that mixtures of bromoform

and a less dense organic solvent (e.g. benzene, carbon tetrachloride or ethanol) were preferable to aqueous (e.g. Toulet) solutions. Although ethanol was the least toxic of the diluting solvents examined by Jeanson-Luusinang (1960) and Monnier et al. (1962), these authors reported that it dissolved portion of the humified soil organic matter.

Preliminary experiments in which a range of diluting solvents were compared established that satisfactory separations were obtained using petroleum spirit (a mixture of saturated hydrocarbons of boiling range 80-100°C). This solvent was relatively non-toxic, and apparently dissolved little of the organic matter (probably mainly lipid material). Consequently the bromoform solution did not have to be redistilled as frequently as when ethanol was used. Like ethanol it produced a firm soil pellet on centrifuging, so that decantation of the LF without contamination by heavier soil particles was possible. However, as it was also more volatile than the bromoform, precautions had to be taken to minimise density changes during fractionation. The solution density was determined by weighing a known volume, and was readjusted before being reused. In order to retard the accumulation of decomposition products, the mixture was best stored in dark bottles over metallic copper turnings. The solvents were readily purified by distillation under reduced pressure from freshly ignited calcium oxide lumps (to remove HBr or Br_2), rapid and even distillation with minimum decomposition of the

bromoform being achieved by the use of a capillary bleed of air which has been successively scrubbed by alkaline pyrogallo and concentrated H_2SO_4. All manipulations were conveniently carried out in an efficient fume hood.

b) Density

Solutions of SG 1.75 - 2.25 have been reported as giving the best separations of "free" (LF) from clay-combined organic materials. However the sharpness of the separation varied with soil type. In practice a solution SG of 2.0 gave satisfactory separations, and this was confirmed in the present study. As shown in Table 11, the use of lower solution densities markedly reduced

TABLE 11. EFFECT OF SOLUTION DENSITY ON YIELDS OF LF FROM A SAMPLE OF URRBRAE SOIL

Sample: Trial, plot, date sampled	C1:29, March 1961		
Depth (cm)	0-6.3		
History	Continuous P		
Solution SG	Yield (g/100 g OD Soil)		
	LF	'Ash'*	'Ash-free' LF
1.8	0.95	0.25	0.70
2.0	1.60	0.52	1.08

*residue after treatment of LF with H_2O_2 and ignition.

both the amount and 'ash' content of the LF. Solutions of SG <2.0 yielded LF material which was often heavily contaminated with soil mineral matter. Thus the choice of a solution SG of 2.0, although arbitrary, generally resulted in the best possible yields of LF material consistent with the inclusion of the least amount of other soil components. To ensure that the solution SG did not exceed 2.0 during fractionation, it was found convenient to adjust the initial density to c. 1.97 g/cc, and to cap the centrifuge tubes containing the suspension.

3. Sample pretreatment and dispersion

Complete dispersion of the soil in the organic liquid is essential if all the LF particles which may be entrained in the sample are to be recovered. The data leading to this conclusion is discussed below.

a) Drying: The use of air-dry samples was found to result in lower yields of ash'free LF than if the soils were thoroughly dried prior to fractionation. For both a red-brown earth (Urrbrae fine sandy loam) and a rendzina (Millicent clay), the yield of ash-free LF was greatest after drying over P_2O_5. Traces of moisture in the samples resulted in increased contamination of the LF with inorganic soil material. The precise mechanism of this effect is not known, but probably involves incomplete dispersion of the soil in the bromoform solution due to the adhesive effect of water films between LF and soil particles (see also p.65 and p.91). These

effects may not have been encountered by earlier workers using ethanol-bromoform mixtures (Monnier *et al.* 1962)

It was considered that this phenomenon was not a serious objection to the use of bromoform-petroleum spirit solutions, as provided the samples were thoroughly dried (at 70°C or over P_2O_5 *in vacuo*) prior to fractionation, satisfactory yields of good reproducibility (see Table 12) were readily obtained from a range of soils of widely contrasting textures.

b) Aggregate disruption and dispersion

Both sample pretreatment and dispersion technique influence the efficiency of the fractionation. A comparison was therefore made of the relative effectiveness of a modification of the procedure proposed by Monnier *et al.* (1962) and of the procedure used above, involving ultrasonic dispersion. The soils, methods and results obtained are given in Table 12.

Except for the podzol, which was essentially non-aggregated, a substantially larger amount of LF was extracted after sonifying the finely ground soil than was obtained by the boiling pretreatment followed by hand shaking. The reproducibility of the sonication technique was indicated by the small standard errors obtained for the red-brown earth and the rendzina. Although similar variations were obtained for samples fractionated by the other technique, no attempt was made to calculate standard errors as the extraction was incomplete.

TABLE 12. COMPARISON OF SAMPLE PRETREATMENT AND DISPERSION TECHNIQUE ON YIELD AND COMPOSITION OF LIGHT FRACTION SEPARATED FROM SOME SOUTH AUSTRALIAN SOILS

(after Greenland and Ford 1964)

Soil Group*	History	g/LF 100g OD Soil		g/100g LF			
		Pretreatment		Organic C		'Ash'	
		A	B	A	B	A	B
Podzol	Virgin heath scrub	4.00	4.00	15.6	14.5	64.4	59.2
Red-brown earth	Continuous wheat	0.88	1.27	23.7	26.5	56.1	55.6
"	Continuous pasture	1.74	2.81 (±0.19)	23.2	22.6	47.2	47.8
Ground water Rendzina	Old pasture	6.66	8.24 (±0.31)	17.9	22.3	49.2	46.6

* Stephens (1962)

(i) Pretreatment A: <0.25 mm soil was boiled for 5 minutes in water, then the water displaced with 4-5 portions of absolute ethanol. The residue was finally dried at 70°C, ground <0.25 mm, and 5.0 g samples shaken by hand for 2 minutes with c. 150 ml bromoform-petroleum spirit solution (SG 2.0).

(ii) Pretreatment B: <0.25 mm soil was dried at 70°C, and 5.0 g samples dispersed with ultrasonics (3 min, 20 Kc/sec, 75 watt) in c. 150 ml bromoform solution.

(iii) Both sets of suspensions were centrifuged (30 min; 2,000 R.C.F.) and their LF obtained by decantation.

(iv) Values in parenthesis are standard errors of tabulated means.

(v) Organic C was determined by dry combustion (Methods B8 and B9).

(vi) 'Ash' = residue after treatment of LF with H_2O_2 and ignition (Methods B2 and B3).

As well as resulting in a more complete, convenient, and reasonably reproducible separation, the sonication technique yielded LF which contained slightly less ash, and similar or slightly greater amounts of organic C. Thus the separate was less contaminated with soil mineral matter.

Notes on the use of the ultrasonic probe

In this, and subsequent studies, a more powerful ultrasonic probe (Branson S-75 Sonifier, 75 watt, 20 ± 0.6 Kc/sec) was used than previously. In order to minimise the temperature increase during the 3 minute sonication it was necessary to construct a water-jacketed insonation vessel, this keeping the temperature rise to $<5^{\circ}C$ (cf. $<10^{\circ}C$ in an uncooled vessel). The use of an ice bath was found to be unsatisfactory, since the condensation of water vapour on the inner surface of the insonation vessel (a 250 ml tall-form beaker) caused the LF particles to adhere tenaciously to both the beaker wall and to the heavier soil particles.

For the soils examined above (except the sandy podzol) the yield of LF was found to depend upon the position of the probe tip in the insonation vessel, presumably because of variations in the efficiency with which the aggregates were disrupted. Thus for a sample from the surface 10 cm of the permanent pasture plot on the Urrbrae soil (C1:29) the mean amount of LF obtained increased from 1.50 to 2.81 g/100 g soil as the probe depth below the liquid was increased from 10 to 50 mm, the total depth of liquid being 70 mm. The 50 mm depth was thus arbitarily selected as standard for future

separations in this type of insonation vessel.

Under these conditions increasing the time of sonication from 1 minute up to a maximum of 9 minutes did not significantly affect the separation, and a 3 minute period was adopted as being convenient and satisfactory.

4. Centrifuging of the soil suspension

a) Soil:liquid ratio

Previous workers (Khan 1959; Jeanson-Luusinang 1960; Monnier et al. 1962) reported that refractionation of the soil pellet, and sometimes also of the LF, was necessary if acceptable separations were to be obtained. Preliminary experiments showed that this problem of the mechanical entanglement of the LF particles in the soil residue was in part due to the incomplete dispersion obtained by these authors, and in part also to the relatively concentrated (5-10 g/100 ml) soil:liquid ratios of the mixtures used.

A suspension of 5 g of soil in 150 ml of bromoform solution was found to minimise occlusion of LF by sedimenting soil particles, so that >95% of the LF separated by repeated fractionation of the same sample was obtained in the first extraction. Thus the need for repeated fractionation was avoided, and the extraction simplified.

b) Centrifuging

Monnier et al. (1962) reported that centrifuging the soil

suspension for 5 min at 1,000xg resulted in a better separation than was obtained by simply allowing the suspension to sediment under gravity. However in order to minimise the mechanical occlusion of LF particles during centrifugation it was found necessary to allow the suspensions to stand (in capped centrifuge bottles) for at least 30 minutes before centrifuging. As shown in Table 13, this resulted in negligible entanglement of the LF within the soil pellet.

The most satisfactory results were obtained by centrifuging for 30 min at 1,600-2,000 R.C.F. in a centrifuge fitted with a swing-out head. This gave a firm pellet which enabled contamination of LF by soil material during decantation to be minimised.

TABLE 13. EFFECT OF SEDIMENTATION PRIOR TO CENTRIFUGING ON RECOVERIES OF LF FROM A SAMPLE OF URRBRAE SOIL

Sample: Trial, plot, date sampled	C1:29, Oct.'63
Depth (cm)	0-10
Rotation	Continuous P
Treatment	**mg LF/5 g OD Soil**
1. a) sonify, centrifuge immediately	93.4
b) resuspend pellet	28.8
Total LF separated:	122.2
2. Sonify, stand >1 hour before centrifuging	126.5

5. Statement of fractionation technique

The soil was ground to pass a 0.25 mm sieve, larger stones or plant parts being rejected. After oven drying at 70°C (or *in vacuo* over P_2O_5), 5.0 g samples were immediately placed in a 250 ml beaker and c. 150 ml of bromoform-petroleum spirit solution of SG 2.0 added. The suspension was sonified (20 Kc/sec, 75 watt) for 3 minutes under standard conditions in a water-jacketed beaker, transferred to a centrifuge tube and allowed to stand for at least 30 minutes. After centrifuging at 1,600-2,000 R.C.F. for 30 minutes, the supernatant together with the LF was quantitatively decanted onto a filter paper (Whatman No. 50) under suction. The LF was washed with petroleum spirit, dried at 70°C, and weighed.

(iii) Application to a Range of Soils

Previous studies (Chapter III, A(i)) demonstrated that the amount of LF separated from a red-brown earth varied with its agronomic history, especially the relative lengths of the pasture and cropping phases in the rotations concerned. The phase of the rotation at sampling and the time during the growing season that the plot was sampled also influenced the amount of LF present (Table 10). This procedure was subsequently modified (Chapter III, A(ii)), and applied to a range of Australian soils (Tables 14 and 15).

Whilst these results confirmed that the amount of LF separated varied markedly both with the soil type and with its

TABLE 14. PROPERTIES OF SOME SOILS AND OF THEIR LF
(after Greenland and Ford 1964)

Group*	Series	History	Depth (cm)	pH	g/100 g OD (70°C) Soil					g/100 g LF					% Soil C or N in LF	
					<2μ	C	N	C:N	LF	C	N	C:N	'Ash'	'Humus'	C	N
Podzol	Mt. Compass	virgin heath scrub	0-10	4.2	<2	0.68	0.028	24.3	4.00	14.5	0.73	19.1	59.2	4.2	85.3	100
Solodised Solonetz	unnamed	(cultivated)	0-5	6.4	9	1.04	0.082	12.7	1.07	22.9	1.16	19.8	48.0	6.6	23.6	15.1
Solonized brown	Waipeup	W after 6 yrs medic	0-8	7.8	9	0.58	0.054	10.7	1.22	23.0	1.42	16.2	ND	3.5	48.4	32.1
Red-brown earth	Urrbrae	Continuous WF	0-10	5.1	20	0.98	0.079	12.4	0.78	31.8	1.10	28.9	ND	4.2	25.3	10.8
		Continuous W	0-10	5.2	20	1.63	0.126	12.9	1.27	26.4	1.14	23.1	55.6	10.2	20.6	11.5
		Continuous P	0-10	5.2	20	2.23	0.194	11.5	2.81	22.6	1.98	11.5	47.8	14.0	28.5	28.7
Ground water Rendzina	Millicent	Old P	0-5	5.7	38	5.81	0.517	11.3	8.24	22.3	1.73	12.9	46.6	4.9	31.6	27.7
Lateritic red earth	Tippera	Cropped (10 yrs)	0-15	5.3	54	0.94	0.058	16.2	0.03	20.1	ND	ND	ND	3.5	0.6	ND
		virgin	0-15	6.1	54	1.69	0.092	18.4	0.15	24.8	ND	ND	ND		2.2	ND

ND = not determined. * after Stephens (1962)

Methods:
(i) pH determined on 2:1 (w/v) suspension of soil in 0.01 M $CaCl_2$
(ii) Organic C determined by dry combustion (Method B9)
(iii) Organic N determined by Kjeldahl procedures (Methods B11 and B12)
(iv) 'Ash' determined by ignition (Method B1)
(v) LF-'humus' estimated from E260 mμ of alkali extract (Method B3).

agronomic history, some doubts as to the reliability of this data nevertheless remained. These arose from the lack of adequate criteria by which the completeness of the separation of the non-humified material could be assessed. As subsequent studies demonstrated that the grinding pretreatment used materially affected the amount of LF separated (Chapter III, B), the data obtained using the above procedure must be treated with reservation. Although the LF estimates given in Tables 14 and 15 will be minimal values, the general trends displayed by the data are probably valid, and as they are of interest to this study they are briefly discussed below.

a) Table 14: In the five temperate soils examined, the LF usually accounted for a significant portion of the total organic matter: it comprised 0.8 to 8.0% by weight of the soil and contained between 20 and 85% of the carbon and 11 to 100% of the nitrogen. In the lateritic red earth from Katherine (N.T.), sampled in August after 4 months of the dry season, it constituted <0.2% by weight of the soil and contained 2.2% or less of the carbon. Although this latter value is probably an underestimate, it is unlikely that the amount of LF separated by the technique ultimately developed (Appendix A) would be more than twice this figure. It would thus be reasonable to infer that the rate of turnover of LF material in this soil is apparently higher than in similar temperate soils.

The amount of LF in the Urrbrae samples varied markedly

with the agronomic history of the sample site, and as the same trend was apparent in the tropical soil it was inferred that this may be a general phenomenon warranting further investigation.

In Table 14 the soils are ranked in order of increasing clay content, and it is apparent that in the temperate soils at least the proportion of the soil carbon in the LF tended to decrease with increasing clay content. The corresponding trend for soil nitrogen was not as clear, presumably as it was influenced to a greater extent by the nature of the vegetation on the sample site, plots under leguminous pastures tending to have comparable proportions of their soil C and N present in the LF. This was supported by the observation that the C:N ratios of the LF separated from the pasture plots were generally lower (c. 11 to 16) than those from cultivated soils (c. 20 to 29), although the variations in soil C:N ratios were much smaller. In general the C:N of the LF tended to be higher than that of the soil from which it was separated, this being in agreement with the results of earlier workers (Table 9). Other properties of these LF samples are discussed in Chapter IV.

b) Table 15: As the relationship between the dynamics of the LF and the turnover of soil N is of major interest to this study, analyses of LF-N and soil N only have been made. Organic C analyses were also rejected as they were subject to interference both from soil carbonates (in the Walpeup and Longerenong samples) and from traces of residual organic solvents in the LF.

TABLE 15:

a) Methods:

(i) LF: separated by method of Greenland and Ford (1964)
(ii) LF-N: calculated from % N in LF (Method B12)
(iii) Soil N determined by Dumas procedure (Method B11)
(iv) All calculations based on appropriate mean values for B.D.

b) Soils: (see Appendices C1 to C4)

(i) Longerenong: means of samples from trial LR2 (Rep. 1); Sept.'64 and Sept.'65.
(ii) Walpeup: means of samples from trial MR2 (Rep.1); Sept.'64 and Sept.'65.
(iii) Urrbrae: means of various samplings from C1 trial of plots at different phases of tabulated rotations
(iv) Truro: Continuous FW = sample from site at initiation of trial (Clarke et al.1967)
Other data refers to samples (from Rep.2) after 3 years of pure swards of species shown; (+N) = 125 kg Urea-N/ha/year. Plant tops were mown and removed from all plots.
R = Annual ryegrass (Lolium rigidum Gaud.)
P = Phalaris tuberosa L.
C = Subterranean clover (Trifolium subterraneum L.)

TABLE 15. EFFECT OF ROTATION ON THE PROPORTIONS AND WEIGHT OF ORGANIC NITROGEN IN THE LF OF VARIOUS SOILS

Soil Group* and Series or Trial Site	Rotation and Phase	Depth (cm)	Approximate Mean Amount (kg/ha)		% Soil N in LF
			LF	LF-N	
Grey soil of heavy texture (Longerenong, Vic.)	Continuous FW: F	0-10	290	4	0.7
	" " : W		310	5	0.9
	Continuous PPFW: P_1		900	18	3.1
	" " : P^1		1950	39	4.6
	" " : F^2		1700	36	4.9
	" " : W		720	16	3.2
Solonized brown (Walpeup, Vic.)	Continuous FW: F	0-10	1600	21	3.4
	" " : W		2080	28	4.1
	Continuous PPFW: P_1		2920	47	5.2
	" " : P^1		3940	63	6.5
	" " : F^2		3840	69	9.1
	" " : W		3120	56	6.0
Red-brown earth (Urrbrae fine sandy loam; Waite Institute, S.Aust.)	Continuous FW	0-10	5950	60	5.0
	" W		11900	133	7.6
	" PPFW		11450	170	8.6
	" PPPPWW		16250	266	13.3
	" P		26200	510	20.1
Red-brown earth (Stockwell loamy fine sand; Truro, S.Aust.)	Continuous FW	0-2.5	955	11	4.1
	then 3 yr R		1320	23	7.6
	" " " (+N)		2220	39	11.0
	" " " P		790	12	4.3
	" " " (+N)		1460	23	8.1
	" " " C		1780	42	10.4

* Stephens (1962)

The principal trends of interest in the data presented in Table 15 are as follows. It would appear that the relative rates of accretion and loss of LF material may differ from the Urrbrae, Walpeup and Longerenong soils, as marked differences were found in the amounts of LF separated from plots under comparable rotations. Generally the greatest amounts of LF were found in the Urrbrae samples, although at each site plots under pasture-wheat rotations contained appreciably more LF than plots which were continuously cropped. The Urrbrae data in particular suggests that the LF tended to accumulate under pastures and decline during cropping. There was some evidence that the mean N content of the LF was higher under pasture than under crop, probably because leguminous residues are relatively richer in N than are graminaceous (grass or cereal) ones. This was supported by the Stockwell data, where the marked interaction of the botanical composition of the pasture sward and the supply of available N in the soil on the amount and N content of the LF was indicated by the two-fold increase in both LF and LF-N resulting from the application of nitrogenous fertilizer to pure grass swards. Even after only 3 years, and despite the influence of two dry seasons, considerable increases in the amount of LF-N had apparently occurred in the surface 2.5 cm of soil. Annual ryegrass contributed more LF and exhibited a greater response to fertilizer-N application than did phalaris (a perennial). As the structural improvement measured over this period was greatest under

ryegrass, it is likely that part at least of the LF may have been acting as a readily available substrate for microorganisms involved in the stabilization of aggregates (Clarke et al. 1967). Similar correlations between the amount of plant residues accumulated under leys and the increase in the proportion of water-stable aggregates have been reported by Clement and Williams (1958) and Monnier (1965), and it is probable that this provides a partial explanation for the generally observed structure-improving effects of leys.

(iv) Conclusions

These preliminary studies have demonstrated that it is possible at least to partially fractionate the organic matter of a wide range of soils relatively simply and rapidly by dispersing the soil in a heavy organic liquid (bromoform-petroleum spirit, of SG 2.0). Ultrasonic dispersion facilitated the separation of organic material which was not combined with the clay fraction. Although the amount of LF so obtained was probably an underestimate of the amount actually present, it was evident that it depended on the soil type and its agronomic history, the greatest amounts generally being found in heavy textured soils and in soils under old pastures. Some evidence for seasonal variations in the amount of LF was obtained for plots under different wheat or pasture-wheat rotations. The LF comprised an important proportion of the soil C and N, and its C:N ratio (generally <25) suggested that it might represent an important reserve of potentially mineralizable N.

In view of these promising preliminary results, it was decided to use this method for the systematic study of changes in the LF-N and mineralizable N contents of soils at various stages of pasture-wheat rotations.

However during the initial stages of the investigation of the seasonal trends in the amounts of LF present in samples of the Walpeup and Longerenong soils, it was found that not only were the amounts of LF separated by this technique small, but the variation between duplicates was more erratic than could reasonably be accounted for by sampling errors. Further, the yield of LF obtained from a <2mm subsample was up to twofold that from a similar sample ground <0.25 mm prior to fractionation. Also, the apparent seasonal fluctuations in LF (Table 16) were smaller than expected, even in 4-course plots recently fallowed from pasture. These differences were not due to variations in the amount of inorganic material separated with the LF, as the 'ash' contents were similar (c.35-45%) for LF obtained by either grinding pretreatment.

This decrease in LF yield as a result of fine grinding was unexpected, as previous authors (Lefebvre-Drouet 1963) had reported that fine grinding (<0.2 mm) of a rendzina released an additional amount of LF over that obtained from a <0.5 mm sample. This additional material presumably represented fine plant residues released when the larger aggregates were ruptured by the grinding treatment. The anomalous nature of this effect was emphasised by the observation that for the 3 soils examined, many of these

TABLE 16. EFFECT OF GRINDING ON APPARENT SEASONAL VARIATIONS IN LF CONTENT OF SOME WALPEUP AND LONGERENONG SAMPLES

Trial and Plot No.	Rotation and Phase	Date Sampled	g LF/100 g OD (70°C) Soil ground	
			<2 mm	<0.25 mm
MR2: 4	PPFW : P_2	Sept.'64	0.39	0.15
	" : "	Oct.'64	0.35	0.25
	" : "	Dec.'64	0.35	0.22
	" : F	June '65	0.32	0.18
	" : "	July'65	0.32	0.19
MR2: 5	FW : W	Sept.'64	0.12	0.07
	" : "	Oct.'64	0.11	0.07
	" : "	Dec.'64	0.11	0.08
	" : F	June '65	0.17	ND
	" : "	July'65	0.20	ND
LR2: 3	PPFW : P_2	Sept.'64	0.70	0.32
	" : "	Oct.'64	0.40	0.17
	" : "	Dec.'64	0.50	0.23
	" : "	May '65	0.62	0.14
	" : F	July'65	0.55	0.21
LR2: 1	FW : W	Sept.'64	0.06	0.05
	" : "	Oct.'64	0.09	0.03
	" : "	Dec.'64	0.11	0.05
	" : "	May '65	0.11	ND
	" : F	July '65	0.10	0.05

ND = not determined.

larger (0.25-2.0 mm) aggregates were not disrupted by the ultrasonic treatment, as they tended to cluster around the probe shaft at the surface of the bromoform solution. The amount of LF separated would thus have been expected to be less than from corresponding <0.25 mm samples. As quantitative separation of the LF was essential to further studies of the dynamics of this fraction, the effects of the drying and grinding pretreatments used in previous studies were re-examined.

(B) Modification of Fractionation Method

(i) Soils

Samples were collected from the surface 10 cm of the long-term rotation trials established at Victorian Department of Agriculture field stations at Walpeup (MR2; Solonised brown soil) and Longerenong (LR2; Grey soil of heavy texture); and also from the surface 6.3 cm of the permanent rotation trial at the Waite Institute, Adelaide (C1; Red-brown earth). The plots sampled represented all stages of F-W and P-P-F-W rotations. In addition, for the red-brown earth, plots under continuous W, P, or under various stages of other P-W or P-F-W rotations were sampled. Further details of the trials and sampling procedures are given in Appendix C. All samples were air dried at 40°C, ground and sieved <2 mm, and stored at room temperature in sealed containers.

(ii) Methods, Results and Discussion

Samples from 4-course plots under pasture were used initially as they contained the greatest amounts of LF.

1. Effects of grinding

The effect of various grinding procedures is shown in Table 17, the data representing the means of duplicate determinations. For both soils any additional grinding of the <2 mm sample reduced the yield of LF. Grinding the whole soil by hand <0.25 mm caused a greater reduction than did gentle hand grinding combined with

TABLE 17. EFFECT OF GRINDING ON RECOVERY OF LF

Sample	MR2:12, Nov.'65	LR2:15, Nov.'65
Grinding Treatment	g LF/100 g OD (70°C) Soil	
A. Whole Soil, <2 mm	0.36	0.26
B. Whole Soil, <2mm — hand grind and sieve — <0.25 mm	0.24	0.19
C. Whole Soil <2 mm — gently grind 1 min, sieve — <0.25 mm	0.28	0.16
C. Whole Soil <2 mm — gently grind 1 min, sieve — grind residue 5 min, sieve — <0.25 mm	0.02	0.03
C. Whole Soil <2 mm — gently grind 1 min, sieve — grind residue 5 min, sieve — <0.25 mm	0.01	0.04
Total LF Recovered	0.31	0.23

frequent sieving i.e. where the <0.25 mm material was ground for only a relatively short time. As the duplicates were in close agreement, subsampling errors were unlikely to account for this difference.

The short (1 minute) grinding treatment yielded a fraction which represented 30-40% of the weight of the original soil sample, and contained most (70-90%) of the LF ultimately recovered from the sample. The >0.25 mm residue after a total grinding period of 6 minutes consisted mainly of sand grains and coarse plant fragments, indicating that all the soil aggregates had been crushed <0.25 mm.

Evidently the nature of the grinding pretreatment, and particularly the time for which the finer fractions were ground together, influenced the subsequent separation.

The recovery of LF originally separated from <2 mm soil after mixing with the soil residue and then grinding the reconstituted sample <0.25 mm prior to refractionation is shown in Table 18. At best only about half of the LF was recovered.

TABLE 18. RECOVERY OF LF SEPARATED FROM <2 mm SOIL AFTER REMIXING WITH EXTRACTED SOIL AND GRINDING <0.25 mm

Sample	LF g/100 g OD (70°C) Soil		% Recovery*
	<2 mm	After Reconstituting and Grinding <0.25 mm	
MR2:4, Dec.'64	0.34	0.17	50
" , June '65	0.40	0.15	37
LR2:3, July '65	0.43	0.24	56
" , Dec.'64	0.11	0.05	45

* Assumes negligible amount of LF released by re-extracting ground residue (cf. Table 19).

In another experiment approximately $^{2}/_{3}$ of the LF was recovered from reconstituted <2 mm and <0.25 mm samples of the Longerenong soil (Samples A and B, Table 19). However grinding of the LF <0.25 mm either separately (Sample C) or after mixing with the residue from the first fractionation (Sample D) further reduced the recovery to <30%. This indicated that portion of the LF was rendered non-separable even by grinding in the absence of soil. Little additional LF (<0.05 g/100 g soil) was obtained by refractionating the residue from the <2 mm soil, even after it had been ground <0.25 mm, so that apparently any LF contained within 0.25-2 mm aggregates was not being recovered either.

It would appear that the above results reflect the combined effects of grinding on both LF and the soil, and that despite the use of ultrasonics the separation of the LF and soil particles in the organic liquid was incomplete. The possibility that the LF material was somehow bonded to soil particles during the grinding process was therefore investigated. The most likely aggregating agents were considered to be lime ($CaCO_3$); polysaccharide gums (e.g. present within partly decomposed plant fragments and exposed when these fragments were ruptured during grinding); or possibly sesquioxide-organic complexes. These alternatives were examined by subjecting <0.25 mm samples to the following pretreatments.

a) grinding in the presence of 0.1 N HCl to remove $CaCO_3$, or

b) grinding in the presence of solvents, both polar (absolute ethanol ± HCl) and non-polar (petroleum spirit), in an

TABLE 19. EFFECT OF GRINDING OF LF AND SOIL ON SUBSEQUENT RECOVERY OF LF

LR2:15, Nov. '65				% Recovery**
1st Separation		2nd Separation		
Pretreatment	LF (%)*	Pretreatment	LF (%)*	
A. Whole soil, <2 mm	0.23	mix LF and residue	0.20	70
B. " , <0.25 mm	0.17	mix LF and residue	0.15	65
C. " , <2 mm	0.25	grind LF <0.25 mm and mix with residue	0.11	28
D. " , <2 mm	0.28	mix LF and residue, grind <0.25 mm	0.10	21
E. " , <2 mm	0.33	residue only	0.04	-
F. " , <2 mm	0.28	grind residue <0.25 mm	0.04	-

* LF expressed as g/100 g OD (70°C) soil

** Adjusted for 0.04% LF released by refractionation of <2 mm or <0.25 mm residue.

attempt to prevent the formation of possible organic-soil mineral bonds, or

c) pretreatment of the soil with 0.02 M $NaIO_4$ for 1 hour at room temperature to rupture any possible polysaccharide bonds (cf. Greenland, Lindstrom and Quirk 1962), or with neutral 0.1 M $Na_4P_2O_7$ for 16 hours at room temperature to remove any iron or aluminium cations which could otherwise flocculate organic materials (cf. Evans 1959).

However, none of these pretreatments materially increased the yield of LF over that obtained from an untreated <0.25 mm sample.

2. Use of dispersing agent in densimetric liquid

As chemical pretreatment of the sample did not overcome the effect of fine grinding, and as it was felt that the evidence suggested that the problem was basically one of achieving a satisfactory dispersion of the soil in the bromoform solution, the use of a dispersing agent was investigated. Irani and Callis (1963) suggest that one of the best available agents for organic liquids is "Aerosol OT" (sodium dioctyl sulphosuccinate). A sample of the concentrated agent ("Aerosol OT-100") was generously supplied by Cyanamid Australia Pty. Ltd., Melbourne, and added to the bromoform solution with the following results.

a) Effect of drying and grinding pretreatments

The effect of drying (air dry cf. oven dried at 70°C overnight) and grinding (<2 mm cf. <0.25 mm) on the yields of LF

obtained with and without the use of 0.05% w/v "Aerosol OT" from samples of the Walpeup (sand) and Longerenong (clay) soils is shown in Table 20.

As previously found, grinding decreased the yield of LF obtained from both soils. However, in the presence of the surfactant the yield of LF was dramatically increased by 50-100% over that from the dried soils (both <2 and <0.25 mm) extracted in the absence of the surfactant. Although the yield of LF from the oven dried <0.25 mm Walpeup sample was some 15% lower than that from the dried <2 mm sample, there was essentially no difference between the correspondingly treated Longerenong samples, suggesting that the surfactant showed promise as a means of avoiding these grinding effects.

In the presence of the surfactant, oven drying of the soil resulted in yields of LF similar to or greater than those obtained from corresponding air dry samples. When the extracted residue from the <0.25 mm air-dry samples was oven-dried and refractionated (both times in the presence of surfactant) the additional amount of LF so obtained accounted for most of the difference observed during the initial fractionation of the air and oven dried <0.25 mm samples.

Thus the use of the surfactant with oven dried <0.25 mm samples resulted in yields of LF essentially the same as those obtained from oven dried <2 mm samples, and substantially higher than those from corresponding samples extracted without surfactant.

TABLE 20. EFFECT OF SURFACTANT ('AEROSOL OT') ON LF YIELDS AFTER VARIOUS DRYING AND GRINDING PRETREATMENTS

Sample	MR2:12, Nov. '65				LR2:15, Nov. '65			
Surfactant concentration	0% 'OT'		0.05% 'OT'		0% 'OT'		0.05% 'OT'	
Pretreatment	Total LF	'Ash-free' LF	Total LF	'Ash-free' LF	Total LF	'Ash-free' LF	Total LF	'Ash-free' LF
A. <2 mm air dry	ND	ND	0.67	0.35	ND	ND	0.48	0.32
B. " oven dry	0.39	0.28	0.80	0.45	0.31	0.21	0.54	0.30
C. <0.25 mm air dry	ND	ND	0.54	0.30	ND	ND	0.35	0.21
D. " oven dry	0.29	0.20	0.68	0.38	0.18	0.13	0.61	0.29
E. Re-extract residue from C	ND	ND	0.09	0.04	ND	ND	0.10	0.07

ND = not determined.

(i) LF results expressed as g LF/100 g OD (70°C) soil.
(ii) 'Ash-free' LF calculated by correcting for 'ash' content of LF as determined by ignition (Method B1).

It was also observed that in the presence of the surfactant the soil suspensions (particularly those from <0.25 mm samples) settled more slowly. After centrifuging (30 min, c. 1,600 R.C.F.) the supernatants tended to be soil coloured and opalescent, indicating that some colloidal material was still in suspension. Further, the surface of the soil pellets tended to break away during decantation, thus slightly contaminating the LF with clay. This markedly increased the filtration time required, and also tended to increase slightly the 'ash' content of the LF (typically from c. 30-40% (-OT) to c. 40-50% (+OT)).

A longer centrifuging period was found to improve the separation, 1 hour at c. 1,600 R.C.F. producing a consistently firm pellet which minimised the contamination of the LF with soil clay during decantation. The extent of this contamination was found to be dependent on the grinding technique used, the usual gentle hand grinding resulting in a suspension which settled more rapidly and produced a firmer pellet than did vigorous mechanical grinding in a Siebtechnik mill. It was therefore decided to standardize on a procedure in which the sample was ground by hand in a mortar and pestle just sufficiently vigorously to ensure that all aggregates were crushed <0.25 mm, any larger plant fragments and sand grains being mixed with the fine soil before fractionation.

Typical results obtained using this gentle grinding technique and the longer centrifuging time for samples of the three soil groups examined are shown in Table 21. This data supports

TABLE 21. EFFECT OF SURFACTANT ('AEROSOL OT') ON LF YIELDS FROM THREE SOILS

Sample Trial, Plot No., Date (0-10 cm)	Rotation and Phase	<2 mm				<0.25 mm			
		0% 'OT'		0.05% 'OT'		0% 'OT'		0.05% 'OT'	
		Total LF	'Ash-free' LF	Total LF	'Ash-free' LF	Total LF	'Ash-free' LF	Total LF	'Ash-free' LF
C1:29, May '65	Continuous P	1.95	1.17	3.67	2.03	1.69	1.04	3.79	2.14
LR2:3, Sept.'64	PPFW:P_2	0.51	0.33	0.55	0.36	0.40	0.28	0.90	0.55
" :7, " "	FW : F	0.06	0.04	0.09	0.06	0.05	0.03	0.12	0.09
MR2:4, Sept. '64	PPFW:P_2	0.34	0.20	0.46	0.26	0.34	0.21	0.66	0.37
" :1, " "	FW : F	0.21	0.13	0.26	0.16	0.15	0.09	0.40	0.24

(i) Abbreviation of rotations as for Table 10.
(ii) LF results expressed as g LF/100 g OD (70°C) soil.
(iii) 'Ash-free' LF calculated as for Table 20.

the general conclusions arrived at above, viz:

1. In the absence of surfactant, grinding <0.25 mm markedly reduced the yields of LF obtained compared to those from <2 mm samples.

2. When surfactant (0.05% w/v) was used, greater LF yields were obtained from finely ground samples. The 'ash' content of the separate was only slightly increased.

3. Thorough drying of the sample before fractionation was necessary for best results, and was readily achieved by oven drying at 70°C overnight.

b) Effect of surfactant concentration and solution density

Results for the three soil types examined are given in Table 22.

(1) Solution density

As previously observed for soils fractionated without surfactant, the amount and 'ash' content of the LF separated varied markedly with the solution density. For the three soils studied, increasing the SG of the bromoform solution from 1.8 to 2.0 (at the optimal surfactant concentration) resulted in a nearly twofold increase in the amount of 'ash-free' LF separated. The 'ash' content of the separate was increased from c. 25-30% up to c. 35-40% of the LF. A further increase in solution SG up to 2.2 resulted in the separation of an additional 150-200% 'ash-free' LF, but this separate contained an appreciable proportion of soil minerals ('ash' content c. 60-65% of the LF).

TABLE 22. EFFECT OF SURFACTANT ('AEROSOL OT') CONCENTRATION AND SOLUTION DENSITY ON LF YIELDS FROM THREE SOILS

Sample Trial, Plot No., Date (0-10 cm)	Rotation and Phase	g LF/100 g OD (70°C) Soil, Separated using Solution of Stated SG and Surfactant Content											
		1.8		2.0								2.2	
		0.10% 'OT'		0% 'OT'		0.01% 'OT'		0.05% 'OT'		0.10% 'OT'		0.10% 'OT'	
		Total LF	'Ash-free' LF	Total LF	'Ash-free' LF	Total LF	'Ash-free' LF	Total LF	'Ash-free' LF	Total LF	'Ash-free' LF	Total LF	'Ash-free' LF
C1:29, May'65	Continuous P	1.36	0.93	2.24	1.12	3.38	1.69	3.43	1.71	4.25	2.47	11.5	4.0
LR2:3,Sept.'64	PPFW:P_2	0.50	0.41	0.33	0.22	0.53	0.34	0.79	0.49	0.83	0.53	3.9	1.3
MR2:4,Sept.'64	PPFW:P_2	0.22	0.16	0.37	0.21	0.45	0.24	0.46	0.30	0.56	0.37	1.8	0.8

(i) Abbreviation of Rotations as in Table 10.
(ii) 'Ash-free' LF calculated as for Table 20.

Although Monnier (1965, p347) has suggested that a solution SG of 1.75 is advisable for calcareous soils, especially if they contain appreciable quantities of LF, the results for the calcareous Walpeup and Longerenong soils indicate that even when the optimal amount of surfactant (0.1% w/v) was used the yields of LF using a solution SG of 1.8 were much lower than when a solution of SG 2.0 was used. Whilst examination with the light microscope suggested that there is probably more finely divided material present in the latter separate, chemical analyses showed that they were not markedly different. The LF of SG <1.8 contained slightly less 'humic' materials and comparable amounts of total netural sugars compared with corresponding samples of LF obtained using a solution SG of 2.0. The relative amounts of the individual neutral sugars were closely similar (see Tables 28 and 29). As the ash content of the LF was only increased by about half whilst the amount separated was increased by up to twofold by the higher density, it was considered that a solution SG of 2.0 would be the most satisfactory, provided an adequate amount of surfactant was present.

(2) Surfactant concentration

For a solution SG of 2.0, the amount of LF separated increased with increasing surfactant concentration, the maximum yield being obtained at 0.10% (w/v) 'Aerosol OT'. For the Urrbrae sample further increases in the surfactant concentration up to 1.0% w/v produced no further increments in LF yield.

Thus the most satisfactory separation was obtained using a solution of SG 2.0 and containing c. 0.1% w/v 'Aerosol OT'.

As portion of the surfactant was adsorbed by the soil during fractionation it was necessary to readjust the surfactant concentration in the bromoform solution before re-use. Determination of the surfactant concentration was conveniently achieved using the rapid methylene blue procedure of Jones (1945) (see Appendix A).

The mode of action of the surfactant is not definitely known, but several mechanisms may be involved. Since this anionic surfactant is adsorbed by the soil to an appreciable extent, in agreement with the findings of other workers (Krishna Murti et al. 1966), it might be expected to displace soil organic material sorbed on the sites involved. However no increase in the amount of 'humic' material associated with the LF was detected in samples fractionated with the surfactant compared to that obtained without it (Table 28). Further, the glucose content of the LF obtained with the surfactant was usually greater than that of comparable material obtained in the absence of surfactant (Table 29). This suggests that the surfactant released plant residues which were on average less decomposed than those obtained without it. It is conceivable that the LF might be bonded to soil mineral grains e.g. by polyuronide gums present within partly decomposed plant residues. This would be consistent with the decrease in LF yields associated with fine grinding, and the surfactant could feasibly break these "gum-soil mineral" bonds and

so permit the LF particles to be separated from the remainder of the soil. However this might not be the only possible mechanism involved as periodate treatment of the ground soil was not found to liberate any of the 'fixed' LF.

Another explanation is based on the ability of this surfactant to markedly reduce the interfacial tension in mixed organic-aqueous systems. If this mechanism was operative, it implies that there were in the oven-dried samples at least some discrete water films - probably discontinuous and perhaps only a few molecules thick. If such films existed they must have been very restricted in distribution, since the relative vapour pressure in the dried sample would have been much less than the 20% or so required for a monomolecular water film (Quirk 1955). However the inorganic salts present could have increased the osmotic pressure of any residual soil moisture sufficiently to account for the failure to desiccate the soil completely with the treatments used. Any such films could have caused the LF particles to adhere tenaciously to other soil colloids, and as the bromoform solution used was immiscible with water these films could have survived the disruptive effects of the ultrasonic vibrations. The reduction of the interfacial tension of such films by the surfactant would permit the LF to be shaken away from the remainder of the soil. Strong adhesive effects have been reported in moist soils between soil particles and 'foreign bodies' (Fountaine 1954), and are readily accounted for by the product of the

moisture tension in the film and its surface area. Similar effects could well exist in dry soils in situations such as those suggested above.

3. Centrifuging of the soil suspension and recovery of LF

As outlined above, it was found necessary to allow the suspension to settle for >30 min before centrifuging it at c. 1,600 R.C.F. for c. 1 hour. A centrifuge fitted with a swing-out head was preferable as this produced a firm compact pellet which was more stable during decantation of the supernatant than was the pellet formed in an angle head. The tubes should be sealed to minimise evaporation and possible density changes.

The LF was recovered most readily by decanting the supernatant into a beaker, and carefully washing down any LF particles adhering to the walls of the centrifuge tube with a fine jet of the bromoform solution. The solution containing the LF could then be readily and rapidly filtered under suction through a "Millipore" filter (nominal porosity 5.0 ± 1.2μ), washed with petroleum spirit, dried at 70°C for c. 15 min, and the LF then brushed off onto a tared piece of aluminium foil and weighed. With care the LF could be recovered without disturbing any clay which may have broken away from the soil pellet surface during decantation and subsequently formed a 'skin' under the LF particles on the filter. Direct weighing of the LF on the filter was found to be unsatisfactory as the weight change after solvent treatment and drying was too variable.

Each filter could be reversed and re-used at least once, although it tended to become very brittle with repeated use.

4. Precision

The results of 12 individual determinations of the LF content of 5.0 g samples from 4-course plots under pasture at each site are given in Table 23. The precision of the technique was satisfactory, the coefficient of variation ranging only from 4-8%.

5. Use of alternative densimetric liquids

The problem of the differential volatility of the bromoform and of the diluting solvent could be overcome either by the use of a solvent having a comparable vapour pressure to bromoform, such as decalin (decahydronaphthalene); or by the use of another organic liquid of suitable density (c. 2 g/cc) which would not require dilution, e.g. 'Nemagon'* which has a SG of 2.06. Brief details of the methods used, and the results obtained when samples from the three trials were fractionated with either organic liquid are given in Table 24.

In general similar or slightly greater amounts of LF were obtained using 'Nemagon', and with both solutions use of the surfactant increased the yields of LF. The LF separated with 'Nemagon' usually contained slightly more 'ash', this possibly reflecting its greater specific gravity. Some slight differences

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* 1,2-dibromo-3,chloropropane; >97%, kindly donated by Shell Chemical (Australia) Pty. Ltd., Adelaide.

TABLE 23. PRECISION OF FRACTIONATION PROCEDURE FOR THREE SOILS

Soil	Sample	Depth (cm)	Rotation and Phase	g LF/100 g OD (70°C) Soil		C.V.**
				Mean	S.E.*	
Urrbrae	C1:32, Sept.'66	0-6.3	PPFW : P_2	3.130	0.034	0.038
Longerenong	LR2:5, April '66	0-10	" : "	0.528	0.009	0.059
Walpeup	MR2:2, April '66	0-10	" : "	0.442	0.010	0.077

* S.E. = standard error for tabulated mean (n=12)
** C.V. = coefficient of variation.

TABLE 24. COMPARISON OF 'NEMAGON' AND BROMOFORM-PETROLEUM SPIRIT SOLUTIONS FOR SEPARATION OF LF FROM SOME SOILS

Soil	Sample	'Nemagon'*				Bromoform-Petroleum Spirit			
		0% 'OT'		0.1% 'OT'		0% 'OT'		0.1% 'OT'	
		Total LF	'Ash-free' LF	Total LF	'Ash-free' LF	Total LF	'Ash-free' LF	Total LF	'Ash-free' LF
Urrbrae	C1:32, Sept.'66	1.66	0.89	3.32	1.42	1.76	0.94	3.18	1.51
Longerenong	LR2:5, April'66	0.80	0.47	0.75	0.40	0.49	0.31	0.53	0.34
Walpeup	MR2:2, April'66	0.68	0.36	0.72	0.36	0.40	0.24	0.49	0.31

*'Nemagon', (>97% 1,2-dibromo-3,chloropropane) was used as received.

LF was separated by a modification of the technique of Greenland and Ford (1964), using solutions and surfactant concentrations as shown.

LF results are expressed as g LF/100 g OD (70°C) soil, and 'ash-free' LF calculated as for Table 20.

in the yields from the various soils were obtained, the bromoform solution yielding a slightly higher amount of 'ash-free' LF from the Urrbrae soil, whilst the converse held for the other two soils. However these differences are considered to be of minor significance. 'Nemagon' has been successfully used for routine studies of carbohydrate material in the extracted soil residue (Oades and Swincer 1968).

(C) Conclusions

Partly humified organic matter can be separated from soils by a densimetric fractionation procedure involving ultrasonic dispersion of the soil in a heavy organic liquid. Examination of the factors influencing the efficiency and reproducibility of the fractionation indicated that for optimum results the procedure had to be rigorously standardised (see Appendix A). In particular, the finely ground sample had to be thoroughly dried before attempting to fractionate it. The efficiency of the separation could be markedly improved by the addition of a small amount (c. 0.1% w/v) of a surfactant ('Aerosol OT') to the bromoform-petroleum spirit solution. This increased the proportion of non-humified material in the LF. A small increase in the 'ash' content of the LF also resulted from the use of the surfactant, but this is regarded as relatively unimportant.

The completeness of the separation of non-humified from humified organic matter cannot be directly assessed, but can only be inferred from the failure to separate appreciable additional

amounts of LF by repeated fractionation of a soil sample. The technique was successfully applied to a number of soils ranging in texture from a sand to a clay, and has enabled the rapid and reproducible separation of non-clay combined organic materials from them. The amounts of LF were found to vary with the soil type and its agronomic history, and to account for a significant proportion of the soil organic nitrogen.

It has been subsequently used in studies of the relationship between the dynamics of the LF and changes in the reserves of mineralizable N in three agricultural soils (Chapter V), and in studies of changes in polysaccharide materials in soils (Oades and Swincer 1968). Other authors have successfully applied this technique, but omitting the surfactant, to the determination of charcoal (Jenkinson 1966b) and carbohydrate (Oades 1967a) material in soil. This procedure thus appears to be a potentially useful preliminary step for studies of the clay-organic complex of soils.

IV CHARACTERIZATION OF THE LF

Two of the principal objectives underlying the development of a procedure for the quantitative separation of LF from soils were to attempt the fractionation of the organic matter into a relatively undecomposed and a humified fraction, and to examine the hypothesis that the former fraction was the more labile. It was thus necessary to establish that the LF consisted essentially of relatively undecomposed plant residues, although material in all stages of humification would also be expected to be present.

Particular emphasis was therefore given to examination of the physical appearance of the LF, and to the estimation of the amount of soil and 'humic' materials which it contained. The relative lability of the 'free' and 'bound' fractions was also examined by determining the amounts of carbohydrate (= 'available C') and protein ('available N') in the LF; by estimating the rate at which LF was decomposed when incubated with soil, and by relating the resultant net loss of N contained in the LF to the net amount of mineral N released during incubation.

(A) Materials and Methods

Details of the samples used are presented below together with the results. The methods cited are described in Appendix B.

(i) Physical appearance

Samples of the LF and of the residue after digestion with

H_2O_2 (Method B2) were examined under the light microscope with both incident and transmitted polarized light.

(ii) Inorganic material in the LF

The total 'ash' content was determined by ignition (Method B1). During the initial stages of this study the mineralogical composition of the residues from digestion of the LF with H_2O_2 was determined by standard X-ray diffraction and infra-red methods.

(iii) Organic material in the LF

1. Solubility in alkali

The amount of humified materials in LF and soil samples was estimated from comparison of the optical density (at 260 mμ or 450 mμ) of alkali extracts of the samples with the extinction at the same wavelength of a purified humic acid sample from Urrbrae loam (Methods B3, B4, B5). The carbohydrate content of some of these alkali extracts of LF material was determined by an anthrone procedure (Method B6).

2. Cation exchange capacity

The number of acidic groups present on the LF was determined by potentiometric titration of a sample of acid-washed LF against 0.1 N KOH to pH 7.0 (Method B7).

3. Organic C

This was determined by dry combustion, using either

Method B8 or B9. Where necessary corrections were made for carbonates as determined by Method B10.

4. Organic N

Soils: Total soil N was determined initially by a macro-Kjeldahl procedure, and subsequently by dry combustion in a Coleman Nitrogen Analyser (see Method B11). Corrections were made where necessary for the mineral N content of the samples, determined as in Appendix C6.

LF: The organic N content was determined by a micro-Kjeldahl procedure, Method B12 being used during preliminary studies and Method B13 for subsequent analyses.

5. Amino acids

The amino acid composition of LF samples was determined by analysis of 6 N HCl hydrolysates on automatic amino acid analysers (per favour Dr. A.C. Jennings; Method B14) and Dr. J.R.E. Wells (Method B15).

6. Carbohydrates

The neutral sugar content of LF samples was determined by analysis of 1 N H_2SO_4 hydrolysates either by a phenol-sulphuric acid procedure, followed by qualitative identification of the mono-saccharides by paper chromatography (Method B16); or by an anthrone procedure, individual neutral sugars in some hydrolysates being subsequently determined by gas-liquid chromatography (Method B17).

(iv) Incubation studies

1. Oxygen uptake studies

Warburg manometry was used to compare the oxygen uptake rates of soil amended with LF, humic acid and fresh plant residues (Method B18).

2. Mineralization studies

The net NO_3^--N release during aerobic incubation of soil amended with humic acid, fresh plant tissue or the LF separated from a range of soils was determined during the initial stages of this study (Method B19). Subsequently unamended soil samples were aerobically incubated, usually for 4 weeks at c. pF 2 and 35°C (Appendix C5). Details of the development of this procedure are discussed on p 134 . The net changes in mineral N during incubation were related to the net loss of LF-N, and in some cases changes in the composition of the LF separated before and after incubation were also determined.

(B) Results and Discussion

(i) Physical appearance

Photomicrographs of LF samples obtained from several soils using bromoform solutions of various densities, with and without surfactant, are presented in Figures 1-7. These show that the LF usually consisted of irregularly shaped particles, many of which had a recognisable cellular structure. Commonly they were within the

FIGURE 1: LF FROM A SOLONISED BROWN SOIL (WALPEUP)

Plate 1: LF (SG <1.8) separated with surfactant (0.1% 'OT') from a 4-course plot under pasture (MR2:4; Table 22) x 100

Plate 2: LF (SG <2.2) separated with surfactant (0.1% 'OT') from the same plot. x 100

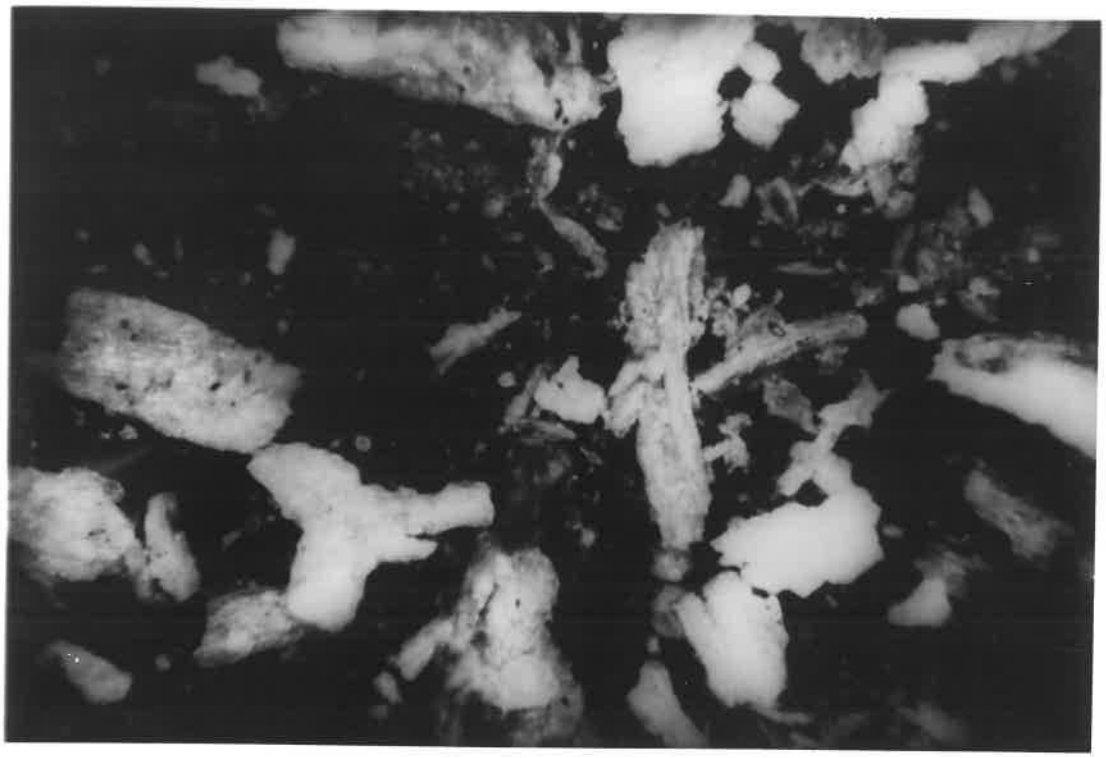


FIGURE 2: LF FROM A GREY SOIL OF HEAVY TEXTURE (LONGERENONG)

Plate 1: LF (SG <1.8) separated with surfactant (0.1% 'OT') from a 4-course plot under pasture (LR2:3; Table 22). x 100

Plate 2: LF (SG <2.0) separated with surfactant (0.1% 'OT') from the same plot. x 100

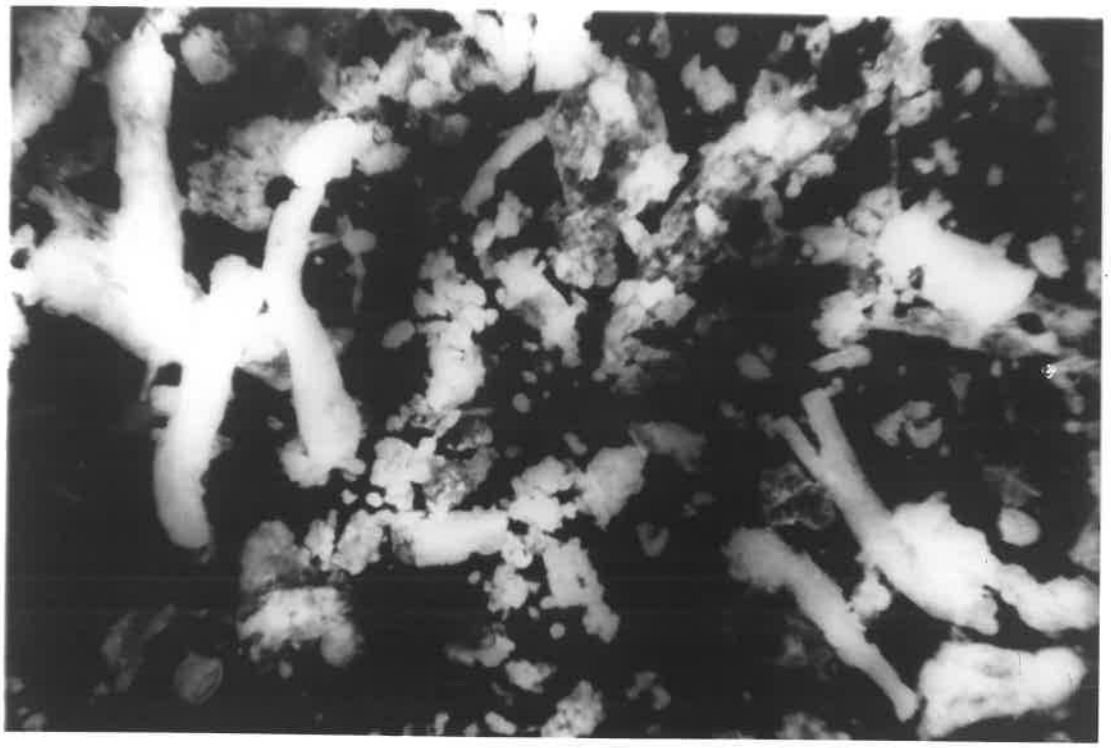


FIGURE 3: LF FROM A RED BROWN EARTH (STOCKWELL)

Plate 1: LF (SG <2.0) separated without surfactant from a plot under a 3-year old *Lolium rigidum* sward (Table 15). x 100

Plate 2: LF (SG <2.0) separated without surfactant from a plot under a 3-year old *Trifolium subterraneum* sward (Table 15). x 100

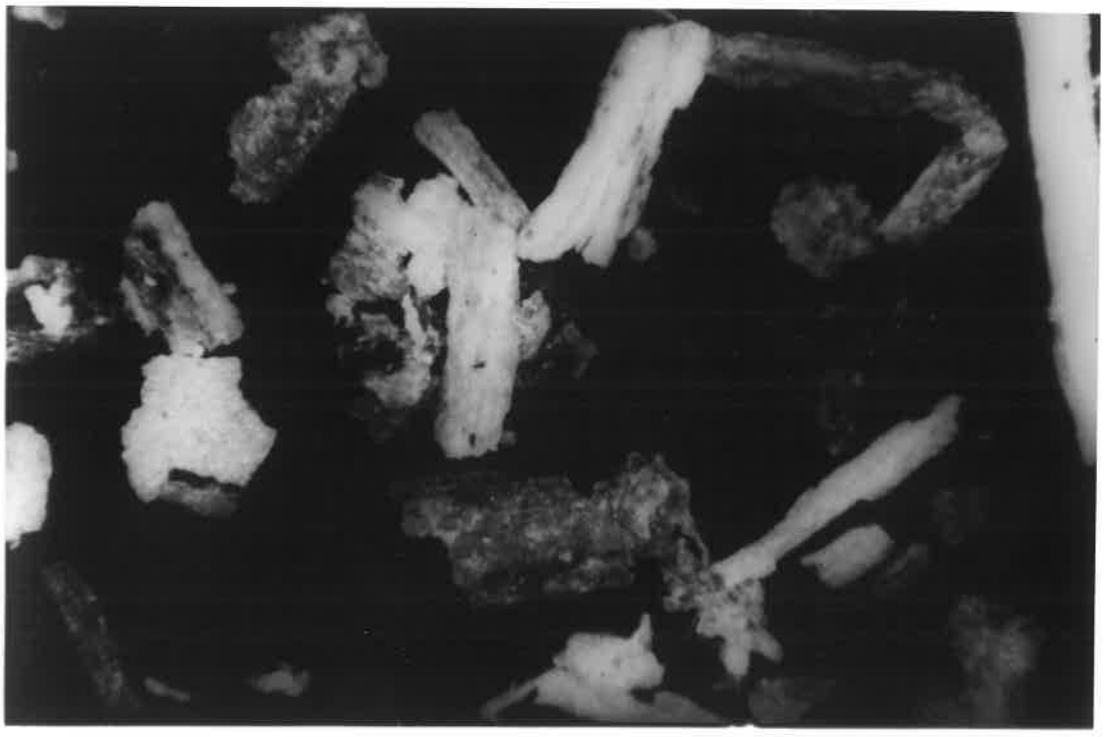
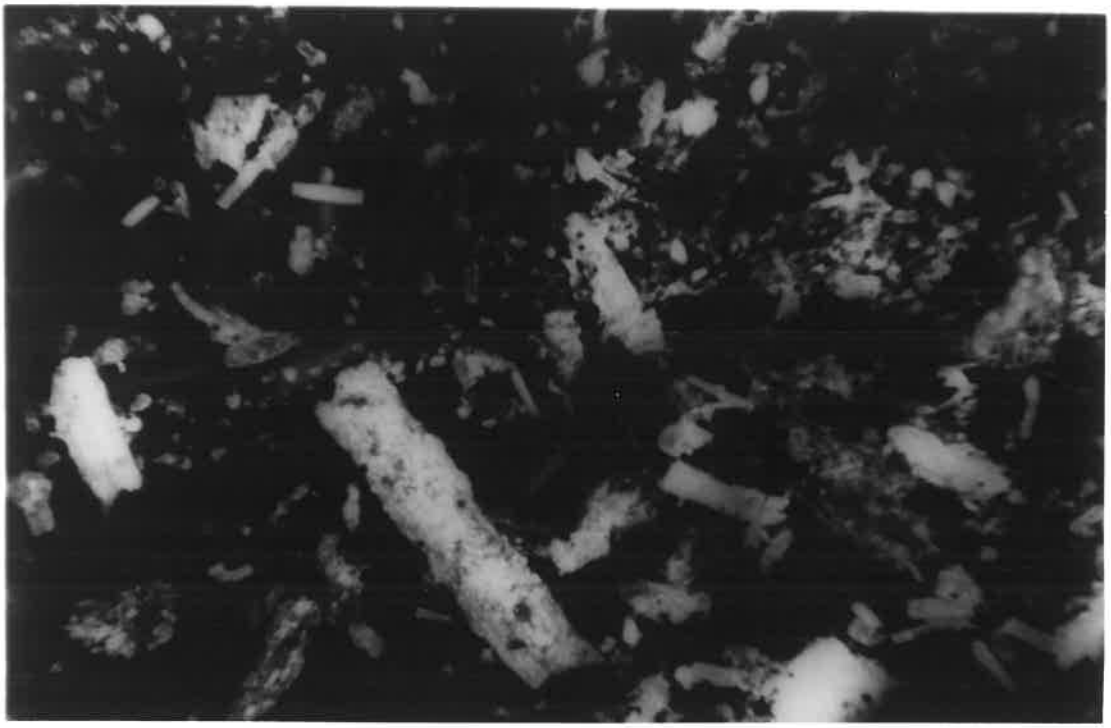


FIGURE 4: LF FROM A RED BROWN EARTH (URRBRAE)

Plate 1: LF (SG <1.8) separated with surfactant (0.1% 'OT') from the continuous pasture plot (C1:29; Table 22). x 100

Plate 2: LF (SG <2.0) separated without surfactant from the same plot. x 100.



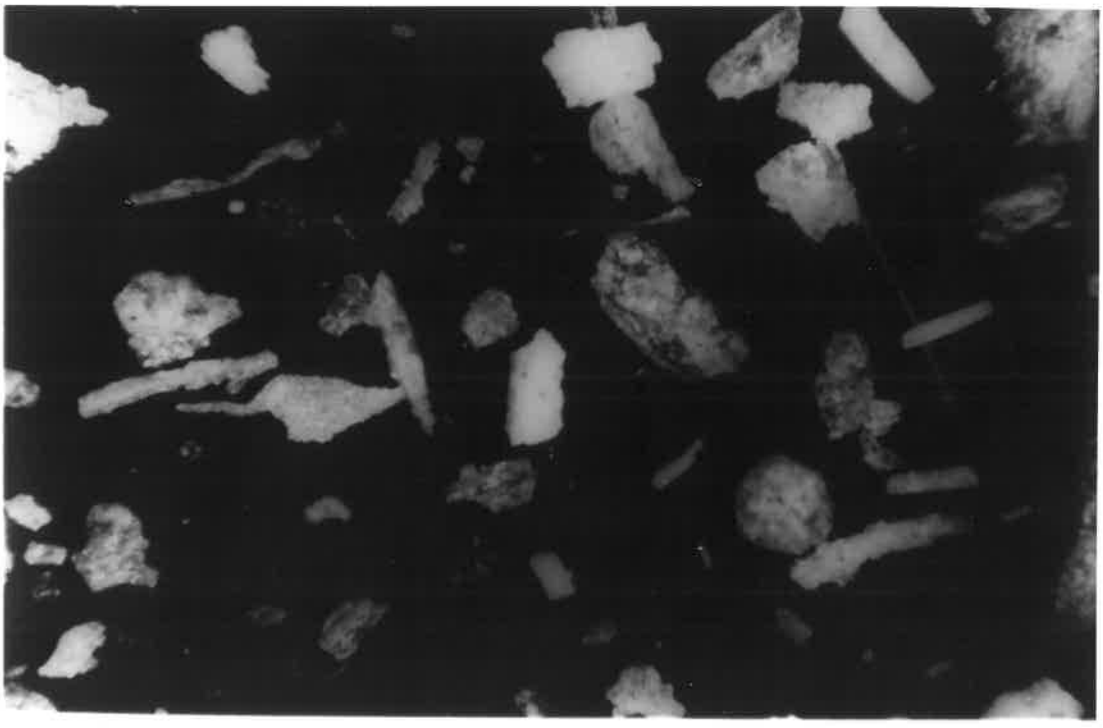


FIGURE 5: LF FROM A RED BROWN EARTH (URRBRAE)

Plate 1: LF (SG < 2.0) separated with surfactant (0.1% 'OT') from the continuous pasture plot (C1:29; Table 22). x 100

Plate 2: LF (SG <2.2) separated with surfactant (0.1% 'OT') from the same plot. x 100

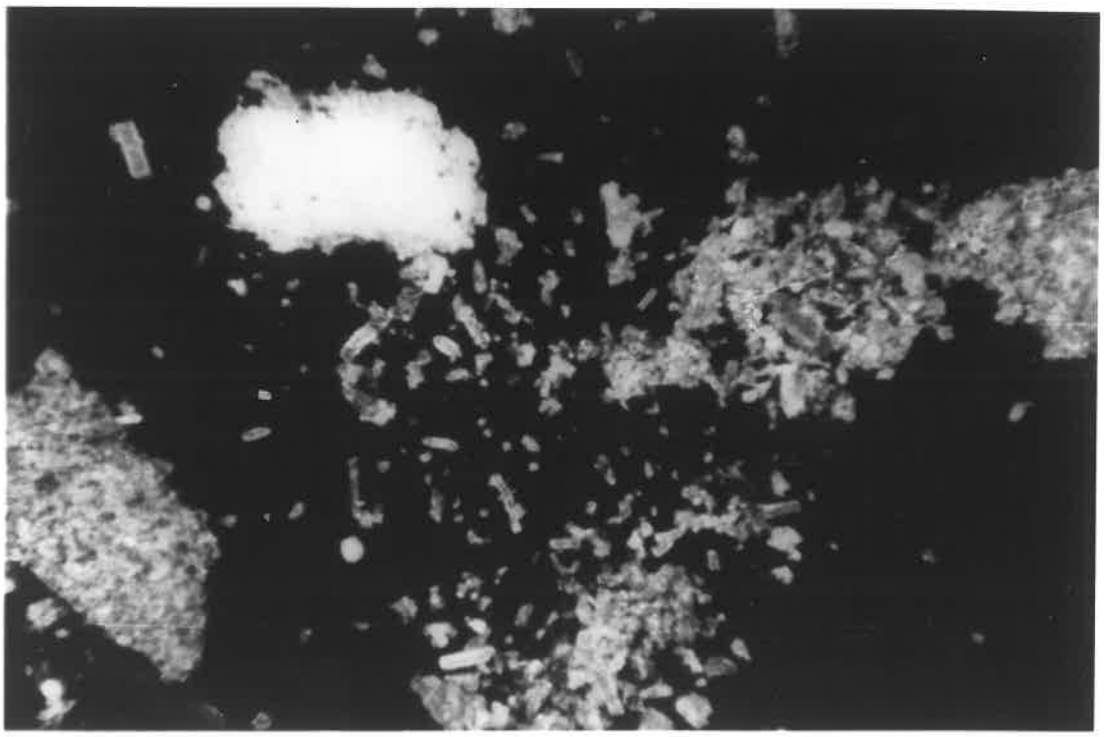


FIGURE 6: LF FROM A RED BROWN EARTH (URRBRAE) AND A BLACK EARTH (WACO)

Plate 1: Graminaceous awn in LF (SG <1.8) separated with surfactant (0.1% 'OT') from the continuous pasture plot (C1:29; Table 22). x 100

Plate 2: LF (SG <2.0) separated with surfactant (0.1% 'OT') from a cultivated site (Appendix E). x 100

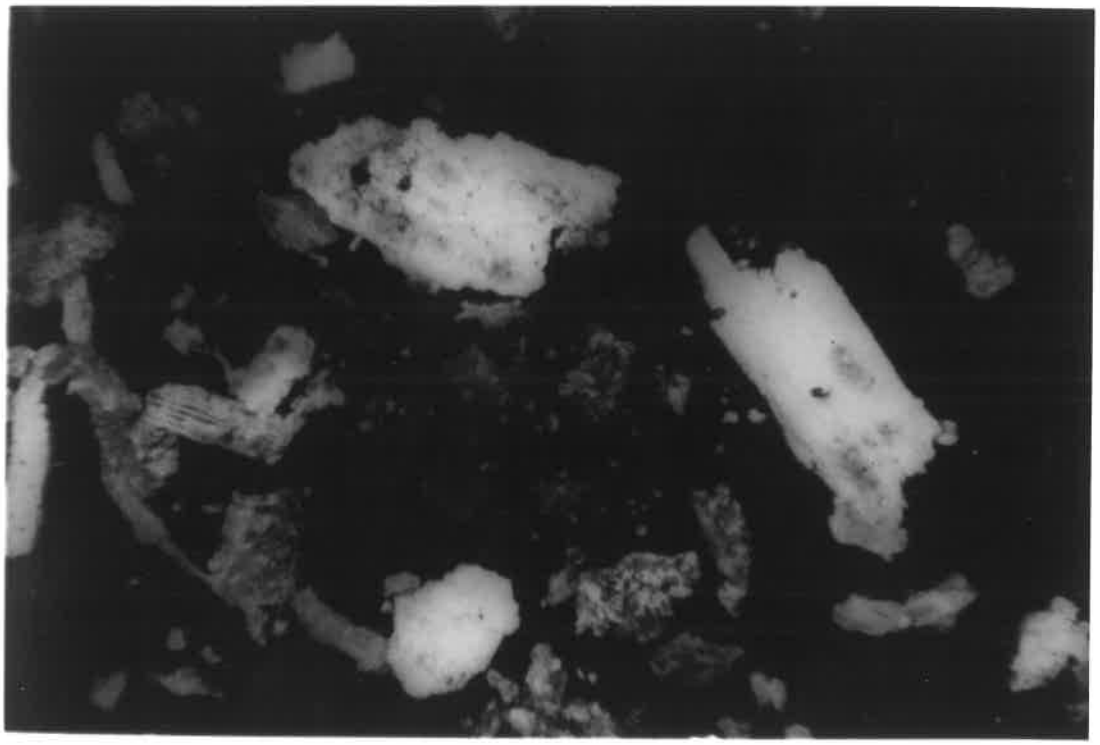
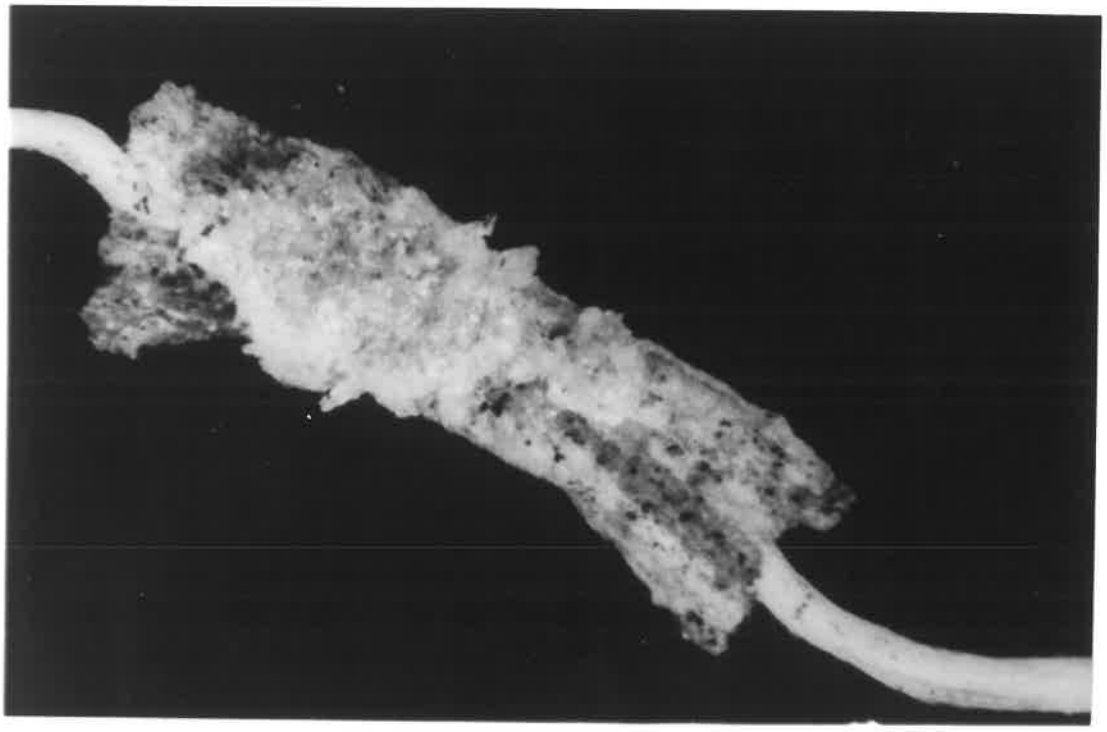


FIGURE 7: LF FROM A KRASNOZEM (GABBINBAR)

Plate 1: LF (SG <2.0) separated with surfactant (0.1% 'OT') from a virgin site (Appendix E). x 100

Plate 2: Root fragment in the same LF sample. x 100



size range c. 100-500 μ, although some larger particles, obviously of plant origin, were also observed (e.g. Fig. 6, Plate 1 and Fig. 7, Plate 2).

In addition there was a variable amount of finer material, usually soil-coloured and of greater than silt size (20μ) and lacking any obvious cellular structure. This finer material tended to be most abundant in the LF separated from a krasnozem (Gabbinbar; e.g. Fig. 7, Plate 1) and from a solonised brown soil (Walpeup; e.g. Fig. 1); and in LF separated with solutions of SG 2.2 (e.g. Fig. 1, Plate 2 and Fig. 5, Plate 2), or with solutions containing surfactant (e.g. cf. Figs. 4 and 5).

The Waco and Longerenong soils, (both clays), contained a relative abundance of larger, straw-coloured fragments, apparently of plant origin (e.g. see Fig. 2 and Fig. 6, Plate 2). As well as particles of plant tissue, it was also possible to identify fragments of charcoal and faunal debris, such as portions of insect exoskeletons. However the nature of much of the LF material is not known, as it generally lacks features which permit its ready identification - although other data, discussed below, suggests that portion at least consists of partly decomposed material.

It was also observed, especially in LF samples from the Urrbrae soil (e.g. Figs. 4 and 5),that some free opalescent bodies having a definite form but no cellular structure were present. They varied considerably in size, and cylindrical forms up to c. 30x200μ

were observed. Similar bodies were present after digestion of the LF with H_2O_2 from a range of soils, and as discussed below it would seem that they are siliceous phytoliths derived probably from Graminaceous plants.

(ii) Inorganic material in the LF

The total content of inorganic material ('ash') associated with the LF usually varied from 30-50% by weight of the LF depending on the soil type, the nature of the grinding pretreatment, and on the presence or absence of surfactant. The ash content was inversely related to the SG of the fractionating solution. However, with careful standardization of the fractionation technique, the extent of variable contamination of the LF with other soil mineral material could be minimised, so that direct comparisons of the amounts of LF separated from different samples from the same trial site could be made.

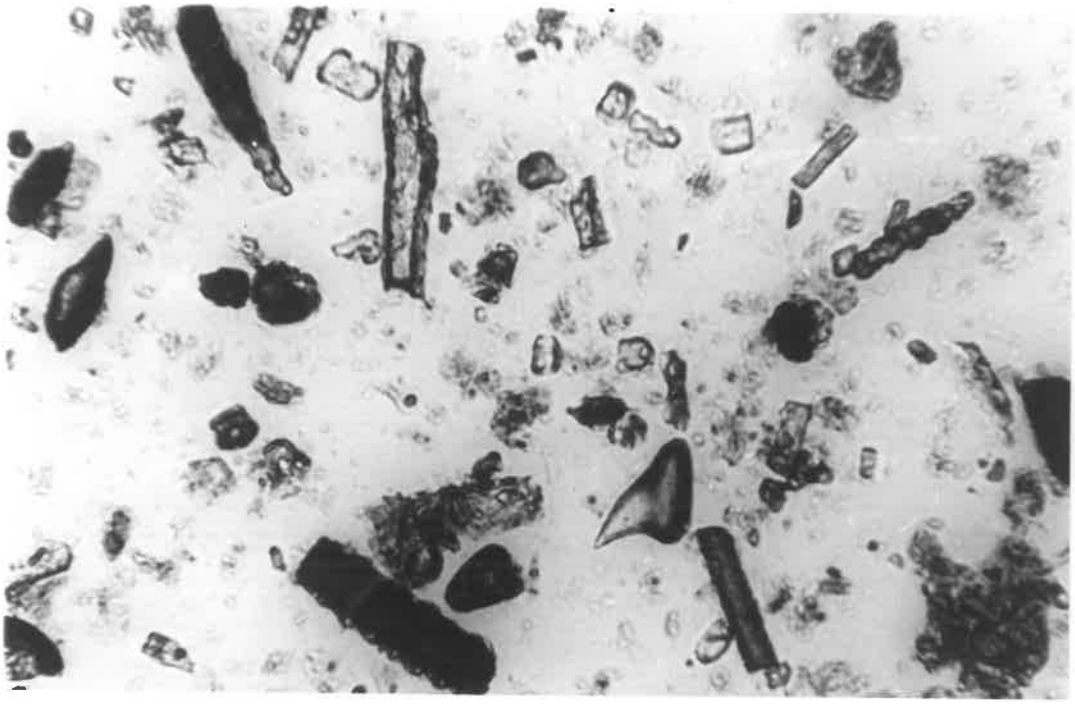
Microscopic examination of the residues after treatment of the LF with H_2O_2 revealed an abundance of discrete well-structured particles, opalescent in reflected light and isotropic in transmitted polarized light. The material from different soils varied in shape, colour, and size, ranging from cylindrical rod-like forms (up to c. 30μ x 200μ) which were often branched or hooked and having either plain or irregular (e.g. serrated) margins, to smaller particles of irregular size (see Figs. 8 and 9).

They closely resembled the insoluble amorphous opaline

FIGURE 8: RESIDUE AFTER DIGESTION OF LF FROM A RED BROWN EARTH (URRBRAE) WITH H_2O_2

Plate 1: Residue from LF (SG <2.0) separated with surfactant (0.1% 'OT') from the continuous wheat plot (C1:17; Appendix D2). x 400

Plate 2: Residue from LF (SG <2.2) separated with surfactant (0.1% 'OT') from the continuous pasture plot (C1:29; Table 22). x 400



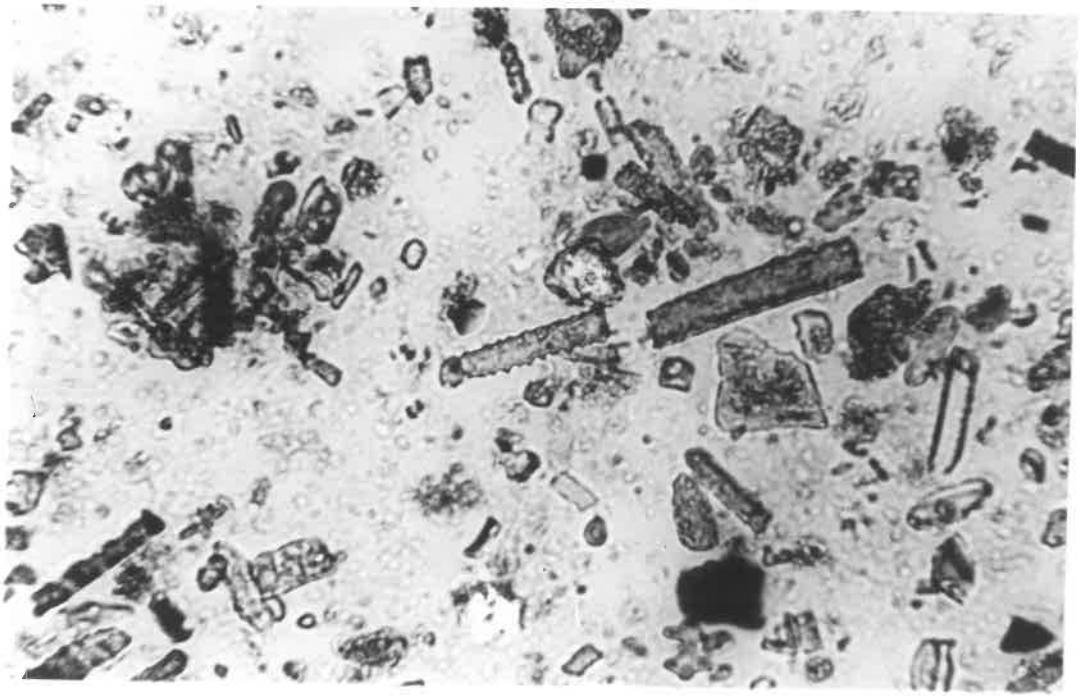
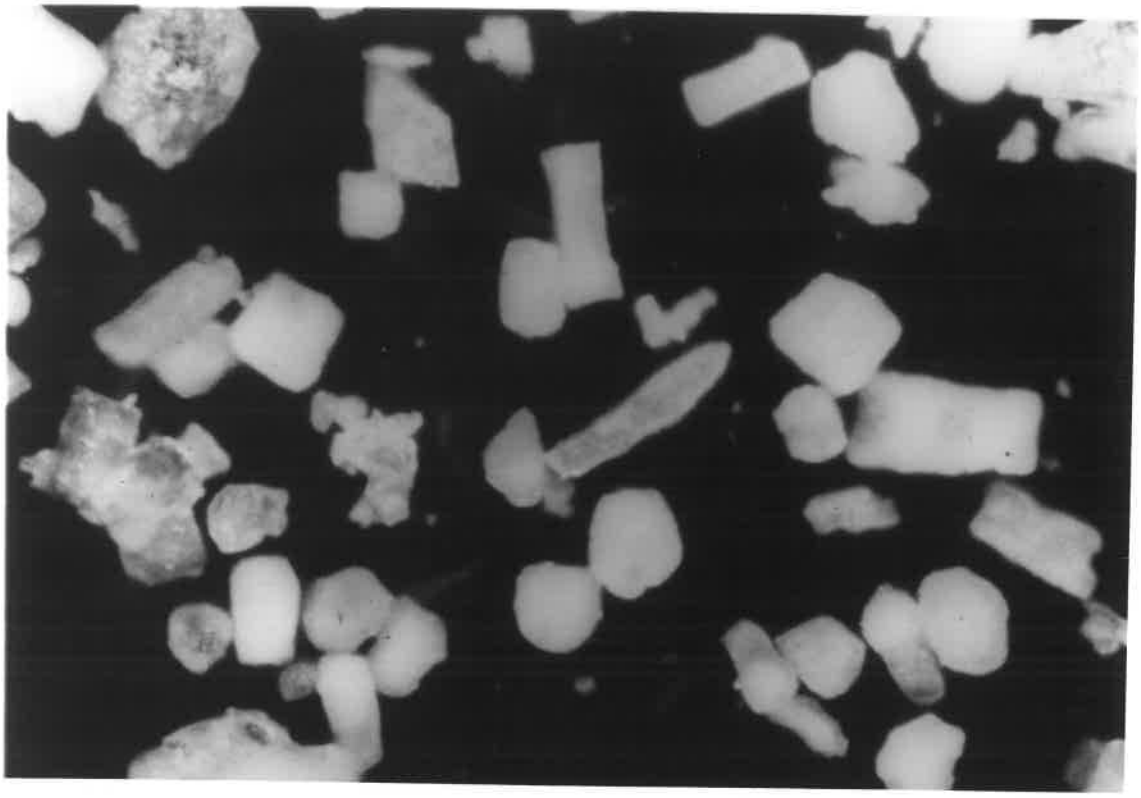


FIGURE 9: RESIDUE AFTER DIGESTION OF LF FROM A LATERITIC RED EARTH (TIPPERA) AND A RED BROWN EARTH (URRBRAE) WITH H_2O_2

Plate 1: Residue from LF (SG <2.0) separated without surfactant from Tippera clay loam (Table 14). x c. 600

Plate 2: Residue from LF (SG <2.0) separated without surfactant from Urrbrae loam. x c. 600.



siliceous structures of SG c. 2.0 found in plants, particularly sedges and grasses (including cereals such as wheat and oats), and generally referred to as opal phytoliths (Baker 1960; Jones and Milne 1963). Such bodies occur less commonly in legumes (Jones and Handreck 1965a, 1965b), and are apparently formed in Graminaceous plants by the deposition of monosilicic acid ($Si(OH)_4$) in intimate association with the cell wall throughout the growth of the plant. A wide variety of forms may be derived even from one type of plant e.g. oats (Jones, Milne and Wadham 1963).

Similar bodies have also been recovered from the fine sand and silt fractions of many soils, where they may comprise up to 1-2% by weight of the soil (Smithson 1958; Baker 1959a, 1959b). Commonly the SG of these phytoliths is within the range 1.50-2.30 with a median value of c. 2.10-2.15, this variation in SG reflecting differences both in the degree of hydration of the silica and in their content of occluded organic material (Khan 1959; Baker 1960; Jones and Beavers 1963). Whilst fine opal phytoliths may be transported long distances by wind (Baker 1960; Folger, Burckle and Heezen 1967), the bulk of such material in most soils is undoubtedly derived from the local vegetation. The relative abundance of the various forms of opal phytoliths have been used in some pedogenetic and paleobotanical studies (Beavers and Stephen 1958; Baker 1959b; Brydon, Dore and Clark 1963; Jones and Beavers 1964a, 1964b; Witty and Knox 1964), and Baker (1959a) has suggested that their average age in soils is probably at most 4000 years.

These findings are consistent with the identification of these particles obtained from the LF separates of SG <2.0 as opal phytoliths. Apparently the bulk of this phytolith material is intimately associated with the fragments of plant material in the LF, as in many LF samples they were not readily apparent until after digestion with H_2O_2. They were most abundant in LF separated from the Urrbrae soil, both in the free state and associated with the LF particles, this being in marked contrast to the other soil types examined. It is possible either that they persist in the free state longer in the Urrbrae soil, or that they are produced in greater abundance by the grasses and cereals growing on this soil, since the concentration of monosilicic acid in the soil solution and thus available for passive plant uptake is known to vary with soil type and pH, lower concentrations occurring in soils rich in sesquioxides or in soils which have a neutral to alkaline reaction (Raupach 1957, p.31; Jones and Handreck 1965b).

X-ray diffraction and infra-red studies of peroxidized LF material (obtained without surfactant) confirmed the predominantly amorphous nature of this material, but also revealed the presence of quartz and traces of clay minerals. A similar investigation of LF material obtained with surfactant has not been made but the general similarity of the 'ash' contents of LF obtained with and without surfactant (Chapter III) suggests that these conclusions are still valid.

(iii) Organic material in the LF

1. Preliminary studies

Undecomposed plant material and humic acid were used as reference materials for initial studies of the chemical nature of the LF as these represent the two extremes of organic materials likely to occur in significant amounts in the LF.

The properties of the LF separated without surfactant from 5 temperate and 1 tropical soil are given in Table 14. For the temperate soils examined the main features were:-

(i) The amount of LF, and the proportion of the soil carbon (20-85%) and nitrogen (11-100%) which it represented, depended both on the soil type and its agronomic history.

(ii) The C:N ratio of the LF was within the range 11-30, and was usually higher than that of the soil from which it was extracted.

(iii) The LF contained 3-14% 'humus' (as estimated from the extinction of a 0.5 N NaOH extract). For the LF separated from the continuous pasture plot on the Urrbrae soil, the estimated 'humus' content of 14% is in good agreement with that expected from its cation exchange capacity (37.2 me/100 g LF at pH 7), since if it is assumed that all the acidic groups are associated with humic materials then some 11-12% of these must be present in the LF (the CEC of purified humic acids separated from the same soil and determined in a similar manner was c. 300-330 me/100 g, Posner 1966). The

average 'ash' content of the LF is 50%, so that c. 25% of the organic material in the LF in this soil is humified material. This proportion is affected by the rotation practised, the LF from heavily cropped plots containing proportionally less 'humus' material.

(iv) The total carbohydrate content of the LF separated from the Urrbrae soil (14.5%) was intermediate between that of fresh plant material (30-50%) and humic acid (7.2%). Although the phenol-concentrated sulphuric acid method used may have overestimated the quantity of sugars in the hydrolysates (Oades 1967a), subsequent analyses of comparable samples by an anthrone procedure has confirmed this conclusion (see Table 26). A range of sugars were detected in the hydrolysates (viz. glucose, galactose, mannose, xylose, and traces of arabinose, rhamnose and fucose), these being similar to those reported in plant and microbial tissues, and in soils (Greenland and Oades 1968).

(v) After acid hydrolysis a higher proportion of the total N was recovered as (amino acid + amide + hexosamine)N from the LF (84%) than from humic acids (45%) separated from the same soil (Urrbrae). Of the nitrogen brought into solution only part (70.6% and 46.2% respectively) was identified as amino acids; and the results are given in Table 25. The values reported are minimal estimates, since undoubtedly some destruction of amino acids and amino sugars occurs during hydrolysis, the ammonia liberated contributing to the relatively high "amide" N values found both in this study and by

TABLE 25. PROPERTIES AND AMINO ACID COMPOSITION OF LF AND HUMIC ACID ISOLATED FROM THE CONTINUOUS PASTURE PLOT ON URRBRAE FINE SANDY LOAM

	LF (SG 2.0, no surfactant)			Humic acid (sample 11R, Posner 1966)		
g/100 g OD soil	2.56			1.15		
Carbon %	29.4			c. 50		
Nitrogen %	2.18			4.18		
C:N	13.5			c. 12		
% Soil C in Fraction	29			25		
% total N in 6 N HCl hydrolysate	88			75		
% total N recovered as (amino acid + amide + hexosamine)N	84			45		
Amino Acids	% N_H	(SE)	%aa-N	% N_H	(SE)	%aa-N
(amide + NH_4^+)N	20.3	(1.17)	-	10.9	(0.21)	-
Basic:						
arginine	8.63	(0.05)	12.2	4.24	(0.05)	9.2
lysine	4.71	(0.25)	6.7	4.63	(0.19)	10.0
histidine	2.97	(0.05)	4.2	3.04	(0.04)	6.6
Acidic:						
aspartate	6.08	(0.05)	8.6	5.99	(0.08)	13.0
glutamate	5.32	(0.12)	7.5	2.90	(0.08)	6.3
Neutral:						
glycine	6.74	(0.03)	9.6	2.98	(0.03)	6.5
alanine	5.89	(0.55)	8.3	2.23	(0.08)	4.8
proline	5.06	(0.05)	7.2	4.55	(0.02)	9.8
serine	4.21	(0.07)	6.0	2.77	(0.10)	6.0
threonine	3.95	(0.13)	5.6	2.43	(0.05)	5.3
valine	3.95	(0.19)	5.6	2.44	(0.02)	5.3
leucine	3.94	(0.07)	5.6	2.37	(0.07)	5.1
iso-leucine	2.32	(0.08)	3.3	1.68	(0.03)	3.6
½-cystine	2.32	(0.13)	3.3	1.21	(0.03)	2.6
phenylalanine	2.29	(0.05)	3.2	1.39	(0.05)	3.0
tyrosine	1.33	(0.12)	1.9	0.47	(0.01)	1.0
methionine	0.83	(0.06)	1.2	0.83	ND	1.8
Hexosamine*	4.35	(0.08)	-	3.50	(0.18)	-
N recovered as % N_H	95.2			60.6		
amino-acid N recovered as % N_H	70.6			46.2		

N_H = total N in hydrolysate. ND = not determined.
SE = standard error (based on 4 analyses of each hydrolysate)
%aa-N = N in amino acid as % total amino acid N recovered
* = two hexosamine peaks were detected but as they were not satisfactorily resolved, a total value only is reported.

other workers (Bremner 1965a). The seventeen amino acids detected in both separates have all been recorded by other authors who have examined the amino acid composition of soil organic matter. Small amounts of other amino acids may also have been present, as ornithine, α- and γ-amino butyric acids, α,ε-diaminopimelic acid, β-alanine, hydroxyproline, and dihydroxyphenylalanine have all been previously reported (Bremner 1965a). The amounts of hexosamines detected, although undoubtedly underestimates, are similar to those reported by other authors.

The relative abundance of the amino acids in the two separates is of some interest, and although some changes in the amino-acid composition may have occurred during the analytical procedures, there are nevertheless some major differences in composition which warrant further discussion. However it is not possible to make comparisons on the basis of the percentages of the total nitrogen in the hydrolysate found in each constituent, since the amino acids form different proportions of the acid-soluble nitrogen. The results have therefore been recalculated, and are also presented in Table 25 on the basis of the N in the amino acid concerned expressed as a percentage of the total amino acid-N identified in the hydrolysate.

Overall the composition of the two separates is similar, both containing similar amounts of the neutral (c. 55-60%), basic (c. 25%) and acidic (c. 15-20%) amino acids, these being similar to

the distributions recorded in whole soil hydrolysates (Bremner 1965a). The dominant amino acids in the LF were arginine, glycine, aspartate, and alanine, whilst the humic acid contained mainly aspartate, lysine, proline and arginine. The LF contained relatively more arginine, glycine, alanine and tyrosine, whilst the humic acid was relatively richer in histidine, lysine, aspartate (probably as asparagine), and methionine. Evaluation of the significance of these differences in the distribution of the nitrogen amongst the various amino acids is not possible at present, since many of the analyses reported in the literature are at best only semi-quantitative. However it is clear that the LF contains a much higher proportion of its nitrogen as amino acid-N than does humic acid, and this is consistent with the conclusion that the LF consists essentially of partly decomposed organic matter, probably mainly of plant origin. It would be of considerable interest to determine the relative amount of proteinaceous material in the LF which is of either plant or microbial origin, since this proportion, as well as the amino acid composition of the LF protein, would be expected to change markedly during decomposition (e.g. during incubation, or in field soils following cultivation or cropping). It is likely that the protein in the LF would constitute the bulk of the readily mineralizable nitrogen in this fraction. Some typical properties of undecomposed plant material, and of LF and humic acid isolated from the same soil are summarised in Table 26.

TABLE 26. TYPICAL PROPERTIES OF ORGANIC MATERIAL IN VARIOUS STAGES OF DECOMPOSITION

Property	Undecomposed Mature Plant Material	Continuous Pasture Plot (Urrbrae loam)		Appendix Reference
		LF	Humic Acid*	
Carbon content (%)	30-40	52	c. 50	Method B9
Nitrogen content (%)	0.5-2	4.0	4.8	Method B11
C:N ratio	20-70	13	c. 12	
Carbohydrate content (%)	30-40	13	<2	Method B17
% total N released by 6 N HCl (20 hours, 110°C) as (amino acid + amide + hexosamine)N	85-95	84	45	Method B14
'Humus' content (%)	<5	24	100	Method B4
Cation-exchange capacity (me/100 g)	<1	74.5	330	Method B7

LF: separated without surfactant; results corrected for an 'ash' content of 50%.

* Humic acid: sample 11R (Posner 1966).

2. Further studies

a) LF samples separated during development of fractionation technique

Some of the data obtained during studies of the fractionation technique is given in Table 27. Although, as discussed in Chapter III, the amounts of LF separated without surfactant are highly dependent on the sample pretreatment and so are of limited value in relation to quantitative studies, the qualitative differences are of interest.

There were obvious differences in the composition of the LF separated from the two Victorian soils examined. The Walpeup (MR2) samples were relatively richer in humic materials and poorer in total sugars, and so were presumably more decomposed than samples from corresponding plots on the heavy Longerenong (LR2) soil. The material separated from <2 mm soil had similar properties to that extracted from <0.25 mm soil, suggesting that the material obtained with either pretreatment was part of a relatively homogeneous fraction. The LF from the Urrbrae soil (C1:29) contained more 'humic' material and less carbohydrate than did the LF from the Walpeup soil, suggesting that relatively decomposed LF may tend to persist longer in the loam (red-brown earth) than in the sand. This point is discussed further subsequently (p. 119).

(1) Evaluation of some methods used to determine degree of decomposition of LF

The data presented in Table 27 also provides information

TABLE 27. PROPERTIES OF SOME LF SAMPLES SEPARATED WITHOUT SURFACTANT FROM VARIOUS SOILS

Sample	LF g/100 g OD Soil	g/100 g OD LF			
		Alkali-soluble 'Humic' Materials (LF-'Humus')		Total Sugars* (Method B17)	Alkali-Soluble Sugars (Method B6)
		From $E_{260m\mu}$ (Method B3)	From $E_{450m\mu}$ (Method B4)		
a) Seasonal fluctuations (all soils <0.25 mm) in 4-course plots (see also Table 16)					
MR2:4, Oct. '64	0.25	ND	14.4	ND	2.2
" , Dec. '64	0.22	"	12.6	(9.7)	3.0
" , June '65	0.18	"	21.8	ND	3.0
" , Aug. '65	0.12	"	26.4	ND	2.7
LR2:3, Oct. '64	0.17	"	6.7	ND	4.0
" , Dec. '64	0.23	"	4.3	(17.0)	3.3
" , May '65	0.14	"	4.3	ND	2.5
" , Aug. '65	0.21	"	4.6	ND	3.3
b) Grinding (see also Table 17)					
MR2:12, Nov. '65 <2 mm	0.36	19.8	10.8	7.9	1.9
" , " <0.25 mm	0.24	ND	11.7	6.8	2.4
LR2:15, Nov. '65 <2 mm	0.26	10.6	6.4	13.2	3.2
" , " <0.25 mm	0.19	ND	7.4	14.6	3.4
c) Miscellaneous samples					
C1:29, Oct. '63 <0.25 mm	2.23	18.6	14.7	5.0(5.4)	1.6
Mature Phalaris tuberosa	-	14.3	5	32-38(37)	10
Humic acids (sample 9, Posner 1966)	-	100	100	2.1(2.2)	ND

* Values in parenthesis obtained by reflux procedure, others by hydrolysis in sealed tubes.

ND = not determined.

for the evaluation of some of the analytical procedures used for the characterization of these LF samples.

(a) The behaviour of the LF as a microbial substrate might be expected to depend upon its content of readily available energy-rich materials (such as carbohydrates) and of protein, provided that other nutrients are in adequate supply. Accordingly an attempt was made to estimate both the total carbohydrate and the hemicellulose (alkali-soluble) fraction, since this latter fraction in particular is usually rapidly attacked when plant material is added to soil (Greenland and Oades 1968). However, a considerable proportion (c. 20-35%) of the total carbohydrate was extracted from both plant tissue and LF samples by the hot alkali treatment used, and it was evident that this technique was unlikely to provide as useful an index of the 'energy' status of the LF as would determination of total sugar content. Also, with the method used it would not have been possible to differentiate between hemicellulose material of plant or microbial origin. The use of an alternative index (LF sugar-C: LF organic-N ratio) is discussed below.

(b) The extent of decomposition of the LF has been assessed by measuring the extinction at either 260 or 450 mμ of alkaline extracts of LF samples. The technique based on measurements at 260 mμ results in an overestimate, probably due to absorption in the ultra-violet by non-humic organic materials, e.g. for Phalaris tissue the E_{260} mμ measurement gave a result approximately 3 times higher than

that based on the E_{450} mμ of a hot alkali extract, which itself may be a slight overestimate if coloured oxidation products are formed (e.g. from carbohydrate material) during extraction. The E_{450} mμ method has the advantage of simplicity and general freedom from such non-specific interference. However although it is recognized to give at best a semi-quantitative estimate of the humic materials present in a sample, no other more satisfactory method is at present available. The agreement between the 'humus' content and the CEC of a LF sample (Table 26) indicates that the estimate so obtained is of the correct order of magnitude. Further, the fact that the 'humus' contents of LF samples separated from the Longerenong clay are similar to that of fresh plant tissue (Table 27) indicates that they are essentially undecomposed.

(2) Carbohydrate composition of LF separated with solutions of SG 1.8 and 2.0

Data relating to the amount and composition of the carbohydrates in various LF samples separated from the 3 soils (see Table 21) is presented in Tables 28 and 29.

Although the lowest amounts of LF were separated from these soils with a solution of SG 1.8, the composition of the LF was not greatly different to that obtained from the same soil using a solution of SG 2.0. The presence of various amounts of surfactant did not produce any differences in this respect. This suggests that within each of these soils the LF was chemically relatively

TABLE 28. EFFECT OF SOLUTION DENSITY AND SURFACTANT CONCENTRATION ON AMOUNTS AND COMPOSITION OF LF SEPARATED FROM 3 SOILS

Trial, Plot No., Date and Depth of Sample	Rotation and Phase	g/100 g OD Soil			Solution SG and % (w/v) 'Aerosol OT'	g LF/100 g OD Soil		g/100 g LF			g/100 g LF ('Ash-free')	
		C	N	'Humus'*		Total	'Ash-free'	'Ash'	'Humus'*	Neutral Sugars	'Humus'*	Neutral Sugars
C1:29, May '65, 0-10 cm (Red-brown earth)	Continuous P	2.25	0.21	0.97	1.8, 0.10%	1.36	(0.93)	33	12.6	8.3	19.1	12.4
					2.0, 0 %	2.24	(1.12)	50	11.9	6.4	23.8	12.8
					" , 0.01%	3.38	(1.69)	50	11.4	ND	22.8	ND
					" , 0.05%	3.43	(1.71)	50	12.8	ND	25.6	ND
					" , 0.10%	4.25	(2.47)	41	12.6	5.3	21.4	11.8
MR2:4, Sept.'64, 0-10 cm (Solonised brown soil)	PPFW : P_2	0.45	0.05	0.23	1.8, 0.10%	0.22	(0.16)	27	9.9	11.6	13.5	15.9
					2.0, 0 %	0.37	(0.21)	45	14.2	13.7	25.8	24.9
					" , 0.01%	0.45	(0.24)	47	12.2	ND	23.0	ND
					" , 0.05%	0.46	(0.30)	34	9.9	ND	15.0	ND
					" , 0.10%	0.56	(0.37)	30	11.0	7.2	15.7	10.3
LR2:3, Sept.'64, 0-10 cm (Grey soil of heavy texture)	PPFW : P_2	1.21	0.13	0.54	1.8, 0.10%	0.50	(0.41)	24	4.1	26.5	5.4	35.0
					2.0, 0 %	0.33	(0.22)	34	5.5	17.0	8.3	25.8
					" , 0.01%	0.53	(0.34)	37	5.9	ND	9.4	ND
					" , 0.05%	0.79	(0.49)	38	4.5	ND	7.3	ND
					" , 0.10%	0.83	(0.53)	36	5.5	16.2	8.6	25.3
Ref. to Method in Appendix		B9	B11	B5			Appendix A	B1	B4	B17		

*'Humus' contents determined by extraction of sample with dilute alkali (Appendix B, Methods B4 and B5).

TABLE 29. CARBOHYDRATE COMPOSITION OF LF SAMPLES SEPARATED FROM 3 SOILS USING SOLUTIONS OF SG 1.8 AND 2.0 AND VARIOUS AMOUNTS OF SURFACTANT

Trial, Plot, Date Rotation and Phase	C1:29, May '65 Continuous P			MR2:4, Sept.'64 PPFW : P_2			LR2:3, Sept. '64 PPFW : P_2		
Solution SG % (w/v) 'Aerosol OT'	1.8 0.1	2.0 0	2.0 0.1	1.8 0.1	2.0 0	2.0 0.1	1.8 0.1	2.0 0	2.0 0.1
Composition: Total Neutral Sugars: g/100 g LF	8.3	6.4	5.3	11.6	13.7	7.2	26.5	17.0	16.2
Monosaccharides* (alditol acetates)	% Total Neutral Sugars identified by GLC								
(i) 6-deoxy-hexoses									
rhamnose	0.72	0.57	0.49	1.03	1.09	0.73	0.42	0.49	0.28
fucose	0.84	0.68	0.57	0.45	0.74	0.60	0.51	0.45	0.30
(ii) pentoses									
ribose	0.22	0.37	0.12	0.38	0.27	0.23	0.14	0.39	0.15
arabinose	4.32	3.68	4.12	3.90	7.74	5.44	4.68	4.19	2.61
xylose	5.73	4.32	6.06	4.51	5.97	5.49	9.25	7.44	5.64
(iii) hexoses									
mannose	11.3	16.5	10.1	10.2	8.13	8.36	5.36	6.67	5.32
galactose	9.61	7.48	10.1	12.8	19.0	11.1	7.92	11.0	8.93
glucose	67.3	66.4	68.5	66.8	57.0	68.1	71.7	69.4	76.8

* Determined by GLC of alditol acetates (Oades 1967b) prepared from sealed tube hydrolysates (Method B17).

homogeneous, although physical examination of the separates showed that more finer particles were present where the higher solution density was used, particularly if surfactant was present.

Despite approximately sixfold differences in the amounts of LF present in the loam (C1) and sand (MR2), the composition of the LF separated from them was remarkably similar, and differed from that isolated from the clay (LR2).

In all the LF samples examined their total neutral sugar complement was composed of predominantly hexoses (c. 90%), together with some pentoses (c. 10%) and small amounts (c. 1%) of 6-deoxy-hexoses. Traces of myo-inositol were also observed in all samples. The predominant monosaccharide was glucose, this accounting for some two-thirds of the total sugars identified. This would be expected as cellulose is the dominant carbohydrate material occurring in plant tissues. Glucose accounts for a much lower proportion (c. 30-35%) of the total neutral sugars identified in isolated soil polysaccharides (J.M. Oades 1967, pers.comm.).

The suite of sugars detected is similar to that reported by other authors who have examined the monosaccharide composition of LF (Greenland and Ford 1964), soils, litter-soil mixtures, or of isolated soil polysaccharides (Greenland and Oades 1968). It is difficult to evaluate the significance of the relative proportions of the various monosaccharides identified, both because it is not

possible to determine the relative amounts of plant and microbial saccharides in the LF samples, and also because of the lack of comparable data in the literature. However the data is consistent with the conclusion that the LF represents partly decomposed material, probably mainly of plant origin.

This conclusion is supported by the data relating to the amount of humic materials (LF-'humus') in these samples. In all 3 soils the total amount of 'humus' in the LF accounted for some 20-25% of the soil organic carbon (assuming 50% C in 'humus'). However, the LF from the Longerenong (LR2) soil was much less humified than that separated from the other two soils. This was in agreement with its relatively high total carbohydrate content (about twice that of the LF from the other soils) and with its physical appearance (predominantly large plant fragments). It would appear that conditions in the Urrbrae soil (C1) favour the accumulation of large quantities of moderately well-humified LF material.

b) Changes in carbohydrate and amino acid composition of LF during incubation

(1) Introduction

The studies reported above were aimed primarily at an overall characterization of the LF separated from a range of soils, and were also used as a means of monitoring the progress of the fractionation technique during the investigations leading to the inclusion of surfactant in the procedure.

Another objective was to determine whether any marked changes occurred in the composition of the LF either in the field (e.g. during the growing season) or in the laboratory during aerobic incubation. Since it is well known that under favourable conditions the microbial decomposition of substrates such as plant residues is largely dependent upon the "available energy" and "available nutrient" status of the substrate (usually expressed in terms of e.g. C:N ratios), it appeared that some attempt to assess the likely susceptibility to microbial attack of the LF by some chemical index based on this concept would be warranted. As the main source of energy rich materials in plant tissues are carbohydrates and the main source of readily metabolisable organic nitrogen is protein, selected samples of LF were analysed for total carbohydrate and organic N.

(2) Materials

The LF samples examined were separated from soil samples which had been aerobically incubated for either 0, 4 or 17 weeks at 35°C, and from samples separated from 4-course plots during studies of changes in LF and mineralizable N at the 3 sites. Further details of the experiments concerned are given below and in Chapter V.

(3) Methods

All carbohydrate analyses were based on acid (1 N H_2SO_4) hydrolysates obtained by reflux (Method B17). Sugar-C was calculated

on the basis of an average carbon content of 40% (w/w) of the total neutral sugars as estimated by an anthrone procedure.

LF-N determinations were by a micro-Kjeldahl procedure (Method B13) and amino acid analyses based on ion-exchange chromatography of acid (6 N HCl) hydrolysates (Method B15).

Since carbohydrates comprise some 10-25% of the LF-C, then the C:N ratio of the LF samples would be c. 4-10 times that of the sugar-C:N ratios quoted below.

(4) Results and discussion

Before discussing the results obtained two factors which complicate their interpretation need to be considered. Firstly, the extent to which random sampling and analytical errors have influenced the results obtained has not been determined. In the present study the analyses were performed on small quantities of LF derived from a subsample of a composite soil sample obtained from a rotation trial plot, and although the amount of LF separated from such subsamples has been shown to be reasonably reproducible (Table 23) the extent to which the composition of such separates varies is unknown. The other difficulty is that the interpretation of data derived from studies of overall (net) changes in the composition of a substrate is complicated by the dynamic equilibrium in which much of the soil organic matter is involved. Greenland and Oades (1968) concluded from their review of studies of the transformations of

^{14}C-labelled saccharides in soil that there is continual degradation and resynthesis of microbial polysaccharide, much of which is derived from plant carbohydrate material. Kuo and Bartholomew (1963) have also suggested that a similar turnover of plant protein occurs, the proportion of microbial protein in the plant residues increasing as their decomposition proceeds. As the methods used in this present study do not permit the differentiation of plant carbohydrate or protein from microbial material, the evaluation of the significance of the net changes observed is largely dependent upon further quantitative studies.

(a) Changes in Sugar-C:LF-N ratio

The results of analyses of various LF samples are presented in Tables 30 and 31. As the 'ash' contents were not determined, changes in the ratio of sugar-C to organic N in the samples are probably a better index of qualitative changes in composition than are changes in the magnitude of either sugar-C or organic N. Also, as the 'ash' content of LF samples separated from the various soils differed comparisons between the soils are best made on the basis of the above ratio, although some comparisons within a given soil type of the LF sugar and nitrogen data may perhaps be made as the variations in 'ash' content involved are probably small.

The data in Table 30 indicates that sugar-C:LF-N ratio tended to be greatest in the LF from the clay (LR2) and least in

(i) C1 samples (0-6.3)cm; MR2 and LR2 samples (0-10)cm
(ii) Rotation abbreviations as in Table 10
(iii) Total sugar content determined by reflux procedure (Method B17); Sugar-C calculated on basis that it was equivalent to 0.4 (total sugars)
(iv) LF-N determined by micro-Kjeldahl procedure (Method B13).

TABLE 30. SEASONAL CHANGES IN THE COMPOSITION OF THE LF IN THREE SOILS UNDER VARIOUS ROTATIONS

Trial, Plot, Date	Rotation and Phase	LF g/100g OD Soil	g/100 g LF			Sugar-C / LF-N
			Total Neutral Sugars	Sugar-C	N	
C1:17, Feb. '67	Continuous W	2.11	3.95	1.58	0.98	1.61
" , Sept. '66	Continuous FW:W	1.72	3.35	1.34	0.89	1.50
C1:35, Feb. '67	" " :"	1.78	3.56	1.42	0.95	1.49
C1:32, Sept. '66	PPFW:P_2	2.73	3.21	1.28	1.12	1.15
" , Feb. '67	" :"	2.30	4.54	1.82	1.22	1.48
C1:31, May '66	" :"	1.95	3.45	1.38	1.27	1.08
" , Sept. '66	" :F	2.25	4.29	1.72	1.25	1.37
" , Feb. '67	" :"	1.96	3.62	1.45	1.25	1.15
C1:30, Sept. '66	" :W	1.91	3.81	1.52	1.26	1.20
" , Feb. '67	" :"	1.95	4.22	1.69	1.24	1.36
C1:29, Sept. '66	Continuous P	4.15	4.89	1.96	1.46	1.33
" , Feb. '67	" "	3.44	5.31	2.12	1.55	1.37
MR2:7, Sept. '64	PPFW:P_2	0.64	4.89	1.96	1.66	1.17
" , June '65	" :F	0.66	8.77	3.51	1.83	1.91
" , March '66	" :"	0.69	7.96	3.18	1.69	1.88
MR2:9, Sept.'64	" :W	0.53	5.92	2.37	1.34	1.76
" , June '65	" :P_1	0.42	5.56	2.22	1.50	1.48
" , March '66	" :"	0.62	7.76	3.10	1.24	2.50
LR2:3, Sept. '64	PPFW:P_2	0.76	16.3	6.51	1.42	4.58
" , May '65	" :"	0.61	14.5	5.80	1.92	3.02
" , March '66	" :F	0.51	13.6	5.46	2.04	2.67
LR2:4, Sept. '64	" :W	0.20	11.8	4.74	1.67	2.83
" , May '65	" :P_1	0.21	14.0	5.58	2.18	2.56
" , March '65	" :"	0.21	13.9	5.57	1.94	2.87

TABLE 31. CHANGES IN THE COMPOSITION OF THE LF IN THREE SOILS DURING AEROBIC INCUBATION FOR 17 WEEKS AT 35°C

Trial, Plot, Date	Rotation and Phase	Weeks Incubated	LF g/100g OD Soil	g/100 g LF			Sugar-C / LF-N
				Total Neutral Sugars	Sugar-C	N	
C1:29, Sept. '66	Continuous P	0	4.03	4.89	1.96	1.46	1.33
" , "	"	17	3.62	4.94	1.98	1.58	1.25
C1:32, "	PPFW:P_2	0	2.63	3.40	1.36	1.13	1.20
" , "	" :"	17	2.35	2.40	0.96	1.21	0.79
C1:35, "	FW:W	0	2.11	3.81	1.52	0.90	1.69
" , "	":"	17	0.88	1.96	0.78	0.88	0.89
MR2:2, April '66	PPFW:P_2	0	0.48	4.92	1.97	1.79	1.09
" , "	" :"	17	0.40	4.21	1.68	1.60	1.05
MR2:5, "	FW:F	0	0.34	4.89	1.96	1.29	1.51
" , "	" :"	17	0.27	5.86	2.18	1.36	1.60
LR2:5, April '66	PPFW:P_2	0	0.51	14.3	5.72	1.93	2.96
" , "	" :"	17	0.27	9.88	3.95	2.20	1.79

Data calculated as for Table 30.

that from the loam (C1), this largely reflecting the relatively greater content of carbohydrate material in the LF from the Longerenong soil. Thus the magnitude of the ratio was in general agreement with the previous finding that the LF in the Longerenong soil tended to be relatively the least humified and that in the Urrbrae soil the most humified.

Within each soil, the ratio tended to be higher in LF from 2-course (FW) than from 4-course (PPFW) plots (Tables 30 and 31). Seasonal variations in this ratio tended to be small, and to parallel changes in the quantity of LF present, particularly in the 4-course plots. The significance of these trends is not known, and further data is required if their relationship to changes in mineralizable N in these soils is to be evaluated.

The data in Table 31 indicates that in nearly all the samples from the 3 soils examined the amount of LF and its sugar-C: LF-N ratio decreased during incubation. Thus the disappearance of part of the LF, and the relative loss of carbohydrate and enrichment in organic N of the remaining LF, provide indirect evidence for the decomposition of the LF. Other authors have reported similar changes in the chemical composition of plant residues during their decomposition (e.g. E.W. Russell 1961; Kononova 1961; Bartholomew 1965).

(b) Changes in monosaccharide composition

The relative amounts of the individual monosaccharides

identified in various LF samples are presented in Table 32. Traces of myo-inositol were detected in all samples. The proportion of glucose present was less, and the proportion of pentoses and 6-deoxy-hexoses greater, than that found previously (Table 29). The greater relative yield of pentoses from the samples hydrolysed under reflux (Table 32) suggests that destruction of labile sugars may have occurred during the sealed tube hydrolysis used previously (see Appendix B17). Whilst there are therefore some reservations about the absolute magnitudes of the values obtained, comparison of the relative amounts obtained from various samples using any one hydrolysis procedure is considered to be valid.

The relative concentrations of the individual sugars were similar for all the unincubated LF samples, hexoses being the dominant constituents (70-80% of total), pentoses accounting for some 20-25% and the deoxy-hexoses for only 102% of the total neutral sugars. Glucose was the major monosaccharide present (45-55%).

In the two LF samples for which data is available, there were some changes in the relative amounts of the various sugars during incubation. These changes may indicate that the LF separated after incubation contains newly synthesised material. Alternatively they may merely be due to removal of part of the original LF. The relative increase in the amounts of rhamnose and fucose suggest that the LF separated after incubation contains new material, as other authors have reported a similar relative accumulation during the

TABLE 32. CARBOHYDRATE COMPOSITION OF LF SAMPLES FROM THREE SOILS:
A. BEFORE AND AFTER AEROBIC INCUBATION FOR 17 WEEKS AT 35°C
B. SAMPLED AT VARIOUS TIMES OF THE YEAR

Treatment	A						B			
Trial, Plot, Date Rotation and Phase Weeks Incubated	C1:32, Sept. '66 PPFW:P_2 0	17	MR2:2, Apr. '66 PPFW:P_2 0	17	LR2:5, Apr.'66 PPFW:P_2 0	17	C1:31 May '66 PPFW:F 0	Feb. '67 0	MR2:7, June'65 PPFW:P_2 0	LR2:3, May '65 PPFW:P_2 0
Composition: Total Neutral Sugars (g/100 g LF)	3.40	2.40	4.92	4.21	14.31	9.88	3.45	3.62	8.77	14.51
Monosaccharides* (alditol acetates)	% total neutral sugars identified by GLC									
(i) 6-deoxy-hexoses										
rhamnose	0.95	2.86	1.49	ND	0.82	1.45	1.26	1.13	1.18	0.68
fucose	0.98	1.55	1.33	ND	1.24	1.85	0.86	1.01	1.32	0.90
(ii) pentoses										
(ribose + arabinose)	6.25	9.83	10.2	ND	8.14	11.2	8.09	8.13	8.53	8.19
xylose	14.7	14.7	10.9	ND	17.0	15.7	14.7	15.5	9.54	17.8
(iii) hexoses										
mannose	14.9	9.83	10.8	ND	6.23	9.95	14.3	9.15	9.64	8.59
galactose	17.5	11.5	14.8	ND	9.92	13.2	15.1	12.3	14.2	16.0
glucose	44.7	49.7	47.7	ND	56.5	46.4	45.8	53.4	55.6	46.0

ND = not determined

* Samples hydrolysed by reflux procedure (Method B17).

decomposition of plant material in soil (Sowden and Ivarson 1962, cited by Greenland and Oades 1968, Table 9). Although this could be due to the 6-deoxy-hexoses being relatively resistant to microbial degradation, the data of Keefer and Mortensen (1963) suggests that they probably participate together with the other saccharides in the microbial transformations associated with the decomposition of plant residues. The lack of change in the glucose figures is surprising, and may indicate that the plant cellulose is stable or has been stabilized against microbial attack, e.g. by lignin encrustations. It is also possible that the microbial polysaccharides synthesised from the plant carbohydrates were present in relatively small amounts and were not sufficiently dissimilar in composition to affect markedly the average sugar composition of the separate.

(c) Changes in amino acid composition

The amino acid composition of 6 N HCl hydrolysates of some LF samples before and after aerobic incubation for 4 weeks at 35°C are shown in Table 33.

The results were different from those reported in Table 25 for a sample of LF from Urrbrae loam. The most striking difference was the lower proportion of the LF-N recovered as amino-acid N, the earlier results indicating that 84% of the LF-N was recovered as (NH_4^+ + α-amino + hexosamine)N, whereas only 35-40% was recovered in these samples. Similar (low) results were obtained when a

LF separated as in Appendix A.

*On basis that ninhydrin-positive material in hydrolysate is predominantly (NH_4^+ + α-NH_2)N, expressed relative to a glutamate standard.

tr = trace.

TABLE 33. AMINO ACID COMPOSITION OF SOME LF SAMPLES BEFORE AND AFTER AEROBIC INCUBATION (c. pF 2, 4 weeks, 35°C)

Trial and Plot Date (some composited) Rotation and Phase	C1:32 Sept. '66 PPFW:P_2		C1:35 Sept. '66 FW:F		LR2:5 (Oct. + Nov.) '65 PPFW:P_2		MR2:11 (Jan. + Mar. + Apr.) '66 PPFW:P_2	
± Incubation	-	+	-	+	-	+	-	+
LF-N % % LF-N as (NH_4^+ + α-NH_2)N* in 6 N HCl hydrolysate	1.13 35	1.12 34	0.90 36	0.91 33	1.80 41	2.10 33	1.57 34	1.65 37
Amino Acids	N in Amino Acid as % Total Amino Acid N Recovered							
Basic:								
arginine	7.2	7.5	8.0	8.0	7.2	5.5	6.9	6.3
lysine	5.7	6.0	5.3	5.3	7.6	6.7	6.5	6.5
histidine	3.6	3.6	3.5	3.0	3.4	3.2	3.3	3.2
ornithine	0.7	2.4	0	3.0	0.3	1.6	2.5	2.7
total	17.2	19.5	16.8	19.3	18.5	17.0	19.2	18.7
Acidic:								
aspartate	9.3	4.4	9.5	8.6	9.4	20.8	9.6	11.0
glutamate	7.6	7.9	8.3	8.3	7.8	6.0	6.8	6.9
total	16.9	12.3	17.8	16.8	17.2	26.8	16.4	17.9
Neutral:								
glycine	18.2	19.4	19.0	19.3	17.7	15.5	18.9	19.2
alanine	12.4	12.7	12.0	10.7	13.3	8.6	11.0	10.6
valine	6.2	6.4	6.3	5.5	6.8	4.2	4.6	4.5
serine	5.8	5.9	5.4	5.8	5.4	5.5	5.8	5.6
threonine	5.1	5.0	5.5	5.0	5.4	4.5	4.8	4.7
proline	5.7	6.0	6.1	6.4	3.3	7.0	6.9	6.5
leucine	5.1	5.3	4.6	4.7	5.3	4.7	5.3	5.1
iso-leucine	3.6	3.6	3.2	2.8	3.6	2.8	3.3	3.4
phenylalanine	2.3	2.3	2.0	2.1	2.0	1.7	2.1	2.0
tyrosine	0.9	1.1	0.9	1.0	1.4	1.2	1.0	1.0
½-cystine	0.5	0.5	0.5	0.5	tr	0.5	0.6	0.7
total	65.8	68.2	65.5	63.8	64.2	56.2	64.3	63.3

sample of LF from the Urrbrae soil was hydrolysed under nitrogen by either a reflux or a sealed tube (Method B15) procedure. The discrepancy between the absolute magnitude of the results in Tables 25 and 33, although surprising, may result from possible losses of amino acids during hydrolysis due to non-enzymic oxidation by sugars and polyphenols (A.C. Jennings 1967, pers.comm.). Evidently such losses did not involve selective destruction of particular amino acids, as other data indicated that similar relative proportions of the various amino acids were present in hydrolysates of a sample of LF from the Urrbrae soil obtained by the sealed tube procedure of either Method B14 or Method B15.

Comparison of the relative amounts of the various amino acids obtained using either hydrolysis procedure is therefore considered to be valid. The differences in the relative proportions of glycine, alanine and aspartic acid for the various LF samples from the Urrbrae soil (Tables 25 and 33) may reflect real differences in the composition of LF separated with and without surfactant. Hexosamines were not detected in the above analyses, undoubtedly due to their destruction during hydrolysis.

In general the absolute amount and relative proportions of the 17 amino acids identified were closely similar to those reported for whole soil samples (Bremner 1965a). The relative proportions of basic, acidic and neutral amino acids were similar for all the samples, the only major difference being the relatively high aspartic acid

content of the incubated Longerenong sample. The only other change which apparently occurred during incubation was an increase in the proportion of ornithine in the Urrbrae and Longerenong samples. This general lack of change in the relative proportions of the amino acids may indicate that the plant protein had been degraded and resynthesised into microbial tissue in the soil before incubation.

(iv) Incubation studies

1. Preliminary studies

Studies of the rate of oxygen uptake by a sample of Urrbrae loam amended with undecomposed plant tissue, LF or humic acid (Method B18) indicated that the LF was decomposed at a rate intermediate to that of the other two substrates examined. After incubation for 40 hours at 30°C, the mean oxygen uptake rates of unamended soil, and of soil amended with LF or humic acid were 2 µl/hr, 7-10 µl/hr, and c. 3 µl/hr respectively.

The effect of amending a similar sample of the Urrbrae soil with comparable amounts of various substrates on nitrate release during short term (14 day) incubation (Method B19) is given in Table 34. Some nitrate was released from each of the LF samples. Material derived from cropped plots, and having relatively wide C:N ratios (>20) produced a smaller net release of nitrate than did material of lower C:N ratio derived from old pastures. Undecomposed plant tissue apparently produced complete immobilization of any mineral-N

TABLE 34. MINERALIZATION OF VARIOUS SUBSTRATES ADDED TO URRBRAE LOAM

Substrate		Approx. ppm N in Added Substrate	Net Mineralization (ppm NO_3^--N)	
Origin and History	C:N		Actual	as % added substrate-N
LF from Ground water Rendzina; old pasture	13	1730	+ 75	4.3
" " Solodised Solonetz; cultivated	20	1160	+ 26	2.2
" " Red-brown earth; continuous pasture	11.5	2000	+ 44	2.2
" " Red-brown earth; cont. wheat	23	1140	+ 17	1.5
" " Podzol; virgin scrub	19	730	+ 4	0.5
Humic acid from Red-brown earth	c.12	2100	- 38	not calculated
Mature Phalaris tuberosa tissue	c.69	350	- 42	not calculated

(i) LF samples obtained without surfactant (Table 14)
(ii) Incubation and analytical procedures are described in Method B19.
(iii) Net N mineralized by amended soil is expressed relative to that of unamended soil (control) with a net mineralization of 40 ppm NO_3^--N (c. 2% of the total N in the soil).

released from decomposition of the native soil organic matter. The effect of the humic acid could be explained as being due to either a stimulated rate of immobilization of mineral-N or to an inhibition of the rate of mineralization of the native soil organic nitrogen, but further data would be required to clarify this point.

Under these incubation conditions it is clear that separated LF material is at least potentially available as a substrate for soil organisms, although no information as to its long-term behaviour is available. A major difficulty in extrapolating from such experiments to field conditions is that changes may occur in the LF particles during their separation, and these may affect their subsequent mineralization. Such changes may be due to

i) oven-drying at 70°C prior to fractionation;

ii) grinding, i.e. reduction in particle size; or

iii) solvent treatment, which may remove lipids or other materials rendering the LF particles hydrophobic, or of organic materials which may exert some biocidal activity (cf. Stevenson 1966).

In addition, in field soils comminution of the LF by the soil fauna, and simultaneous mixing with the active microflora in their digestive tract, may also influence breakdown of the LF. These problems are largely avoided by following changes in the mineral-N and LF-N contents of comparable sets of unamended soils, one set being incubated and the other not.

2. Further studies

These were designed to determine for each of the 3 soil types examined:

i) What incubation conditions (moisture content, temperature, and duration) were required to achieve satisfactory mineralization rates,

ii) What seasonal variations in LF-N and mineralizable N occurred in plots at various stages of 2-course (FW) and 4-course (PPFW) rotations,

iii) What changes in the composition of the LF occurred during incubation.

a) Determination of incubation conditions required

The pretreatment which a soil receives prior to incubation strongly influences its subsequent mineralization behaviour (e.g. Bremner 1965c). Consequently all samples were subjected to the same drying (40°C) and grinding (<2 mm) treatments, and stored for at least 1 month at room temperature in sealed containers before incubation.

In a preliminary experiment duplicate samples of soil from the two Victorian trials (Walpeup and Longerenong) were incubated at various moisture contents at 35°C for 4 weeks, essentially as in Appendix C5. The results obtained are presented in Table 35.

Maximum mineralization was obtained for the clay (LR2)

TABLE 35. CHANGES OCCURRING ON INCUBATION AT VARIOUS MOISTURE CONTENTS FOR 4 WEEKS AT 35°C

Trial, Plot	LR2:7					LR2:5					MR2:1					MR2:2				
Date	Nov. '65					Nov. '65					Nov. '65					Nov. '65				
Rotation	FW					PPFW					FW					PPFW				
Phase	W					P_2					W					P_2				
Initial soil N (%)	0.077					0.111					0.038					0.054				
" " N_i (ppm)	0					2					4					12				
" " LF (%)	0.08					0.23					0.20					0.37				
" " LF-N (ppm)	19					40					25					69				
" " " (% soil N)	2.6					3.6					6.6					12.8				
" " pH	7.7					7.6					7.6					7.4				
Initial soil moisture (%)	24	34	44	54	64	24	34	44	54	64	10	14	18	22	32	10	14	18	22	32
Final soil N_i (ppm)	5	8	11	10	6	27	56	93	73	72	13	26	28	30	30	31	50	51	57	55
" " LF (%)	0.05	ND	0.05	ND	0.04	0.23	ND	0.17	ND	0.14	0.18	ND	0.12	ND	0.20	0.33	ND	0.43	ND	0.70
" " LF-N (ppm)	6	ND	7	ND	7	45	ND	33	ND	27	21	ND	11	ND	23	76	ND	58	ND	81
Net change N_i (ppm)	+5	+8	+11	+10	+6	+25	+54	+91	+71	+70	+9	+22	+24	+26	+26	+19	+38	+39	+45	+43
" " LF-N (ppm)	-13	ND	-12	ND	-12	+5	ND	-7	ND	-13	-4	ND	-14	ND	-2	+7	ND	-11	ND	-12

i) all samples (0-10) cm
ii) pH determined on 1:5 (w/v) suspension in 0.01 M $CaCl_2$
iii) N_i = inorganic N = $(NH_4^+ + NO_3^-)N$

ND = not determined

when the initial moisture content was adjusted to c. 45% (w/w), this resulting in the mineralization of some 1.5% and 8.2% of the soil nitrogen in the 2-course (FW) and 4-course (PPFW) plots respectively. The corresponding values for the sand (MR2), when adjusted to 15-25% moisture, were some 6% and 7% of the soil nitrogen. As these moisture contents approximated those obtained by wetting up air dry (<2 mm) aggregates under 100 cm suction, they are subsequently referred to as those corresponding to c. pF 2. The corresponding moisture content for the Urrbrae fine sandy loam samples was c. 25%.

There was no evidence that aeration was limiting during incubation, and during subsequent experiments gas chromatographic analysis of the atmosphere above some moist soil samples incubated in a similar fashion in sealed polystyrene containers (volume c. 200 cc) established that in all cases <0.1% CO_2 (v/v) was present, and that the (N_2 + O_2) content was essentially that of normal air. Evidently diffusion through these containers was sufficient to maintain adequate aeration. However when comparable samples were incubated in glass vessels fitted with plastic aeration devices ('Res Caps') no loss of water vapour occurred but appreciable amounts of CO_2 (up to 0.5-1.0% v/v) accumulated in the atmosphere above the samples. These devices have been found to be satisfactory by other authors (Keeney 1965; Bremner 1965c) but it would seem that they do not aerate the samples as efficiently as do the polystyrene containers

used in this study.

Although some loss of moisture always occurred during incubation (up to 2.5 ml per sample), mineralization did not appear to be adversely affected. Keeney (1965) has reported similar results in Table 14 (p. 71), where even the loss of 25% of the water added initially did not significantly reduce the amounts of nitrogen mineralized.

Nitrification at this temperature (35°C) evidently proceeded normally and more rapidly than ammonification, as <1 ppm NH_4^+-N was detected in the incubated soils. Also negligible change in soil pH (<0.1 unit) occurred during incubation.

Appreciable losses of the LF-N initially present occurred during incubation, the losses at the "optimal" moisture contents being similar in both soils for corresponding plots, i.e. some 17% and 50% for the 2-course and 4-course plots respectively. These losses of LF-N accounted for appreciable proportions (8-100%, mean 38%) of the net nitrogen mineralized, implying that since the LF-N initially present only accounted for 3-13% of the soil nitrogen, the LF-N was apparently a relatively labile fraction of the soil organic matter.

There was no evidence that the 'humus' content of the LF increased during incubation, the values for LF from the Longerenong 2-course and 4-course plots before and after incubation being 5.4 cf. 8.2% (w/w), and 3.9 cf. 4.0% respectively. Corresponding values for the Walpeup plots were 16.4 cf. 17.0% and 12.4 cf. 11.6%. This

could indicate either that the humic materials formed during decomposition were more closely associated with the soil colloids than with the LF residues, or possibly that portion of the LF material was completely decomposed, so that the LF remaining had actually undergone relatively little decomposition.

(b) Determination of changes occurring during long-term incubations

Sets of 25.0 g samples of each soil were moistened and incubated as in the routine method (Appendix C5), for periods of up to 17 weeks. At selected intervals a complete set was analysed for its LF-N and mineral-N content. Since differences in the behaviour of subsamples of any given soil are included in the results relating to various incubation periods, only the major trends apparent in the results will be discussed. The results obtained are presented in Table 36.

In all cases appreciable mineralization occurred, the mean rate tending to decrease fairly rapidly after the first 2 to 4 weeks of incubation. Similar asymptotic mineralization-time relations have been obtained by other workers, and have generally been ascribed to the accumulation of soluble materials which may either stimulate immobilization or inhibit ammonification (e.g. Harmsen and Van Schreven 1955; Lewis 1963). Moisture losses especially during the last 9 weeks of incubation, may also have contributed to this decline in the mineralization rate. It is also probable that fractions of

NB:

i) Abbreviations as in Table 10

ii) "non LF-N" calculated from difference between total soil N (N_t) and initial LF-N.

iii) - = not calculated as result confounded by apparent immobilization of portion of LF-N.

TABLE 36. CHANGES IN LF AND SOIL N ON INCUBATION FOR VARIOUS PERIODS (c. pF 2, 35°C)

Trial and Plot No.	C1:29				C1:32				C1:35			
Date	September '66				September '66				September '66			
Depth (cm)	0-6.3				0-6.3				0-6.3			
Rotation	Continuous P				PPFW				FW			
Phase	P				P_2				F			
Initial soil N (N_t; %)	0.216				0.132				0.083			
" " N_i(ppm)	12.6				2.1				3.8			
" " LF (%)	4.03				3.13				2.11			
" " LF-N (ppm)	588				354				190			
" " " (% soil N)	27.2				26.8				22.9			
Weeks incubated	2	4	6	17	2	4	6	17	2	4	6	17
Final soil N_i (ppm)	60	90	98	132	24	32	40	79	12	15	27	37
" " LF (%)	4.20	3.57	4.00	3.62	3.28	2.79	2.77	2.35	2.11	2.25	1.83	1.95
" " LF-N (ppm)	534	550	556	572	344	313	310	284	198	167	171	149
Net change N_i (ppm)	+47	+77	+85	+119	+22	+30	+38	+77	+8	+12	+23	+33
" " " (% soil N)	2.2	3.5	3.9	5.5	1.7	2.3	2.9	5.8	0.9	1.4	2.7	3.9
" " " (% initial "non LF-N")	0.4	1.8	3.4	6.6	1.2	(-1.4)	(-0.6)	0.7	1.2	(-1.7)	0.6	(-1.2)
" " LF-N (ppm)	-54	-38	-32	-16	-10	-41	-44	-70	+8	-23	-19	-41
" " " (% initial LF-N)	9.1	6.5	5.4	2.7	2.8	11.6	12.4	19.8	-	12.1	10.0	21.6
Approx. mean N mineralization rate (ppm/week)	24	15	4	4	11	4	4	4	4	2	5	1
Approx. Relative Mineralization Rate of LF-N cf. "non LF-N"	22.7	3.6	1.6	0.4	2.3	-	-	28.3	-	-	16.7	-

Trial and Plot No.	MR2:2				MR2:5				LR2:5				LR2:1			
Date	April '66								April '66							
Depth (cm)	0-10								0-10							
Rotation	PPFW				FW				PPFW				FW			
Phase	P_2				F				P_2				F			
Initial soil N (N_t; %)	0.036				0.025				0.107				0.064			
" " N_i (ppm)	11.6				3.8				20.3				23.0			
" " LF (%)	0.48				0.34				0.51				0.11			
" " LF-N (ppm)	87				43				98				21			
" " " (% soil N)	24.2				17.2				9.2				3.3			
Weeks incubated	2	4	6	17	2	4	6	17	2	4	6	17	2	4	6	17
Final soil N_i (ppm)	26	33	39	48	18	24	27	28	59	87	93	149	30	39	41	50
" " LF (%)	0.43	0.35	0.24	0.40	0.29	0.24	0.22	0.27	0.31	0.25	0.25	0.27	0.10	0.08	0.08	0.08
" " LF-N (ppm)	67	53	37	64	36	29	24	37	59	45	50	59	16	16	14	14
Net change N_i (ppm)	14	21	28	37	14	20	23	25	38	67	72	129	7	16	18	27
" " " (% soil N)	3.9	5.8	7.6	10.1	5.3	7.7	8.8	9.4	3.5	6.2	6.7	11.9	1.1	2.4	2.8	4.2
" " " (% initial "non LF-N")	(-2.4)	(-5.1)	(-8.7)	5.5	3.4	2.9	1.9	9.2	(-0.1)	1.4	2.5	9.3	0.3	1.8	1.8	3.2
" " LF-N (ppm)	-20	-34	-50	-23	-7	-14	-19	-6	-39	-53	-48	-39	-5	-5	-7	-7
" " " (% initial LF-N)	23.0	39.1	57.5	26.5	16.2	32.6	44.2	14.0	39.8	54.0	49.0	39.8	23.8	23.8	33.4	33.4
Approx. mean N mineralization rate (ppm/week)	7	4	4	1	7	3	2	0.2	19	15	3	6	4	5	1	1
Approx. Relative Mineralization Rate of LF-N cf.	-	-	-	4.8	4.8	11.2	23.2	1.5	-	38.6	19.6	4.3	79.3	13.2	18.5	10.4

varying degrees of ease of decomposition exist within the LF and within the humified fraction, but examination of this problem will require the use of isotopic tracers.

In all the samples examined there was a net loss of LF-N during incubation. However, in some of the Urrbrae samples, particularly that from the continuous pasture plot (C1:29) there was an apparent increase in the amount of LF present after incubation for 2-6 weeks, although the amount of LF remaining after incubation for 17 weeks was in all cases appreciably lower than that initially present. The LF-N content of the C1:29 sample tended to increase throughout the incubation period, whereas in the other two samples the LF-N content generally decreased. Similar apparent increases in the LF and LF-N contents of some of the Walpeup and Longerenong samples were also observed during incubation. These increases in LF and LF-N may be artifacts resulting from experimental error, the magnitude of which cannot be estimated as the experiment was not replicated. They may nevertheless be real, and reflect the occurrence of the net assimilation of LF material during incubation, possibly resulting from the development of microbial tissue in and on LF particles (cf. p. 126). Further experiments, possibly employing ^{14}C and/or ^{15}N-tagged LF material are required to clarify this point.

However there were obvious differences between these 3 soils in the proportion of their total N which was mineralized,

although this was generally lowest in the 2-course plots. Despite their relatively high initial N status, even after 17 weeks, samples of the Urrbrae (C1) soil had mineralized a much lower proportion of their total N (4-6%) than had corresponding plots from either the Walpeup (9-10%) or Longerenong (4-12%) soils. Similar differences between these soils in the proportion of their LF-N which disappeared during incubation were observed, the greatest relative loss occurring in the heavy Longerenong soil where 30-40% of the LF-N initially present had disappeared after 17 weeks incubation. Even though the LF accounted for only 3-9% of the total nitrogen in this soil, this disappearance of LF-N accounted for some 25-30% of the mineral nitrogen released during the 17 week period. Corresponding figures for the Walpeup soil were 17-24% of the soil N and 25-60% of the mineralized nitrogen, and for the Urrbrae soil 23-27% and 13-124% respectively. Further evidence for the relative lability of the LF is provided by the estimated relative rates of mineralization of the LF-N and "humified N" (taken as "non LF-N") in these samples. Although these estimates have been based on data subject to appreciable experimental error, their magnitude is of considerable interest to the present study. The calculations are derived from net mineralization data, and thus probably represent underestimates of both the rates involved.

In most cases a much higher proportion of the LF-N than of the "humified N" was mineralized during incubation. In the

Urrbrae samples this "relative net mineralization rate" varied from <1 to 28. It tended to be higher in the 2-course and 4-course plots than in the plot under continuous pasture, this reflecting the generally higher proportion of the initial LF-N mineralized in these plots. The values of the "relative net mineralization rate" decreased as incubation proceeded in the continuous pasture plot, whereas they increased in the 4-course plot. This may indicate a rapid initial mineralization of labile LF in the continuous pasture sample, or less probably a progressive increase in the mineralization rate of the "humified N" and/or a decrease in the rate of immobilization of mineral N derived from the LF. The data from the 4-course plots probably indicates a progressive decrease in the net mineralization rate of the "humified N" and/or a decreased rate of immobilization of the LF-N mineralized. The Longerenong data shows a similar decline in this relative rate with time (e.g. from 39 to 4). The Walpeup data exhibits no consistent trend, although values of up to 23 were obtained for the 2-course sample.

Overall the LF is clearly more labile than the "humified N", their relative lability varying with soil type, agronomic history and duration of incubation.

This data is broadly in accord with the previous suggestions as to the relatively labile nature of the LF. However such estimates of the turnover of LF-N during incubation and of the relative rates of mineralization of LF-N and of humified N, must be

regarded as probably being minimal, as the LF-N remaining after incubation may represent material which has been subjected to several cycles of microbial degradation and resynthesis (Kuo and Bartholomew 1963; Bartholomew 1965). It is also evident that both the LF and the more humified material are involved in the turnover of N in these soils.

The major conclusions from these incubation studies may be summarized as follows:

1. For the soils studied incubation for 4 weeks at c. pF 2 and 35°C provides a satisfactory and convenient means of estimating the relative rates at which the LF-N and the soil N mineralize, longer incubation periods contributing relatively little additional information.

2. The LF appears to be an important source of mineralizable N in these soils, as even in the Longerenong clay where it represents only 3-9% of the soil N the net loss of LF-N during incubation accounts for some 30-80% of the mineral N released during a 4 week period. A higher proportion of the LF-N was mineralized in plots under 4-course than in plots under 2-course rotations.

The LF-N mineralized at a much faster rate (up to 20-40 times) than did the "humified N"; this relative net mineralization rate varying with the type of soil, its agronomic history, and with the duration of the incubation treatment used.

The influence of soil type, rotation and seasonal

factors on the dynamics of the LF in relation to changes in the mineralizable N reserves of these soils needs to be studied further if the agronomic significance of the LF under field conditions is to be evaluated.

(C) Summary

The organic material obtained by ultrasonic dispersion and densimetric fractionation of soil in a heavy organic liquid (the 'light fraction') has been identified as consisting essentially of finely comminuted and partly humified plant fragments, together with phytoliths and trace amounts of soil minerals.

Chemical studies showed that although some 'humic' materials were also present, the gross composition of the LF more nearly approximated that of fresh plant material than that of 'humic' extracts of soils. Smaller amounts of 'humic' materials were associated with the LF separated from a clay (Longerenong) than with that separated from a sand (Walpeup) or a loam (Urrbrae). The CEC of a sample of LF from the latter soil was largely accounted for by its 'humus' content.

The total content of hydrolysable neutral carbohydrates was intermediate between that of undecomposed plant material and humic acid, and was highest in LF samples separated from the clay and least in LF from the loam. The monosaccharides identified by GLC were glucose, galactose, mannose, xylose, arabinose, ribose, rhamnose and fucose. Trace amounts of myo-inositol were detected.

Hexoses (notably glucose) accounted for the largest proportion (c. 75%) of the total neutral sugars, pentoses accounting for c. 25% and 6-deoxy-hexoses for 1-2%.

Some 35-80% of the nitrogen contained in the LF was released as amino acids on acid hydrolysis. Seventeen amino acids were identified by ion-exchange chromatography, the relative proportions being similar to those reported for soil samples. There was virtually no change in the amounts of the individual amino acids during incubation, suggesting that the LF-N may represent microbial protein synthesised prior to incubation. However the increase in the relative proportions of rhamnose and fucose in the LF separated from these soils after incubation suggested that it contained newly synthesised material.

The degree of decomposition of the LF was examined by estimation of the amount of humified materials associated with it, and also by determination of its sugar-C:LF-N ratio. The LF from the clay contained the lowest proportion of humified materials and had the greatest sugar-C:LF-N ratio (2.6 - 4.6) suggesting that it was relatively undecomposed. The LF from both the sand and the loam was apparently more humified. In all 3 soils the sugar-C:LF-N ratio was greatest in 2-course plots. Seasonal fluctuations in this ratio were apparently small, although it decreased during incubation.

Short term incubation studies indicated that the LF was

a potentially available substrate for soil organisms, and that its decomposition was accompanied by a net release of mineral N. When samples of the 3 soils (Urrbrae, Walpeup and Longerenong) were incubated under conditions promoting rapid mineralization (i.e. at c. pF 2 and 35°C) for up to 17 weeks, the resultant loss of LF-N accounted for 25-100% of the mineral N formed from the soil organic matter. As the LF accounted for only 3-27% of the total soil N, it would appear that it was a relatively labile fraction. During a 4-week incubation period the LF-N appeared to mineralize from 4-40 times more rapidly than did the remainder of the soil N, although this relative net mineralization rate varied with soil type, agronomic history and the duration of incubation.

Partly humified plant residues (the LF) thus appear to be a labile part of the soil N, and may represent an important component of the mineralizable N reserves of these soils. However both LF and more humified material appear to be involved in the accumulation and release of N in these soils.

V STUDIES OF THE DYNAMICS OF LF-N IN SOME FIELD SOILS UNDER PASTURE-WHEAT ROTATIONS

The fertility of Australian wheat soils is largely dependent on the organic matter reserves accumulated whilst the land is under pasture. As the contribution of the LF to the reserves of mineralizable N in such soils is not known, it has been investigated in 3 soils of contrasting texture under F-W and P-F-W rotations.

(A) Methods

(i) Collection of field samples

1. Details of trials sampled

The long term rotation trials sampled were located at the Waite Institute, Adelaide (S.Aust.); the Mallee Research Station, Walpeup (Vict.); and Longerenong Agricultural College, Dooen (Vict.). These trials are referred to as C1, MR2, and LR2 respectively.

a) Climate

Brief details of some of the principal features of the climate experienced at each site are given in Table 37. Generally the distribution of the rainfall received at each site shows a pronounced winter-spring maximum, although summer storms may contribute appreciable amounts in some seasons. For both Victorian centres the period studied included successive relative wet (1964) and dry (1965) years.

TABLE 37. SUMMARY OF CLIMATIC DATA FOR TRIAL SITES

Site	Mean Elevation (m)	Mean Air Temp. * Min Max	Approx. Growing Season Duration	Rain (cm)	Annual Rainfall (cm)
1. Waite Inst., S.Aust.	130	W: 7.9, 14.6 S: 15.3, 26.6 (42 years)	April to October	1966: 46.2 mean (42 years): 48.2	64.3 62.1
2. Walpeup, Vict.	100	W: 5.0, 14.8 S: 14.4, 29.7 (12 years)	April to October	1964: 31.9 1965: 19.5 mean (36 years): 21.0	40.0 27.5 31.7
3. Longerenong, Vict.	150	W: 3.7, 13.5 S: 11.9, 28.2 (19 years)	May to November	1964: 46.1 1965: 26.5 mean (56 years): 28.2	53.1 35.3 41.6

* W = winter (June-August), S = summer (December-February).

b) Soils

The soils examined are listed in Table 38 with some analytical data and references to more complete descriptions.

TABLE 38. TYPICAL PROPERTIES OF SOILS AT TRIAL SITES

Trial	Group*	Texture	Depth (cm)	g/100g OD Soil		pH	C:N
				<2μ	$CaCO_3$		
C1	Red-brown earth	fine sandy loam	0-6.3	19	nil	5.2	c. 14
MR2	Solonised brown	sand to sandy clay loam	0-10	10-25	0.1-0.4	6-7	c. 11
LR2	Grey soil of heavy texture	clay	0-10	c.50	0.1-2	7-8	c. 12

* Stephens (1962); see also Appendix C1

pH is of 1:2 (w/v) suspension in 0.01 M $CaCl_2$.

c) Design and management

Details of the design, management and botanical composition of the pastures at the 3 sites are given in Appendices C2 and C3.

2. Sampling procedures

C1: Samples were collected at regular intervals throughout the 1966 growing season and subsequent summer. Each sample was a composite of 8 random cores taken with a Coile sampler (Coile 1936)

from the surface 6.3 cm of plots at various stages of a range of rotations. The apparent (bulk) density of each sample was calculated in the usual manner. The samples were air dried (40°C), ground and sieved <2 mm (larger plant fragments and stones were rejected), and stored in air-tight containers at room temperature.

MR2, LR2: Samples were collected during the last 3 months of 1964, and subsequently at frequent intervals throughout 1965 from immediately after the opening rains until the following autumn. Each sample was a composite of 30 individual c. 2.5 cm diameter cores from the surface 10 cm of plots representing all stages of both F-W and PPFW rotations. Subsamples of the field moist soils were either preserved immediately in 0.08 M $CuSO_4$ solution for subsequent extraction and estimation of their mineral-N content, or air dried, ground and stored as above. Further details of the sampling procedures followed, and of the method used for the estimation of apparent (bulk) densities from the weight and moisture content of each 30 core sample are given in Appendix C4.

(ii) Analytical procedures

These were based on those developed during the earlier stages of this investigation, and are briefly as follows.

1. Light fraction

This was separated as described in Chapter III and Appendix A.

2. Nitrogen

a) Total N: Total soil N (N_t) was determined by dry combustion in a Coleman Nitrogen Analyser (Method B11) corrections being made where necessary for mineral-N. The N content of LF samples was determined by a micro-Kjeldahl technique (Method B14), and the amount of organic N in the LF (LF-N) calculated from the relationship:

LF-N (ppm) = 100 (% LF in soil)(% N in LF)

b) Mineral-N: The total mineral N (N_i, = $(NH_4^+ + NO_3^-)N$) content of the soils at sampling (field N_i) was determined by steam distillation of N Na_2SO_4 equilibrium extracts (soil:solution ratio c. 1.5; shaken for 1 hour) with MgO and Devarda's alloy (Appendix C).

3. Incubation studies

25.0 g samples of <2 mm soil (from MR2 and LR2 only) were moistened to c. pF 2, and incubated in closed polystyrene containers for 4 weeks at 35°C in the dark. No additional aeration was required. Duplicate sets of samples (± incubation) were analysed for total mineral-N (N_i) and LF-N (Appendix C5), and hence the decrease in LF-N (ΔLF-N) and the mineral-N released on incubation (ΔN_i) calculated.

Mineralizable-N was taken as the difference between the total mineral-N content of the soil at sampling (field N_i) and after incubation (final N_i). Except for some samples, notably those obtained from LR2 during the summer and autumn of 1965 from

recently-fallowed 4-course plots where there was an appreciable increase in N_i between sampling and the commencement of incubation in the laboratory (i.e. due to air-drying and storage), mineralizable N was closely approximated by ΔN_i.

4. Calculation of results

a) The data obtained from these analyses was calculated both on the basis of the proportion of the variate concerned in the soil analysed (e.g. mg LF-N/kg OD soil), and on the actual quantity contained in the weight of soil present per unit area to the depth sampled (e.g. kg LF-N/ha 10 cm). The values adjusted for the bulk (apparent) density (BD) provided the better basis of comparison as appreciable differences in bulk density were found both between different plots and within the same plot (e.g. sampled at different times of the year). Typical values for the 3 soils are given in Table 39 (see also Appendices D1 and D2).

TABLE 39. MEAN BULK DENSITY VALUES FOR TRIALS SAMPLED

Trial	Depth (cm)	Approx. BD (g OD Soil/cc)			kg OD Soil/ha in Sampled Depth
		Consolidated		Cultivated	
		Pasture	Wheat		
C1	0-6.3	1.45	1.30	1.25	= 0.635 BD x 10^6
MR2	0-10	1.45	1.45	1.25	= 1.015 BD x 10^6
LR2	0-10	0.9-1.2	0.9	0.7-0.9	

The Longerenong samples in particular had BD values which were much lower and more variable than those found for the other trials, but these were in generally close agreement with other values obtained for comparable plots (Rooney 1966; pers.comm.).

Where the required BD values were not available, values were estimated either by interpolating from the values found for the same plot from a previous and a subsequent sampling, or from the appropriate mean values as tabulated above.

Such calculations are not entirely satisfactory, but are preferable to comparisons of data expressed on a concentration (mg/kg) rather than absolute (mg/ha) basis. It is likely that the practice of sampling all plots to a constant depth (0-10 cm) could lead to an overestimate of N_t and LF-N in the soil under pasture relative to those under fallow as the LF profile is probably more uniform in fallow plots than in pasture plots. Under pastures plant residues accumulate particularly in the surface few cm of soil (Russell 1960; Clarke et al. 1967) and following cultivation would be redistributed through the ploughed layer. Skene (1966, 1967) and Henzell, Fergus and Martin (1967) have described some approaches to the problem of estimating the accumulation of organic matter under pastures, but have not considered the problems associated with sampling cultivated plots.

b) In order to group the data for the evaluation of e.g. the overall effects of a change of rotation phase, it was sometimes

necessary arbitrarily to assign data from a given sampling to a particular phase of the rotation concerned.

The date of transition between successive phases of a rotation was taken to coincide either with the first cultivation (for plots being fallowed or sown), or for pasture plots with the opening autumn rains.

(iii) Statistical analyses

1. Evaluation of errors applicable to MR2 and LR2 data

Complete analysis of all the samples collected from both trials would have required more time than was available. Samples from one replicate only of each trial were therefore analysed, as it was considered that this would serve to indicate the general nature of the relationship between changes in LF-N and mineralizable N in these soils. Consequently, as most of the data obtained are derived from single analyses of composited samples from one replicate of each trial, appropriate estimates of the errors applicable to between plot (treatment effects) and within plot (seasonal effects) comparisons were required. The derivation of these is described in Appendix D4.1, and the results are summarized in Table 40.

2. Relationships between total, LF and mineralizable N

These were studied by conducting simple regression analyses on appropriately pooled data from the 3 soils examined, with particular emphasis on the influence of soil type, rotation and stage of rotation. Seasonal trends included in these results were disregarded.

LF = Light fraction (SG <2.0)

N_t = Total organic N in sampled depth of soil

LF-N = Total organic N in LF separated from unincubated sample

ΔLF-N = Loss of LF-N

ΔN_i = Mineral-N released

} on incubation

adjusted for actual seasonal variations in bulk density

LSD = Approx. least significant difference for between plot comparisons, the values in parenthesis applying to ratios of values to be compared.

ND = not determined.

TABLE 40: SUMMARY OF ERROR ESTIMATES FOR MR2 and LR2 DATA

Trial	MR2			LR2		
Error Estimate	LF g/100g Soil	LF-N kg/ha 10 cm	ΔN_i kg/ha 10 cm	LF g/100g Soil	LF-N kg/ha 10 cm	ΔN_i kg/ha 10 cm
a) SE of a single determination on a 30-core composite	0.05	15	ND	0.04	14	ND
b) LSD (p=0.05) for:						
1. Within plot comparisons of data:						
(i) from 2 different samples,	0.16	47	ND	0.12	20	ND
(ii) averaged from each of successive phases of rotation.	0.11	32	ND	0.08	14	ND
2. Between plot comparisons of data:						
(i) from 1 determination per plot,	0.18	(1.74)	(1.75)	0.15	(2.56)	(6.05)
(ii) averaged:						
from 1 season only	0.15	(1.57)	(1.58)	0.10	(2.16)	(4.35)
from all samples	0.08	(1.27)	(1.27)	0.07	(1.52)	(2.25)

Derivation of estimates is given in Appendix D4.1. Values in parenthesis are applicable to ratios of values to be compared.
SE = standard error
LSD = least significant difference
ND = not determined.

(B) Results and Discussion

The results discussed below are derived from various plots of the C1 trial and from one replicate each of the MR2 (Replicate 2) and LR2 (Replicate 1) trials. Samples from Replicate 1 of MR2 were not used extensively as some plots had other trials superimposed on them. For the studies outlined in Chapter V, A(iii)2 and Chapter V, B(i)2, the results obtained from the incubation of samples from the other two replicates of each trial (viz: MR2, May and August 1965; LR2, June and August 1965) were also included (see Table 46).

(i) Mean levels of total, LF and mineralizable N

1. Effect of soil type and rotation

a) LF-N

The mean values for the total soil organic N (N_t) LF and LF-N contents of plots at each site during different stages of all rotations sampled are given in Table 41.

The amounts of LF-N were generally higher in the Urrbrae loam (C1) samples than in the other two soils. However the relative amounts present in different rotations followed a constant pattern, continuously cropped plots containing less than plots under rotations including a pasture phase (viz. 3-course (PPW), 4-course (PPFW) or 6-course (PPPPWW)). A plot under continuous grass-clover pasture contained the highest amounts - some 33 metric tons of LF (500 kg N)/ha 6.3 cm. This represented 26% of the soil N. The

TABLE 41. MEAN LF, TOTAL N, LF-N AND MINERALIZABLE N CONTENTS OF THREE SOILS

Trial	Depth (cm)	Rotation and Phase	LF (metric tons per ha)	N_t	LF-N (kg N/ha)	ΔLF-N	ΔN_i	$\frac{LF\text{-}N}{N_t}$	$\frac{\Delta LF\text{-}N}{\Delta N_i}$
MR2 (Rep.2)	0-10	Continuous FW:F	5.4	453	68	9	23	0.15	0.39
		Continuous " :W	6.3	507	91	7	23	0.16	0.30
		Continuous PPFW:P_1	8.6	628	124	22	36	0.20	0.61
		" " :P_2	9.4	647	151	25	47	0.23	0.53
		" " :F	9.8	711	165	22	50	0.23	0.44
		" " :W	8.3	597	123	12	36	0.21	0.33
LR2 (Rep.1)	0-10	Continuous FW:F	1.1	647	18	6	24	0.03	0.25
		" ":W	1.0	647	18	7	18	0.03	0.39
		Continuous PPFW:P_1	2.2	934	44	21	57	0.05	0.37
		" " :P_2	4.4	1061	80	34	77	0.08	0.44
		" " :F	3.8	1054	77	30	74	0.07	0.40
		" " :W	2.5	1004	53	20	63	0.05	0.32
C1	0-6.3	Continuous FW:W	15.4	755	141	ND	ND	0.19	ND
		Continuous W	17.8	937	177	ND	ND	0.19	ND
		Continuous PPW:P_2	19.6	1200	210	ND	ND	0.18	ND
		" " :W	21.5	1250	270	ND	ND	0.22	ND
		Continuous PPFW:P_2	22.4	1232	259	ND	ND	0.21	ND
		" " :F	19.3	1220	240	ND	ND	0.20	ND
		" " :W	16.2	1047	200	ND	ND	0.19	ND
		Continuous PPPPWW:P_4	21.8	1315	260	ND	ND	0.20	ND
		" " :W_1	23.0	1155	280	ND	ND	0.24	ND
		" " :W_2	25.4	1220	350	ND	ND	0.29	ND
		Continuous P	32.6	1926	497	ND	ND	0.26	ND
LSD (p=0.05)		MR2	1.2	ND	(1.27)	ND	(1.27)	ND	ND
		LR2	0.7	ND	(1.52)	ND	(2.25)	ND	ND

proportion of the soil N contained in the LF was similar for the different rotations, despite relatively large differences in total N.

The trends were similar for the Walpeup sand and Longerenong clay, despite the effects of successive wet and dry years. The sand contained 5-10 metric tons of LF (70-154 kg N/ha 10 cm), representing 15-23% of the total soil N, whilst the corresponding figures for the clay (1-4 metric tons LF (20-80 kg N)/ha 10 cm, 3-7% of N_t) were much lower. For both soils the amount of LF-N was significantly* higher in 4-course than in 2-course plots. Within the 4-course plots there was a significantly lower amount of LF-N in plots under wheat compared to plots under pasture. Rather unexpectedly no significant decrease in mean LF or LF-N content was observed in 4-course plots fallowed from pasture at any of the 3 sites. This may be due to a systematic overestimate of the BD and thus the absolute amounts of N_t and LF-N/ha in the fallow plots, due to the greater probability of compaction occurring during sampling of fallow than of pasture plots. Alternatively, it is possible that confounding effects of simultaneous accretion and loss processes may be involved, the gradual comminution of larger (<2 mm) fragments of incorporated pasture residues (by soil fauna and/or the normal decomposition processes) tending to offset losses of LF material initially present under the pasture prior to its cultivation. Consequently although the mean net change in LF-N might be small during the fallow phase, decomposition of LF material could still be contributing

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* 5% level of significance is used throughout text, and is based on errors estimated in Appendix D4.1.

significantly to the soil reserves of mineralizable N.

The amounts of LF-N accumulated after more than 18 years of comparable rotations were in the sequence loam>sand>clay, whilst the corresponding levels of soil N were in the sequence loam>clay>sand. Thus the relative rates of accretion and loss processes must differ markedly between these soils, the Urrbrae loam in particular tending to attain a relatively higher equilibrium level of both total and LF-N.

b) Mineralizable N

Detailed incubation studies were confined to samples from the two Victorian trials. Although the mean values presented in Table 41 mask some appreciable seasonal fluctuations in mineralizable N (see p.167) some general patterns were nevertheless apparent. For both trials, ΔN_i was significantly higher in 4-course than in 2-course plots. Some 7% of the total N of the 4-course plots under second year pasture or fallow mineralised during incubation compared to only 3-5% in the 2-course plots. Within the 4-course plots those under wheat or first year pasture tended to mineralize less N during incubation but only in the Walpeup samples was this difference significant.

Significantly more LF-N disappeared during incubation of samples from 4-course plots, the absolute magnitude of the loss being generally similar for comparable plots in both trials. The

lability of the LF-N relative to the humified-N may be estimated if the net loss of LF-N accounted for a corresponding amount of the mineral N released during incubation. This assumes that the decomposition of the LF does not promote immobilization of N.

In the sand, the loss of LF-N during incubation accounted for 30-60% of the mineral-N released, even though it only represented 15-23% of the soil N. The relative contribution of the LF-N was apparently greatest in 4-course plots, notably those under pasture. In the clay, the loss of LF-N accounted for a somewhat lower proportion (25-45%) of the mineral N released than in the sand, but as the LF only represented 3-7% of the soil N it was apparently relatively more labile in the clay than in the sand. The difference between the 2-course and 4-course rotations was less pronounced than in the sand.

In both soils it is evident that more humified fractions of the soil N represent an appreciable proportion of the mineralizable N reserves, but that the relative importance of these two fractions varies with soil type, and possibly also with rotation.

2. Relationships between total, LF and mineralizable N

The results of the regression analyses are summarized in Table 42 (see also Appendix D4.2). For both trials N_t and LF-N were highly significantly ($p < 0.001$) correlated, the relation being better for 4-course ($r=0.7$) than for 2-course ($r=0.5$) plots. Although both N_t and LF-N were significantly correlated with the

Footnotes to Table 42 (continued)

When LF-N = 0, $N_t = \bar{X} - \bar{Y}/a$, where $\bar{X}$ and $\bar{Y}$ are the respective mean values of N_t and LF-N. The error in the constant (b) is given by

$$\sqrt{\left[\frac{SE(\bar{Y})}{\bar{Y}}\right]2 - \left[\frac{SE(a)}{a}\right]2}$$

, where $SE(\bar{Y})$ and SE(a) are the standard errors of $\bar{Y}$ and the slope (a) respectively.

3) * = $p<0.05$
** = $p<0.01$
*** = $p<0.001$

NS = not significant
ND = not determined.

TABLE 42. RELATIONSHIPS BETWEEN TOTAL N, LF-N AND MINERALIZABLE N FOR C1, MR2 AND LR2

Trial	Rotation	Depth (cm)	Correlation (unit=kg N/ha)			
			r	Constant (SE)	r	Constant (SE)
			X=LF-N and Y=N_t		X=ΔN_i and Y=N_t	
C1	(1) Continuously cropped	0-6.3	NS	ND	ND	ND
	(2) P-W rotations		0.35*	55(43%)	ND	ND
	(3) all rotations		0.85***	351 (9%)	ND	ND
MR2	2 course	0-10	0.53**	276(45)	NS	ND
	4 course		0.71***	263(41)	0.53***	376(43)
	whole trial		0.80***	270(21)	0.68***	317(25)
LR2	2 course	0-10	0.48**	487(42)	NS	ND
	4 course		0.73***	630(42)	-0.37*	650(27)
	whole trial		0.87***	525(22)	0.46***	678(39)

1) Data from the various C1 rotations sampled (see Appendix D2) were grouped as follows:
   (i) those involving continuous cropping (i.e. W or FW rotations),
   (ii) those including a pasture phase in the rotation (i.e. 3-course (PPW), 4-course (PPFW) or 6-course (PPPPWW) rotations), and
   (iii) all rotations sampled (i.e. (i) + (ii) + continuous P rotation).

2) The results of the regression analyses tabulated above were derived from an analysis of the fit of the pooled data to the simple linear relation

$$Y = a X + b$$

where a and b were both constants.
The values of the constant (b) presented above, together with the appropriate standard error (SE), were calculated in the normal manner with the exception of the results from the C1 trial. In this case the values of the constant (b) were calculated as below from the fit of N_t(X) on LF-N(Y), as this gave a better estimate than was obtained from the reverse fit, since more measurements per plot were made of LF-N than of N_t.

results of the incubation tests (ΔN_i and final N_i), there was no significant effect of rotation at either site, except for the 4-course plots at Walpeup. ΔLF-N was also poorly related to the incubation results. Thus either LF-N or ΔLF-N would appear to be of little value for the prediction of mineralizable N in these soils. This may reflect the combined effects of the mineralization of more humified materials, seasonal variations in LF-N and mineralizable N, and also of any net assimilation associated with the decomposition of the LF.

The mean size of the 'inert' pool of N in these soils may also be estimated from these results if it is postulated that, on the average, 'inert' N will not participate in the equilibrium between 'active' N and either LF-N or mineralizable N. The average values of the constants obtained from the highly significant correlations between LF-N and N_t at the 3 sites are reasonably reliable, and in the 2 Victorian trials these values are of similar magnitude to those obtained from the relationship between ΔN_i and N_t. The relative magnitudes of the 'inert' pool of N in these soils is thus apparently LR2 (450-650 kg N/ha 10 cm) > C1 (350 kg N/ha 6.3 cm)> MR2 (250-300 kg N/ha 10 cm), these representing some 50-70%, 30% and 40-50% of their mean total soil organic N reserves respectively (see Table 41). When allowance is made for the relative amounts of LF-N in these soils, then the size of the "active, non-LF" soil N pool is apparently <40% of the total soil N. This pool may well include proteinaceous material

of microbial origin.

It is also of interest to note that the apparent size of these 'inert pools' is less than that estimated by using the data of Table 41 and assuming that the "active" pool is approximated by the appropriate mean value of ΔN_i. These latter estimates are undoubtedly too large, as the proportion of the total soil N mineralized during the 4 week incubation period is probably appreciably lower than that mineralized in the field during a growing season. Also the incubation data refers to net changes only, and the relative rates of the mineralization and immobilization processes involved probably differ for soils incubated in the field or in the laboratory.

Whilst the above estimates are at best very approximate, they serve to illustrate further the profound influence of soil texture and environment on the apparent distribution of soil N between these active (LF and non-LF) and passive pools. It is highly unlikely that the system is as simple as assumed above, as active and passive pools probably exist within the LF, as well as within various other fractions of the soil N.

(ii) Seasonal changes

1. Field levels of mineral N (LR2, MR2)

Changes in the mineral N content of cultivated soils provide little information as to the seasonal patterns of N mineralization, due to the confounding effects of plant uptake, microbial assimila-

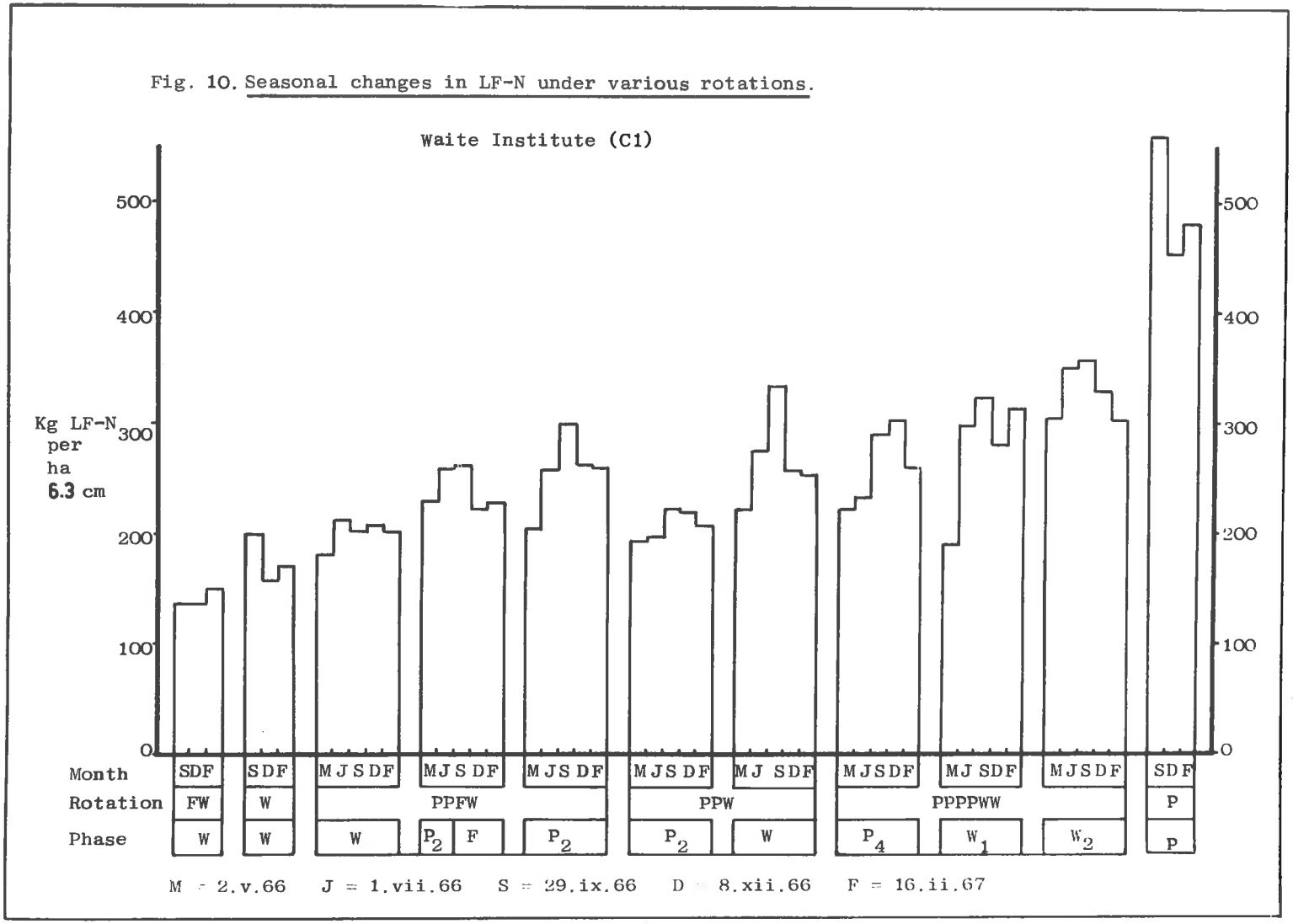
tion, leaching and denitrification. However as this data is of agronomic interest the mean levels under the various treatments are tabulated in Appendix D1. In general the levels in the clay were higher than those in corresponding plots on the sand. During the growing season <5 ppm ($NH_4^+ + NO_3^-$)N was usually present, appreciable accumulation only occurring in plots under fallow on crop stubble during late summer to early autumn. There was also some accumulation during this period in plots under pasture, indicating a decrease in plant uptake and/or microbial assimilation. There was no evidence of rapid net mineralization following the cultivation of the pastures (cf. Penman 1949), although this may simply reflect the dominance of immobilization associated with decomposition of the pasture residues.

2. LF-N and mineralizable N

a) C1 samples

The amounts of LF-N present under the various rotations sampled are shown in Fig. 10 and tabulated in Appendix D2. There were appreciable differences in the amounts accumulated in the various plots, the extremes being represented by a three-fold difference in the amount of LF-N after 19 years continuous pasture as opposed to more than 40 years of continuous cropping. This confirmed the earlier results discussed on p. 57 . Some evidence for seasonal variations in LF-N was also obtained, as it tended to increase during spring in plots under pasture or crop, and to

Fig. 10. Seasonal changes in LF-N under various rotations.



decline during the summer months. There were also indications of a loss of LF-N in the 4-course plot after fallowing, although the actual change in LF-N may have been greater, as the estimated BD of the cultivated plot could have been too high (see p. 158).

b) LR2 and MR2 samples

Further information relating to the actual seasonal changes within individual plots under successive stages of 2-course and 4-course rotations at these two sites is presented in Figures 11, 12 and 13, and is tabulated in Appendix D5. The period covered includes the spring of 1964 and the whole of the 1965 growing season from immediately after the opening rains until the following autumn. After an initial ploughing to 10 cm the plots were cultivated as indicated. The mineral N content at sampling (field-N_i) is shown for each plot, together with the amount of LF-N present before incubation and the total mineral N (final N_i) present after incubation. (This was principally NO_3^--N, as <1 mg NH_4^+-N/kg soil was present after incubation). Mineralizable N is given by the difference between the field-N_i and the final-N_i (incubation) curves. The appropriate LSD (p=0.05) values for comparison of any two estimates of LF-N within a given plot (see Table 40) are shown on each graph.

Although samples were not collected during the 1964-5 summer and autumn, the net changes over this period were evidently generally small. The apparent dramatic increase in LF-N during the late spring of 1964 in the 4-course plot under pasture at

Fig. 11. Seasonal changes in LF-N and mineralizable N

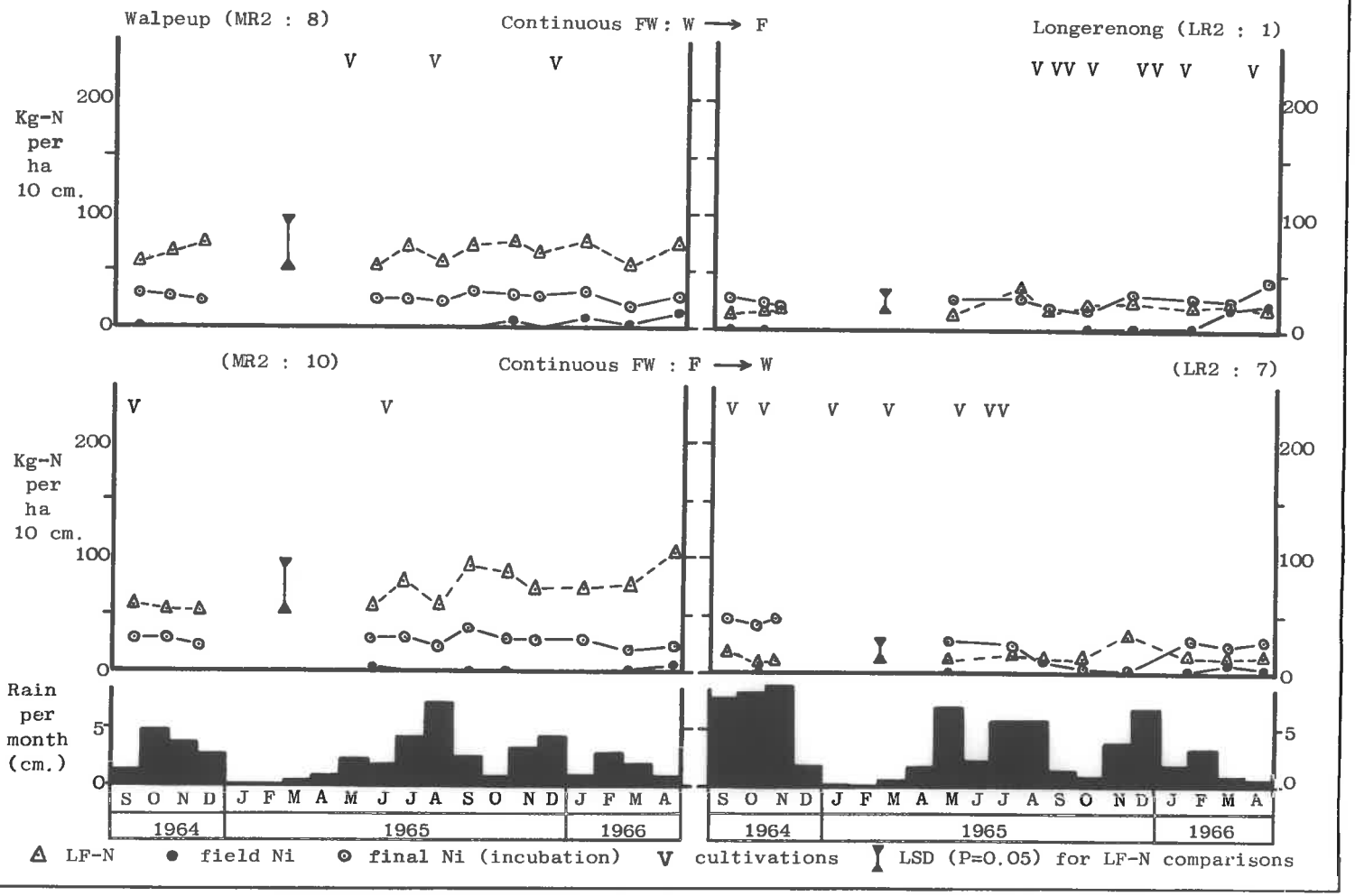


Fig. 12. Seasonal changes in LF-N, mineralizable-N; Longerenong

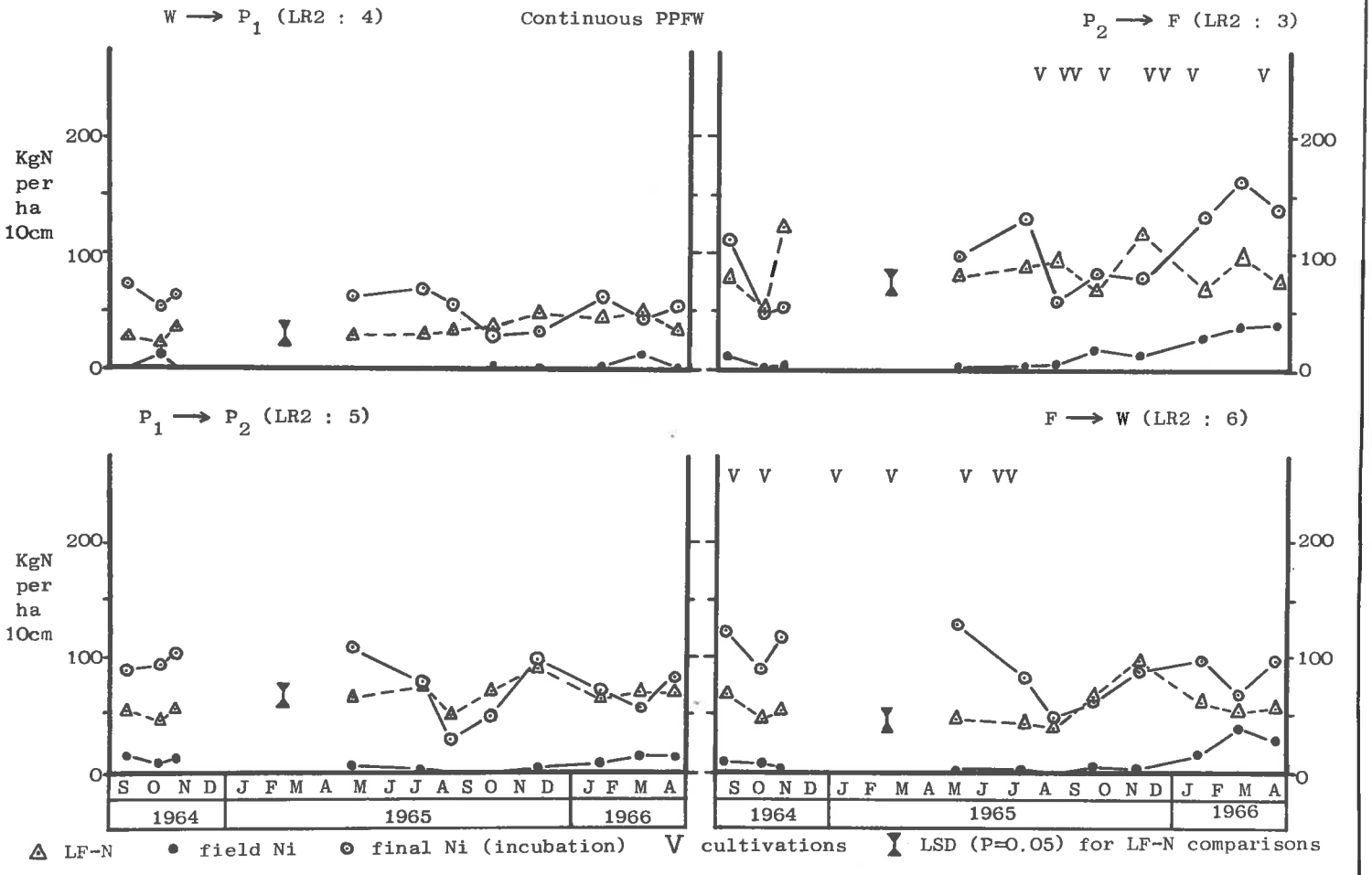
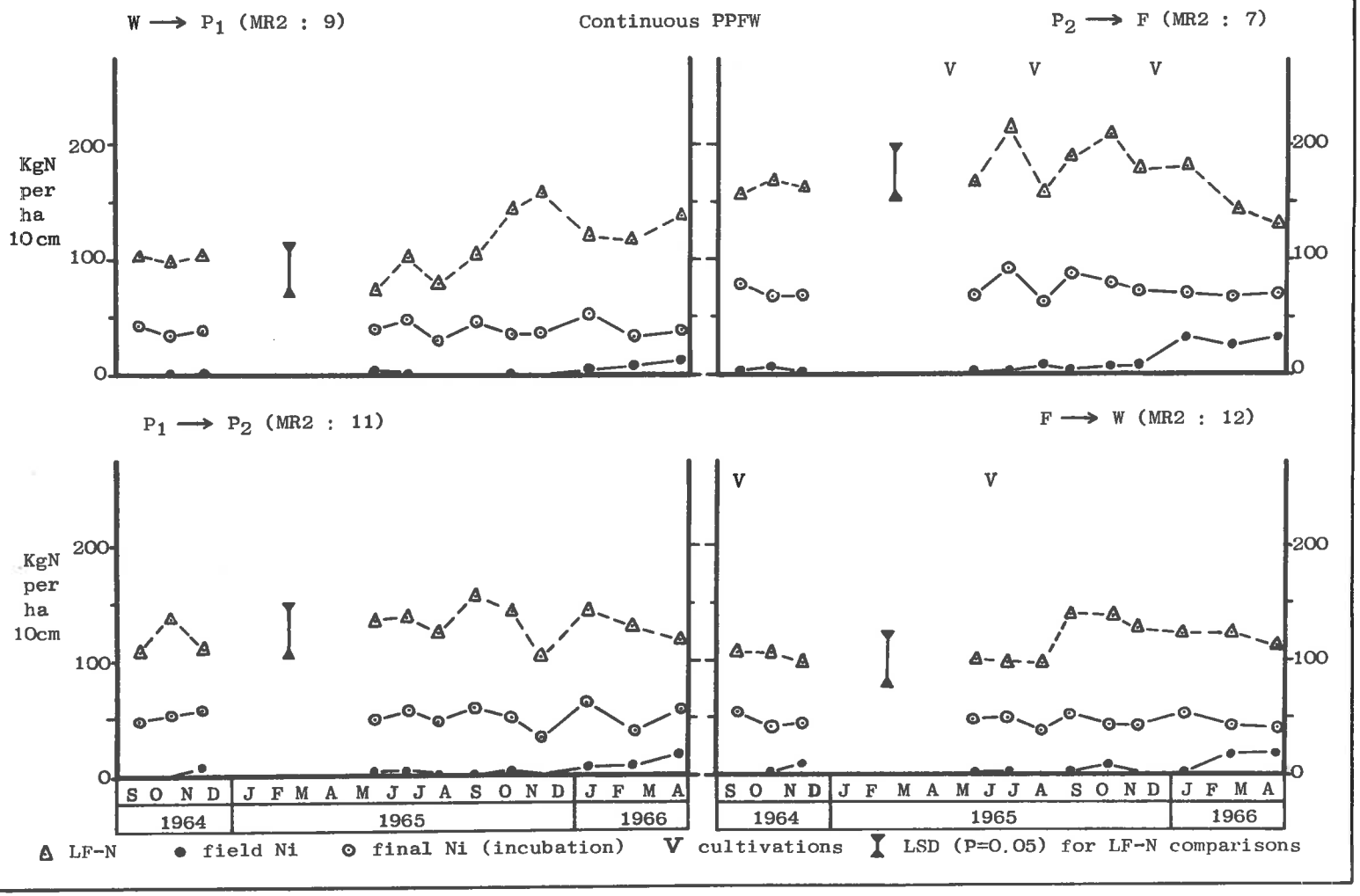


Fig. 13. Seasonal changes in LF-N and mineralizable-N; Walpeup.



Longerenong (LR2:3) is probably an artifact, reflecting the incorporation of an unusually high amount of plant top growth material in the sample as a result of a slashing treatment some 3 weeks before the December sampling. This effect was not apparent in 1965, possibly due to seasonal conditions being less favourable for the growth of the annual grasses controlled by this treatment.

(1) Field N_1: As discussed above, the field levels of mineral N were higher in the 4-course than in the 2-course plots, the levels in the clay tending to be slightly higher than those in the corresponding plot on the sand. Appreciable accumulation, particularly in 4-course plots under fallow or wheat, occurred mainly in late spring and summer.

(2) LF-N: The relative amounts of LF-N were very different for the two soils, plots under 2-course and 4-course rotations on the sand containing respectively 3-4, and 2-3 times as much LF-N as did corresponding plots on the clay. In both soils the 4-course plots contained greater (2 to 3-fold) amounts than did 2-course plots.

Seasonal variations in the amount of LF-N were generally greater in 4-course than 2-course plots. In both trials no significant changes in LF-N were detected in 2-course plots, although there was a tendency for some increase under the wheat crop. While the patterns within the 4-course plots exhibited some general similarities, striking differences were also evident. In the sand there was a significant increase during late spring and summer under

the first year pasture, which was followed by a period of loss; however no significant increase was observed under a second year pasture during this period. The converse was true for corresponding plots on the clay, a significant increase in LF-N only being detected during the second year of pasture. Plots fallowed from pasture also exhibited a different pattern in the two soils, although the changes in the clay may have been partly obscured due to sampling errors resulting from the frequent cultivations. There was some indication of a net accretion of LF-N during the late spring at Longerenong, followed by a period of net loss. The greatest loss of LF-N was observed for the corresponding Walpeup plot, where evidence was obtained of an overall net decrease of some 50-70 kg LF-N during the fallow period. At both sites, particularly Longerenong, there was a tendency for a net accretion of LF-N (up to 50 kg) to occur during late spring and summer under the wheat crop, this presumably reflecting the increased return of root residues as the crop matured. Some indication was also obtained of a subsequent period of net loss in the Longerenong plot.

(3) Mineralizable N: The pattern of seasonal changes in the net amount of mineral N released during incubation differed markedly between the two soils. In the sand there was generally little change in the amount of mineralizable N, except for the 4-course plot in fallow where there was a decrease during the summer period which coincided with the increase in field N_i and decrease in LF-N. On the other hand, in the clay - especially in 4-course

plots - there was a pronounced decrease in mineralizable N during late winter and spring which was followed by a rapid rise during summer, these changes matching the smaller seasonal trends in LF-N. The reason for these seasonal variations in mineralizable N is not clear, but changes in amount or composition of substrates other than the LF may be involved, as preliminary investigations suggested that the composition of the LF (as assessed by its sugar-C:LF-N ratio) did not dramatically change over this period (Table 30). However, as only a few analytical results were available, further evaluation of such changes in the composition of the LF was not possible.

The relative magnitudes of the LF-N and mineralizable N curves also differed for the two soils. In the clay they were generally of comparable magnitude, and exhibited similar patterns of seasonal variation. However in the sand, the amount of LF-N was generally 2 to 3 times greater than the mineralizable N reserves, and except in the 4-course fallow plots, displayed different patterns of seasonal variation.

(C) Summary

The influence of soil type and rotation on the mean levels of LF-N, and on the seasonal changes in these levels, have been examined for a loam (Red brown earth), a sand (Solonised brown soil) and a clay (Grey Soil of heavy texture). The LF represented from 20-30% of the total soil N (N_t) in the loam. Corresponding values for the sand and clay were 15-23% and 3-7% respectively. In general plots under rotations including a pasture phase contained more LF-N

than did plots under a F-W rotation. Plots under pasture contained significantly greater amounts of LF-N than did similar plots under wheat. No significant decrease in LF-N was detected in 4-course plots fallowed from pasture, presumably because this was offset by the large addition of plant material when the pasture was cultivated.

The size of the pools of 'inert' N in these 3 soils was estimated from the relationship between LF-N and N_t. These pools represented some 30%, 40-50% and 50-70% of the mean N_t of the loam, sand and clay respectively.

Changes in the amounts of LF-N in plots on the sand (Walpeup) and clay (Longerenong) were examined in relation to changes in mineralizable soil N. Seasonal variations in LF-N and mineralizable N occurred on both the sand and the clay, these being greatest in 4-course plots. In general the changes in LF-N were relatively small, and not obviously related to the pattern of seasonal changes in mineralizable N. As the carbohydrate to nitrogen ratio of the LF did not appear to change markedly during the period studied, changes in the amount or composition of substrates other than the LF may have caused the observed fluctuations in mineralizable N. The nitrogen to carbohydrate ratio of the LF was sufficiently high for its decomposition to be accompanied by a net release of mineral N.

In general a greater proportion of the soil N was released during incubation of samples from 4-course than from 2-course plots; typical values were 7% and 3-5% respectively. The greatest loss of

LF-N during incubation occurred in 4-course plots. In the sand this loss of LF-N accounted for 30-60% of the mineral N released. The corresponding value for the clay was 25-45%, despite the fact that the LF represented only 3-7% of the total soil N.

Thus the original hypothesis that the LF was a more labile part of the soil N was confirmed, although clearly other fractions of the soil N also contributed to the reserves of mineralizable N.

Although LF-N thus provides a measure of the available N accumulated in a soil, its value for prediction purposes varies markedly with the soil group concerned. Further studies designed to locate and identify the labile N in both the LF and the remainder of the soil N are therefore required.

VI DISCUSSION AND CONCLUSIONS

This thesis has examined the hypothesis that relatively undecomposed plant residues accumulated in soils under pastures represent an important reserve of available N. It is based on the observation that there is a rapid loss of plant residues from soil following the cultivation of virgin or old pasture land (Saunder and Grant 1962) and that this is usually associated with an appreciable net mineralization (Sears 1953; Wetselaar 1967) or immobilization (Nye 1950) of soil N. The practical implications of this phenomenon are of considerable agronomic significance, because although this loss of organic matter is generally associated with a decline in soil fertility, ley farming practices may reverse this trend. However, although legume-based pastures are known to result in the net accretion of organic N, little is known of the form in which this available N accumulates, or of the manner and efficiency of its release to subsequent wheat crops.

Many empirical procedures for estimating the size of the available N pool in soils have been proposed (Bremner 1965c) but these have generally been of limited use, and have contributed little information as to the nature of the labile N. A more rational approach would be one based on the recognition that there are 3 broad but biologically distinct groups of organic materials in soils. These are the relatively stable amorphous colloidal material ("humus"), living microbial and faunal tissue (the "biomass"), and recently added plant and animal residues. It is

probable that each of these groups contains relatively labile and relatively resistant (passive) components, and it is the contribution of the labile N of plant residues to the pool of active soil N which has been examined in the present study.

Soil humic substances are synthesised during the microbial degradation of plant lignins and tannins, and are only slowly decomposed in soil (Dubach and Mehta 1963; Felbeck 1965). Similar materials are also synthesised by some soil fungi (Martin, Richards and Haider 1967). The resistance of the humified N to decomposition has been investigated by many authors, and has generally been ascribed to its complex chemical form (Bremner 1965a). However it is possible that it includes peptide or protein components which may be decomposed by the soil microflora (Martin et al. 1967; Simonart, Batistic and Mayaudon 1967; Ladd and Brisbane 1968).

The biomass represents a highly active part of the organic matter. Although there are few estimates of the contribution of faunal tissue to the active soil N (E.W. Russell 1961), microbial tissue has been shown to account for up to 2-3% of the total organic matter. This fraction contains relatively active (zymogenic) and passive (autochthonous) components which turn over at different rates (Jenkinson 1966a). The size, composition and activity of this microbial population is largely governed by the frequency and rate at which fresh plant residues are added to the soil.

The amount of relatively undecomposed plant residues in soils, and its contribution to these active and passive pools of

soil N has not previously been examined critically due to the lack of a suitable procedure for estimating the amount of each material in soils. The physical fractionation of organic matter into "free" (LF) and "bound" fractions appears to be more likely to yield biol-
(2) ogically significant fractions than the chemical fractionation procedures used by most other investigators.

The usefulness of such physical fractionation procedures depends on their ability to quantitatively separate the humified-N in the clay-organic complex (Greenland 1965b) from the non-humified or only partly humified N in plant residues not intimately associated with other soil minerals. The densimetric fractionation procedure developed during the present study yields reproducible amounts of "free" material (the LF), although the completeness of the separation can only be inferred from the failure to recover additional LF on repeated fractionation of soil. The LF has the physical form of plant residues, is generally light coloured, and has a C:N ratio generally greater than that of the soil. Its carbohydrate and amino acid content and composition are more like those of plant tissue than those of humic acids. It appears to contain relatively little (c. 10-20% (ash-free basis)) humified material, and its ash content was largely accounted for as phytoliths. The LF is therefore regarded as consisting essentially of partly humified plant residues. The distribution of the microbial tissue between the LF and the humified N is not known, but it is probable that it is present both in and on the LF particles and also intimately associated with soil

colloids.

In order to evaluate the contribution of the LF-N to the soil reserves of available N, it is necessary to demonstrate that it may be decomposed by soil microorganisms, that its decomposition may result in the net release of mineral N, and that in field soils under pasture-fallow-wheat rotations there is a relationship between changes in the amount of LF-N and mineralizable soil N.

Incubation studies indicated that the LF is a potentially available substrate for soil microorganisms, and that it is decomposed at a rate intermediate between that of fresh plant tissue and humic acid. Its decomposition was usually accompanied by the release of mineral N, and over a 17 week period the LF-N was mineralized up to 4-40 times faster than the remainder of the soil N (Table 36).

The results obtained from a detailed study of 3 soils under fallow-wheat and pasture-fallow-wheat rotations show that the mean levels of both LF-N and mineralizable N vary with soil type and rotation, but in a less dramatic manner than anticipated. In particular the decline during the fallow phase of the 4-course (PPFW) rotation (Table 41 and Figs. 10-13) is smaller than expected, presumably because it was offset by the incorporation of large amounts of organic residues at the start of the fallow. Nevertheless the changes in the amount of LF-N are proportionally greater than those in total soil N. However for the Walpeup and Longerenong soils the seasonal variations in LF-N are generally much smaller than those of the readily mineralizable N, although the loss of LF-N during a 4 week

incubation period accounts for an appreciable proportion (30-60% and 25-45% respectively) of the mineral N released. The estimated relative decomposition rates of the LF-N and humified N during incubation (Table 36) also suggest that the LF is the more labile, and that its contribution to the readily mineralizable soil N is relatively greater than that of the humified N. Despite the relative lability of the LF, it does not dominate the nitrogen release pattern after short term pastures, although it may well do so after longer pasture periods.

Although the LF represents relatively readily decomposable material, it clearly does not represent the whole of the "active-N" in soils. Jansson (1958) has estimated that this may constitute up to 10-15% of the soil N. Microbial tissue accounts for at most 3% of the soil N. If the mean loss of LF-N during incubation (Table 41) is taken as an estimate of the labile LF-N, then this would account for a further 1-3% of the soil N. However this is certainly an underestimate of the contribution of the labile LF-N to the pool of readily mineralizable N, as immobilization of N derived from both the LF and from the soil humus almost certainly occurs during incubation. In addition mineralizable N as estimated by the incubation procedure used would also underestimate the actual N available during a season for crop growth.

The results of this study suggest that the LF-N is not uniformly labile, nor the humified N uniformly passive. It is

therefore not surprising that although LF-N is a better measure of readily mineralizable N in these soils than is total soil N (Table 42), it still only accounts for 25-60% of the mineral N formed from the soil organic matter. Nevertheless the partial success of this physical fractionation of soil N compares favourably with attempts to obtain a measure of available N by chemically fractionating the total soil organic N. After a series of extensive studies of such methods, Keeney and Bremner (1966b) concluded that "any chemical method of assessing the availability of soil N based solely on determination of a particular form of soil N will prove unsatisfactory".

The physical fractionation technique developed in this study may therefore be of value as a preliminary step in many studies of soil organic matter (cf. Oades and Swincer 1968). Its usefulness for future studies aimed at determining the size and chemical nature of the active N within both the LF and the remainder of the soil N will necessitate both a critical evaluation of the efficiency with which it recovers plant material from soil, and further chemical fractionation of both the LF and the soil residue, e.g. into humic acid, fulvic acid and humin fractions and/or the ammonium-N, amino acid-N, and hexosamine-N fractions of Bremner (1965b). Such studies would be facilitated by the use of ^{15}N and/or ^{14}C labelled plant material. This could be decomposed with soil for various periods under controlled conditions, and the distribution of the label between the mineralized C or N and the remaining LF and non-LF material determined. The relative turnover rates of these various

stages of decomposition could also be estimated e.g. the turnover rate of LF-N (cf. Kuo and Bartholomew 1963) could be estimated from changes in the size and ^{15}N:^{14}C ratio of the exchangeable NH_4^+-N pool during decomposition of ^{15}N-labelled plant material in the presence of a nitrification inhibitor (e.g. 2-chloro-6(trichloromethyl) pyridine). Such studies may also provide information as to the physical distribution of the biomass between the LF and the clay-organic complex. Plant residues of various types could be used (e.g. grass or legume tops or roots), as the relative rates of decomposition of these materials will be largely determined by their chemical composition and physical form (e.g. particle size), and in practice most LF material in field soils is derived from mixed grass-legume swards, together with cereal residues. Another facet of this study of the role of the LF in nutrient cycling under pasture-wheat rotations would be an investigation of its contributions to the S and P cycles.

However it should be noted that such studies assume that the rate of decomposition of the various components of the soil organic matter largely reflects the complexity of their chemical composition. The physical accessibility of these substrates is also obviously important (e.g. Rovira and Greacen 1957; Waring and Bremner 1964b), and will vary not only with the soil type and its management, but also with the size, composition and activity of the soil faunal population.

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APPENDIX A: PROCEDURE FOR SEPARATION OF LF FROM SOILS

1) Preparation of soil samples and bromoform solution

a) Soil samples: Approximately 10 g of air dry, <2 mm soil was subsampled by making random 'scoops' (c. 1 g each) from the bulk sample, and gently ground by hand so that all visible aggregates passed a 0.25 mm (60 mesh) sieve. Larger sand or plant particles were thoroughly mixed with the finer material, and the sample dried either for at least 16 hours at 70°C or for several days over P_2O_5 in a vacuum oven at 40°C and <1 mm mercury pressure. 5.0 (±0.02) g subsamples of the dried soils were then weighed into glass vials, and replaced in the oven for at least several hours before fractionation.

b) Densimetric liquid

(1) SG: The density of the bromoform-petroleum spirit solution was determined by weighing in a tared 100 ml volumetric flask, and was adjusted by trial and error to 1.97 ± 0.01 g/cc.

(2) Surfactant concentration: This was adjusted to c. 0.1% (w/v) 'Aerosol OT'. The surfactant concentration was estimated by the modified methylene blue assay procedure of Jones (1945). A 1.0 ml portion of the densimetric liquid was pipetted into a 10 ml volumetric flask and made up to volume with petroleum spirit. 1.0 ml aliquots were used for subsequent comparison with 1.0 ml portions of a standard 0.01% (w/v) solution of 'Aerosol OT-100' in petroleum spirit. Each 1.0 ml portion was pipetted into a 100 ml separating funnel containing 20 ml distilled water, 3 drops 6 N HCl, 1.0 ml

methylene blue solution (0.1% w/v in water) and 20 ml chloroform. After shaking vigorously for 20-30 seconds, the emulsion was allowed to break and the chloroform layer drained off. This was then washed by reshaking with 20 ml distilled water, and after standing the chloroform layer was again drained off, filtered through a small pad of dry absorbent cotton wool in a thistle funnel, and some of the filtrate transferred to a 1 cm glass cuvette. The extinction of the unknown and standard solutions at 650 mμ were determined in a Unicam SP600 Spectrophotometer within 1 hour of colour development. As the extinction vs concentration plot was linear over the range 0-0.10 mg 'Aerosol OT', the approximate surfactant concentration was determined from the ratio of the extinctions of the standard and unknown solutions.

Thus

$$\%(w/v) \text{ 'Aerosol OT'} = 0.1(E_{650} \text{ unknown})/(E_{650} \text{ standard})$$

The appropriate volume of a stock solution of the surfactant (10% w/v in petroleum spirit) was then added to the bromoform solution.

2) Fractionation procedure

The dry soil sample (5.0 g) was removed from the oven, immediately placed in a 250 ml tall-form beaker, and c. 145 ml of bromoform solution added. The beaker was fitted into a cold-water jacket, and the suspension sonified for 3 minutes with a Branson S-75 Sonifier (75 watts, 20 ± 0.6 Kc/sec), the probe tip being positioned c. 2 cm from the bottom of the beaker. The beaker was removed from its water jacket, the outside carefully dried with paper tissue,

and the suspension quantitatively transferred into a 250 ml pyrex (M.S.E.) centrifuge tube. After balancing, the tubes were sealed with rubber caps and allowed to stand for at least 30 minutes before being centrifuged at c. 1,600 R.C.F. for c. 1 hour in a centrifuge (M.S.E. ' Major') fitted with a swing-out head. The supernatant and LF were carefully decanted into a beaker, and the walls of the centrifuge tube washed down with a fine jet of bromoform solution. The bulked supernatant and washings were then filtered through a 5μ 'Millipore' filter, washed with a few ml petroleum spirit, and dried for 15-30 min at 70°C. The LF was brushed off the filter onto a tared piece of aluminium foil, care being taken with the Longerenong and Urrbrae samples to avoid disturbing the 'skin' of clay immediately adjacent to the filter surface. The LF was weighed to the nearest 0.05 mg; whence

$$\text{g LF/100 g OD (70°C) soil} = 0.02\ (\text{mg LF}).$$

The bromoform solution was filtered through a fluted filter paper (Whatman No. 1) and reused after readjustment of its density and surfactant content as above. The 'Millipore' filters could be reused at least once. When required, the bromoform solution was purified by distillation under reduced pressure from calcium oxide. The bromoform solutions were best stored in dark glass bottles over metallic copper turnings.

APPENDIX B: METHODS USED FOR THE CHARACTERIZATION OF SOIL AND LF SAMPLES

1. Inorganic material in the LF

METHOD B1: Ash

10-30 mg LF were weighed into a tared, previously ignited silica crucible, and ignited for 1-2 hours in a muffle furnace at 550°-600°C. The residue was generally either brick red or a pale grey to brown in colour. After cooling in a vacuum desiccator over P_2O_5 the crucible was reweighed.

METHOD B2: Residue after digestion with H_2O_2

A suitable quantity of LF was repeatedly digested at 70°C with successive 5 ml portions of 30% (v/v) H_2O_2, until digestion was judged to be complete - usually after several overnight treatments. The residue was repeatedly washed by centrifuging with distilled water to remove soluble material, and finally dried at 70°C.

2. Organic material in the LF

a) Solubility in alkali

Estimates of the amount of humic materials in samples of LF (LF-'humus') or soil (soil-'humus') were based on comparisons of the optical density (at 260 mμ or 450 mμ) of 0.5 N NaOH extracts with the extinction at the corresponding wavelength of the purified humic acids isolated from the surface 6.5 cm of the permanent pasture plot (C1:29) on Urrbrae fine sandy loam.

METHOD B3: LF-'humus'

This was estimated by shaking 10-15 mg LF with 25 ml 0.5 N NaOH at room temperature for 6 hours, centrifuging (c. 2,000 R.C.F., 4-5 min) then diluting 20 ml of the supernatant to 50 ml with 0.5 N NaOH. The optical density of this solution at 260 mμ(E_{1cm} 260 mμ) was determined in 1 cm silica cells in a "Uvispek" or "Shimadzu" spectrophotometer against an appropriate blank. The 'humus' content was calculated on the assumption that the extracted materials were similar to those extracted from Urrbrae fine sandy loam and having a specific extinction coefficient ($E^{1\%}_{1cm}$ 260 mμ) of 398 (Dr. A.M. Posner 1964, pers.comm.), i.e. that

% 'humus' in LF = 196 (E_{1cm} 260 mμ)/mg LF

METHOD B4: Micro-method for LF-'humus'

This technique was developed to permit rapid estimation of the 'humus' content of small amounts of LF. Interference due to absorption at 260 mμ by aromatic non-humic materials was avoided by measuring the E 450 mμ (Roulet, Mehta, Dubach and Deuel 1963) of an alkali extract of the LF. A 1-5 mg sample of LF was shaken with 5.0 ml 0.5 N NaOH at 70°C for 30 minutes, the mixture cooled in ice, centrifuged (c. 2,000 R.C.F., 2-3 min), and the E_{1cm} 450 mμ of the supernatant (diluted as required) determined in a Unicam SP600 spectrophotometer.

The value of $E^{1\%}_{1cm}$ 450 mμ of humic acids isolated in a similar manner from Urrbrae fine sandy loam (sample 9, Posner 1966)

was determined by measuring the values of E_{1cm} 450 mμ of a series of 8 solutions containing from $5x10^{-4}$ to $2x10^{-2}$ g humic acids (dried over P_2O_5) per 100 ml 0.5 N NaOH. The mean value was 74 (range 68-77). Whence

% 'humus' in LF = 67.6 (dilution factor)(E_{1cm} 450 mμ)/mg LF

METHOD B5: Soil-'humus'

This was estimated by shaking 2.0 g soil (<0.25 mm, oven dried at 70°C) with 20 ml 0.5 N NaOH at 70°C for 24 hours, centrifuging (c. 61,500 R.C.F., 30 min, 15°C), then diluting the supernatant as required. Whence, by analogy with Method B2 above,

% soil-'humus' = 0.135 (E_{1cm} 450 mμ)(dilution factor)

METHOD B6: Alkali-soluble carbohydrate in LF

The extent of decomposition of some of the LF samples obtained during the development of the densimetric fractionation method (Chapter III,B) was also estimated by determination of their alkali-soluble carbohydrate content.

A 4.0 ml portion of the alkali extract obtained by Method B4 was pipetted into a clean 15 ml centrifuge tube, acidified with 0.30 ml 8 N H_2SO_4, centrifuged (swing-out head, c. 2,000 R.C.F., 4-5 min), and 1.0 ml of the fulvic acid preparation analysed by the anthrone procedure of Oades (1967a).

b) METHOD B7: C.E.C. of LF

A 50 mg sample was washed repeatedly with 0.1 N HCl, washed free of excess acid (until the washings were chloride-free), oven dried at 70°C, reweighed and suspended in 0.1 N KCl and titrated to pH 7.0 against 0.1 N KOH with a 'Titrigraph' (Radiometer, Copenhagen).

c) Organic carbon

METHOD B8: Dry combustion

A catalyst tube was prepared as described by Mann and Saunders (1952), and the CO_2 evolved estimated volumetrically by absorption in 0.1 N $Ba(OH)_2$ (0.1 M in $BaCl_2$), and back titration with 0.1 N HCl to a phenolphthalein end point (Rixon 1948). Generally duplicate analyses using 10-20 mg LF, or c. 0.2 g soil were made. Recoveries were checked with sucrose (c. 10 mg).

METHOD B9: Combustion in induction furnace

A Fisher carbon induction furnace was used as described by Jackson (1958), except that the absorption tube was packed with both granular (12-20 mesh) self-indicating soda-lime (B.D.H. 'Carbosorb') and magnesium perchlorate. This latter reagent was placed in the outlet arm of the U-tube to prevent significant losses of water vapour occurring during absorption of the CO_2. Usually duplicate analyses were made, using c. 30-50 mg LF or c. 1.0 g soil. The results generally agreed to within 1-2% of the determined values. Where necessary corrections were made for carbonate.

METHOD B10: Carbonate determination

Carbonate was determined gravimetrically by collecting the CO_2 evolved on treatment of 5-10 g of soil with 50 ml of c. 2 N HCl, the absorption tube being packed as above (Method B9). Recoveries were checked with A.R. grade $CaCO_3$ (c. 0.1 g of OD powder).

d) Organic N

METHOD B11: Soil N

Although some initial determinations were made using a modification of the standard macro-Kjeldahl procedure of Bremner (1960) generally soil N was determined by dry combustion in a Coleman Nitrogen Analyser 11 (Model 29A). 1-2 g of air dry soil was thoroughly mixed with c. 3 times its volume of ignited copper oxide powder before combustion at c. 860°C (Stewart, Porter and Beard 1964). The nitrometer was charged with a non-frothing KOH (c. 12 N) solution made by treating the hot alkali with barium hydroxide (Pregl 1937). In order to obtain consistent blank values (usually c. 100-200 µl N_2) it was found necessary to modify the recommended procedure and to regenerate the wire form CuO catalyst (B.D.H., L.R. grade) by washing with 5% (v/v) acetic acid, rinsing thoroughly with distilled water on a 0.5 mm sieve to remove fines, and then igniting at c. 900°C for 1-2 hours in a muffle furnace. Duplicate analyses usually agreed to within 5% of the determined value. Where necessary corrections were made for mineral N.

LF-N: This was determined by a micro-Kjeldahl procedure using either

(a) METHOD B12:

This was essentially a modification of the procedure proposed by McKenzie and Wallace (1957). 20-100 mg LF were digested with 1.7 g Cu-Se catalyst (Bremner 1960) and 3.0 ml of concentrated sulphuric acid for approximately 1 hour. After dilution of the hydrolysate, an aliquot was steam distilled with excess 40% (w/v) NaOH in a Markham still. The ammonia was collected in 5 ml of a 4% (w/v) boric acid solution (containing 20 ml/l of a 3:1 (v/v) mixture of 0.1% (w/v) methanolic solutions of bromocresol green and methyl red), and titrated with 0.010 N $KH(IO_3)_2$.

or (b) METHOD B13:

Subsequently this technique was further modified to facilitate the rapid routine analysis of small samples of LF material. Samples of LF material (5-20 mg, ± 0.05 mg) were weighed into dry pyrex test tubes (c. 15 x 2 cm), 1.0 g Cu-Se catalyst (Bremner 1960) added via a long-stemmed thistle funnel and 1.0 ml concentrated sulphuric acid added from a burette. The samples were digested for 20 minutes after clearing, the test tubes being fitted with cold finger condensers reaching to within c. 5 cm of the bottom of the test tubes. This was found to give satisfactory refluxing of the small volume of digest, and permitted the use of gas-heated micro-digestion units. After cooling, the digests were cautiously diluted with a few ml distilled water, and then quantitatively transferred to a Markham still. The sample was distilled after c. 1 g heavy MgO

powder had been cautiously added, the ammonia liberated being collected in 5 ml of boric acid solution and titrated with 0.005 N acid (see Appendix C6(b)). This procedure gave recoveries which were at least 98% of those obtained with the Coleman Nitrogen Analyser for a sample of wheat flour, and was found to be satisfactory for samples containing only c. 100 µg N.

e) Amino acids

6 N HCl hydrolysates were analysed by automatic amino acid analysers by either of the following procedures:-

METHOD B14: (after Dr. A.C. Jennings)

LF and humic acid (sample 11R, Posner 1966) samples isolated from the permanent pasture plot on Urrbrae loam were hydrolysed for 20 hours at 110°C in an evacuated sealed tube.

Total N in the samples and hydrolysates was determined by the micro-Kjeldahl procedure of McKenzie and Wallace (1957).

Determination of amino acids: The method of Moore and Stein (1954) was used; an automatic 9-column amino acid analyser (Simmonds and Rowlands 1960; Simmonds 1962) being used for the determinations. The procedures followed have been described in detail by Jennings and Morton (1963). Four analyses were made of each hydrolysate.

METHOD B15:(after Dr. J.R.E. Wells)

The amino acid composition of LF samples isolated from 3 soils before and after incubation (4 weeks, 35°C, c. pF 2) was determined as follows. Their total N contents were determined by Method B13.

Hydrolysis: 15-60 mg LF were weighed into dry pyrex test tubes (c. 15 x 1 cm), 6 ml of 6 N HCl added, the tubes constricted and oxygen-free nitrogen bubbled through the suspensions for several minutes. The tubes were immersed in liquid nitrogen, evacuated with a rotary oil pump and sealed under vacuum. After hydrolysis at 105°C for 22 hours, the hydrolysates were evaporated to dryness by vacuum distillation at <45°C, and the remaining acid removed by repeated additions of distilled water and rotary evaporation to dryness. The residues were finally extracted with distilled water and made to 25 ml.

Ninhydrin analyses: The hydrolysates were analysed for total (NH^+ + α-amino)N by a ninhydrin method (Dr. P.E. Stanley 1967, pers.comm.). 1.0 ml samples, containing 0-4 µg N, were mixed with 0.5 ml 0.1 M citrate buffer (pH 4.80) and 1.2 ml of freshly prepared reagent (recrystallised indane trione hydrate (1.0% w/v) in redistilled methyl cellosolve containing distilled water (3.5% w/v) and L(+)ascorbate (c. 35 mg/100 ml solution)). Glutamic acid standards were included with each batch, all analyses being performed in duplicate. A loose fitting glass stopper was placed in each test tube, and the reaction mixtures heated for 10 minutes in a boiling water bath, and then cooled rapidly in tap water. 3.0 ml of 60% (v/v) ethanol was added and after standing for at least 10 minutes the extinction was measured at 570 mµ in 1 cm cuvettes. The colour was stable for several hours, and if required solutions containing up to 100 µg N could be quantitatively diluted after colour development with 60% ethanol.

The (NH_4^+ + α-amino)N contents of each sample were then calculated and expressed as a percentage of their total N.

Determination of amino acids: Suitable portions of the hydrolysates (containing 40-50 µg N) were evaporated to dryness, taken up in 0.5-1.0 ml of a sucrose solution (12.5% w/v) containing 0.2 µ moles nor-leucine/ml (as an internal standard) and 0.1 N with respect to HCl, and analysed by ion-exchange chromatography with a Technicon Amino-acid Auto-analyser.

f) Carbohydrates

METHOD B16: The total carbohydrate contents of samples of LF and humic acid (Sample 12, Dr. A.M. Posner) from the permanent pasture plot on Urrbrae loam (C1:29, 0-10 cm) were estimated and compared with that of undecomposed plant tissue (dried and ground mature tops of Phalaris tuberosa L.) The samples were hydrolysed with 1 N H_2SO_4 for 8 hours at 100°C, and the total neutral sugar content of the samples determined colorimetrically using the phenol-concentrated sulphuric acid method of Dubois et al. (1956). After desalting the hydrolysates with Amberlite MB1 resin, qualitative identification of the component neutral sugars was achieved by one dimensional descending paper chromatography, using ethyl acetate:pyridine:water, 40:11:6 (v/v) (Jermyn and Isherwood 1949). The chromatograms were developed with acetone-$AgNO_3$ and alcoholic NaOH (Trevelyan, Procter and Harrison 1950).

METHOD B17:

Subsequent studies involved acid hydrolysis either under reflux or in sealed tubes as follows:

Reflux hydrolysis: The procedure followed was essentially that of Oades (1967a). 5-50 mg LF were treated with 0.5 ml 72% (v/v) H_2SO_4 for 2 hours at room temperature, the acid concentration reduced to 1 N and the samples refluxed for 16 hours.

Sealed tube hydrolysis: The reflux method was adapted for batch analyses as follows. 2-50 mg LF were pretreated with 72% (v/v) H_2SO_4 for either 2 (or 16) hours at room temperature, the acid diluted to 1 N and the tubes sealed and heated in an oven at 105°C for either 16 (or 5) hours respectively.

Total neutral sugars in the hydrolysates were estimated by the anthrone procedure of Oades (1967a), the charcoal clarification pretreatment generally being omitted. Individual neutral sugars were determined by gas-liquid chromatography (GLC), after the sugars had been reduced to the corresponding alcohols and acetylated under acid conditions (Oades 1967b). The relative proportions of the various sugars were estimated from the areas under the appropriate peaks on the GLC traces, assuming an equal detector response for the various alditol acetates.

Comparison of hydrolysis procedures

Although the data presented in Tables 29 and 30 were derived from sealed tube hydrolysates which gave values for the total neutral sugar contents of the LF samples concerned closely similar to those

obtained when comparable samples were refluxed (Table 32), erratic (low) recoveries were subsequently obtained when other LF samples were hydrolysed in sealed tubes. Low recoveries (45%-90%) were also obtained when 0.5-2.0 mg samples of cellulose powder (Whatman, standard chromatographic grade) were hydrolysed in sealed tubes, whereas hydrolysis of similar samples under reflux gave consistently satisfactory results. Destruction of sugars during the sealed tube hydrolysis must have been involved, as the anthrone procedure used was known to quantitatively estimate soluble polysaccharides. The reason for this destruction is not known, as other authors have found similar sealed tube procedures satisfactory e.g. for the complete acid hydrolysis of polysaccharides (Hirst and Jones 1955, p.286).

In view of these results, the reflux procedure was therefore used for all subsequent studies.

3. Incubation Studies

METHOD B18: Oxygen uptake studies

Warburg manometry was used initially to compare the oxygen uptake rates during the microbial decomposition of various substrates added to a fresh sample of soil from the permanent pasture plot on Urrbrae loam. The substrates included the LF and humic acids separated from the same plots, and dried and ground Phalaris tuberosa tissue.

A fresh sample of the surface soil of the permanent pasture plot on Urrbrae fine sandy loam (C1:29, 0-5 cm, sampled August 1963) was preincubated aerobically for 4 days at 30°C at c. 50% field

capacity and then sieved <2 mm. 1.0 g subsamples were mixed with 100 mg of the required substrate, and quantitatively transferred to calibrated 15 ml Warburg flasks. After adjusting the moisture content of each sample to slightly less than field capacity (c. 25% w/w) the flasks were incubated at 30°C in a water bath without shaking for up to 40 hours. Alkali (0.5 ml of 4 N KOH) was placed on a paper wick in the centre well of each flask to absorb CO_2. The oxygen uptake rates were calculated from the observed gas flux (Umbreit, Burris and Stauffer 1957). Each treatment was duplicated and unamended soil samples were included as controls.

METHOD B19: Mineralization studies

(i) The net release of nitrate during incubation of a similarly prepared soil sample in the presence of various substrates (c. 5% w/w, ash-free basis) was also determined. Amended samples were incubated in triplicate at c. 70% field capacity and 30°C for 14 days. The initial and final nitrate contents of acid extracts (Lewis 1961) were determined by the chromotropic acid method of Clarke and Jennings (1965) and the net nitrification compared with unamended controls.

(ii) Subsequent studies were confined to the measurement of the net changes in mineral N and LF-N when unamended samples were aerobically incubated for 4 weeks at c. pF2 and 35°C (Appendix C5).

APPENDIX C1: SOILS AT TRIAL SITES

(i) Waite Institute (Red Brown Earth; C1)

The following outline of the major features of Urrbrae fine sandy loam, the dominant soil type occurring on the C1 trial, is based on the descriptions of Piper (1938) and Aitchison, Sprigg and Cochrane (1954).

Drainage: moderate

Geology: Developed on argillaceous alluvial or colluvial material derived principally from the moderately calcareous Pre-Cambrian shales and slates.

Landform: Alluvial-colluvial fan from foothills of Mount Lofty Ranges.

Elevation: c. 100 metres above sea level

Relief: gently sloping

Vegetation and Land Use: Originally open savannah woodland, commonly cultivated for wheat.

Profile description: a typical profile (Type RB3) would be:

A 0-(20 to 35) cm. Brown, greyish-brown or light reddish-brown (e.g. 5 YR 5/3-3/2) loam to fine sandy loam. Gravel may be present in limited quantities in the lower A horizon, which may exhibit slight bleaching.

B_1 (20-35) to (82-98) cm. Red-brown (e.g. 2.5 YR 3/4-3/6) medium to heavy clay. Structure is variable in the upper B horizon, but is characteristically prismatic lower. Clay readily fractures into strong polyhedral structural units with distinct surface sheen.

B_2C (82-98) to (250-275) cm. Brown medium clay with visible lime; lime decreasing and becoming more uniform with depth giving a light-brown clay with pockets of lime. Structure friable and granular; clay exhibits well developed fissures or cleavage planes.

When dry the entire profile shows marked cracking. Wide vertical cracks occur in the B horizon, and a horizontal crack between the A and B horizons.

ANALYTICAL DATA (Profile U151; Piper 1938)

Depth (cm)	pH	Chlorides (NaCl) (%)	$CaCO_3$ %	org.C %	N %	Particle Size				Exchangeable Cations				
						CS %	FS %	Si %	C %	sum	Ca	Mg	K	Na
										m-equiv/100g				
0-10	6.0	0.013	0.02	1.32	0.103	2	44	35	18	7.0	4.5	1.4	0.8	0.3
10-23	5.8	0.008	trace	1.07	0.091	2	41	34	22	7.4	4.9	1.7	0.7	0.2
23-46	6.3	0.008	0.01	0.76	0.084	1	26	23	47	15.1	8.9	4.8	1.0	0.5
46-69	6.7	0.010	nil	0.67	0.077	1	19	19	60	22.9	13.2	7.8	1.3	0.7
69-91	7.2	0.012	0.13	0.66	0.073	1	19	19	59	25.1	15.1	8.0	1.4	0.7
91-114	8.4	0.015	4.80	0.41	0.046	1	24	28	41	25.0	17.7	5.6	1.0	0.6
114-137	8.6	0.016	3.96	0.30	0.035	1	25	30	37	23.6	15.9	5.9	1.0	0.8

Clay Mineralogy: Predominantly interstratified illite-montmorillonite, with some kaolinite.

(ii) Walpeup (Solonised Brown Soils: MR2)

The principal soil types present at the Mallee Research Station, Walpeup, have been described and mapped by Newell (1961). The trial is located in the S.W. corner of Paddock 6, on a mosaic of three soil types (Walpeup sandy loam, Type D and Type E). Although no profile descriptions or analytical data are available which relate specifically to samples from the trial site, the range of morphological features and of physical and chemical properties of these soils is indicated by more recent data relating to profiles of two of the three major soil types occurring at the station. This information is summarised below, based on unpublished data kindly provided by Mr. J.K.M. Skene of the Victorian Department of Agriculture, Melbourne.

A. Midmallee sandy loam (Site 10, Newell 1961; resampled May 1966)

Drainage: external-slow, internal-moderately fast.

Geology: Aolian calcareous clayey and sandy deposits laid down during the Pleistocene and since resorted.

Landform: In swales between dunes.

Elevation: 98 metres above sea level.

Relief: very gently sloping or flat.

Micro-relief: flat.

Vegetation and Land Use: originally low sclerophyll forest, now sparse introduced annual pasture following cultivation for wheat.

Profile description:

A	0-7 cm	Dark brown (5 YR 3/4-4/4) sandy clay loam; weak platey to fine subangular blocky structure; slightly hard dry, friable moist; abrupt boundary with
B_1	7-10 cm	Dark reddish brown (2.5 YR 3/5) medium clay; moderate medium subangular blocky structure; porous peds; hard dry, sticky and plastic wet;
	10-20 cm	as above, abrupt boundary with
B_{Ca}	20-30 cm	Reddish-brown (5 YR 4/5-4/4) medium clay; structure and consistence as above; light amount of soft $CaCO_3$.
$B_{Ca}C$	30-60 cm	as above but more $CaCO_3$.
D	60-90 cm	Greyish brown (7.5 YR 5/5-6/5) medium clay; moderate soft and panned $CaCO_3$.
	90-120 cm	Brown (5 YR 5/4-6/4) heavy clay; light soft and panned $CaCO_3$.
	120-164 cm	Brown (5 YR 4/6) heavy clay; decreasing $CaCO_3$.
	164-180 cm	Transitional
	180-210 cm	Reddish brown (5 YR 4/7-5/7) very weakly mottled with yellow-grey, sandy clay; slight $CaCO_3$.

B. Ridge Sand (Site 2, Newell 1961; resampled May 1966)

Drainage: external-rapid, internal-rapid.

Geology: as for Midmallee sandy loam.

Landform: Dune mass

Elevation: 116 metres above sea level

Relief: On crest of high, moderately sloping N-S ridge comprised of hummocks and hollows

Micro-relief: flat

Vegetation and Land Use: Tall (12 metres) sclerophyll shrub woodland. Similar adjacent areas are commonly cultivated for wheat.

Profile description:

A_{11} 0-4 cm Brown (5 YR 3/4-4/4) loamy sand; very weak crumb structure; much coarse organic matter; abrupt boundary with

A_{12} 4-10 cm Brown (5 YR 3/5-4/5) loamy sand; no apparent structure, loose.

10-20 cm as above

A_2 20-24 cm Light brown (5 YR 5/5-6/5) sand; no apparent structure, loose; abrupt boundary with

B_{11} 24-30 cm Dull reddish brown (5 YR 4/6-5/6) light sandy clay loam; weak medium subangular blocky structure; porous peds; slightly hard dry, friable moist;

B_{12} 30-37 cm Dull reddish brown (5 YR 4/8-5/6) sandy clay; structure and consistence as above; slight $CaCO_3$.

B_{Ca} 37-42 cm Dull reddish brown (5 YR 4/4-5/4) sandy clay; hard dry; light soft and concretionary $CaCO_3$.

42-60 cm as above, very weak structure;

C 80-90 cm Light brown (7.5 YR 5/6) sandy clay; decreasing $CaCO_3$.

90-125 cm as above

D 125-210 cm Light reddish brown (5 YR 5/6-6/6) sandy clay; slight $CaCO_3$;

210-240 cm Weakly mottled yellow-grey and light brown sandy clay; slightly hard dry; friable moist, sticky wet; slight soft $CaCO_3$, light concretions.

ANALYTICAL DATA: WALPEUP PROFILES

Depth (cm)	pH 1:5 (H_2O)	pH 1:2.5 ($CaCl_2$)	Total Soluble Salts %	Chloride (NaCl) %	$CaCO_3$ %	Org.C %	N %	Particle Size CS %	FS %	Si %	C %	Exchangeable Cations Total	Ca	Mg	K	Na
													m-equiv/100 g			
				A. MIDMALLEE SANDY LOAM												
0-7	8.0	6.7	0.06	0.01	0.08	1.12	0.09	27	46	6	19	15.7	12.0	4.7	1.7	0.28
7-10	8.2	6.7	0.05	0.01		0.92	0.08	16	27	21	33	31.5	19.3	9.4	2.3	0.93
10-20	9.0	7.3	0.08	0.01	0.61	0.69	0.06	14	23	19	40	34.3	28.0*	13.5*	2.1	1.9
20-30	9.4	7.6	0.12	0.03	8.0		0.06	13	21	18	38	27.3	30.2*	15.4*	1.7	4.3
30-60	9.6	7.8	0.28	0.13	21			9	17	17	34					
60-90	9.5	7.9	0.43	0.20	33	0.18	0.02	14	15	7	39	23.5	27.6*	17.4*	1.7	9.0
90-120	9.4	7.9	0.51	0.27				11	16	11	42					
120-164	9.2	7.6	0.62	0.33	7.8		0.02	11	16	12	49					
180-210	9.3	7.7	0.51	0.28				33	21	5	34					
				B. RIDGE SAND												
0-4	6.6	5.4	0.03	0.003		2.23	0.15	29	58	1	9	10.5	6.7	2.8	0.6	0.02
4-10	6.9	5.8	0.03	0.005		1.16	0.09	33	54	3	9	9.8	6.9	2.3	0.7	0.01
10-20	7.9	6.7	0.03	0.008			0.06	33	51	4	11	9.8	6.3	2.8	1.2	0.13
20-24	7.9	6.4	0.05	0.02	0.52	0.34	0.02	35	57	2	6					
24-30	8.9	7.4	0.22	0.10			0.03	30	49	2	18	13.6	4.3	6.0	0.8	3.2
30-37	9.3	7.6	0.26	0.13				30	43	5	20					
37-42	9.4	7.8	0.35	0.17	9.7	0.53	0.05	24	38	5	22	15.5	24.6*	11.1*	1.2	5.1
42-60	9.5	7.9	0.34	0.17				20	34	6	19					
60-90	9.6	8.0	0.35	0.17	17.3	0.16	0.02	24	37	5	16	14.3	16.0*	11.0*	1.0	4.4
125-210	9.7	7.9	0.39	0.16	4.6			26	37	4	26					
210-240	9.6	7.9	0.39	0.18	6.5			29	37	1	26					

*Includes significant soluble Ca^{++} and Mg^{++} from the $CaCO_3$ present in these horizons.

(iii) Longerenong (Grey Soil of Heavy Texture; LR2)

This trial is located in Paddock 27 at the Longerenong Agricultural College, Dooen. Although there is no published soil survey relating to this area, unpublished data relating to a site approximately 1 km N.E. of LR2 has been kindly made available by Messrs. G. Blackburn, A.R.P. Clarke, and J.G. Pickering of the Soils Division, C.S.I.R.O., Adelaide. This information is summarised below, and is considered to satisfactorily indicate the morphology and properties of the local soil.

Drainage: External-impeded, internal-moderate

Geology: Quaternary clays

Landform: Plain

Elevation: c. 150 metres above sea level

Relief: gently sloping (c. 6 metres in surrounding 1 km).

Micro-relief: very gently sloping or flat

Vegetation and Land Use: Originally grassland, now introduced grasses and weeds. Site seldom cultivated although similar adjacent areas are cultivated for wheat.

Profile description:

A 0-3 cm Dark grey brown (10 YR 4/2) granular self-mulching clay; slight soft $CaCO_3$; diffuse boundary with

3-20 cm Dark grey brown clay with brown (10 YR 5/4) inclusions; sub-angular clods (1-3 cm); friable, slight soft $CaCO_3$; diffuse boundary with

B 10-20 cm Dark grey brown and brown clay; angular clods grading into smaller, friable aggregates; increasing amount

soft $CaCO_3$;

20-30 cm Brown (10 YR 5/3) coarsely mottled with dark grey brown clay; blocky with fine aggregation patches; light soft and occasional concretionary $CaCO_3$ in scattered pockets or veins; many voids;

B-C 30-40 cm as above with occasional grey brown inclusions.

40-50 cm as above

50-60 cm as above, diffuse boundary with

C 60-70 cm Brown (10 YR 5/3) clay; angular clods, friable; occasional clay skins visible; low amounts soft $CaCO_3$.

70-80 cm as above; friable to hard angular clods with visible vertical cracks and distinct clay skins.

ANALYTICAL DATA: LONGERENONG

Depth (cm)	pH 1:5 (H_2O)	pH 1:2.5 ($CaCl_2$)	Total Soluble Salts %	Chloride (NaCl) %	$CaCO_3$ %	Org.C %	N %	Particle Size CS %	FS %	Si %	C %	Exchangeable Cations Total	Ca	Mg	K	Na
													m-equiv/100g			
0-3	8.0	7.1	0.07	0.02	1.4	3.1	0.20	15	17	5	53	34	38	8.6	3.5	0.49
3-10	8.2	7.2	0.06	0.01	1.6	2.2	0.16	14	16	11	50	36	40	11	2.6	0.80
10-20	8.6	7.3	0.07	0.01	4.9	1.0	0.09	9	15	6	59	37	40	4	1.9	2.6
20-30	9.0	7.4	0.11	0.02												
30-40	9.3	7.4	0.13	0.03	4.9	0.5	0.04	10	17	5	58	35	30	17	1.7	7.4
40-50	9.4	7.5	0.19	0.05												
50-60	9.4	7.6	0.26	0.09	4.6	0.3	0.03	10	17	12	52	28	25	18	1.8	11
60-70	9.4	7.7	0.35	0.13												
70-80	9.3	7.8	0.46	0.19	5.4	0.2	0.02	5	15	14	55	35	24	23	2.1	15

Clay Mineralogy: Predominantly illitic; little kaolinite.

APPENDIX C2: DESIGN AND MANAGEMENT OF TRIALS

(1) Design

C1: A non-replicated trial; plot size c. 0.05 ha, with each phase of each rotation studied being represented each year. Trial commenced in 1925, and rotations including pasture were introduced in 1948. Further details are given in the various annual reports of the Waite Institute.

MR2: Randomised block, 3 replications, each plot of c. 0.34 ha. Commenced in 1946 on land that had been intensively cropped for previous 25 years. All phases of both 2-course (FW) and 4-course (PPFW) rotations are represented each year.

LR2: Randomised block, 3 replications, each plot of c. 0.11 ha. Commenced in 1946 on land previously intensively cropped for many years. All phases of both 2-course and 4-course rotations present each year; only 'stubble-burnt' treatment on 2-course plots sampled as this is usual district practice, generally resulting in higher yields and grain protein levels.

(2) Management

C1: Pastures: Sown after opening rains (c. June). Top dressed annually with superphosphate (94 lb/ac; c. 106 kg P/ha). Intermittently and heavily grazed as required (c. 4 periods each of 1 week per year).

Wheat: (c.v. 'Seewari 48') sown after opening rains (c. June) with superphosphate (c. 106 kg P/ha). Stubbles grazed.

Fallow: Ploughed to 8-10 cm in July-August, and subsequently to c. 6-9 cm as required for weed control.

MR2: Pastures: Not sown as hard seed reserves of volunteer medics are adequate, and not topdressed. Intermittently and heavily grazed as required.

Wheat: (c.v. 'Insignia') sown after opening rains (c. May-June) with superphosphate (90 lb/ac; c. 100 kg P/ha). Stubbles grazed.

Fallow: Ploughed to 10 cm in July-August; sown with oats ('covercropped') in September (without superphosphate), and lightly grazed over summer in order to prevent wind erosion. Cultivated after rains in February-March.

LR2: Pastures: Undersown annually (c. July) with wheat crop, and topdressed each autumn with superphosphate (40 lb/ac; c. 45 kg P/ha) Intermittently and intensively grazed. Slashed in November for control of annual grasses.

Wheat: (c.v. 'Olympic') sown after opening rains (c. July) with superphosphate (1 cwt/ac; c. 125 kg P/ha). Stubbles grazed in 4-course plots and burnt (c. March) in 2-course plots.

Fallow: Ploughed in winter to 10 cm and subsequently to c. 6-9 cm as required for maintenance of suitable surface mulch.

APPENDIX C3: BOTANICAL COMPOSITION OF PASTURES

The pastures at these 3 sites were all mixed grass-legume swards, their botanical composition being essentially as follows:

C1: Sown with *Trifolium subterraneum* L (a mixture of 3 commercial strains) and *Lolium rigidum* Gaud; the permanent pasture plot (C1:29) also being sown with *Medicago sativa* L and *Phalaris tuberosa* L. The dominant weed species were *Hordeum leporinum* Link in the long term pasture and *Oxalis pés-cáprae* L and *Avena fatua* L in the rotations lacking a fallow phase (i.e. continuous W, PPW, and PPPPWW).

MR2: Volunteer medics (*Medicago trunculata* Gaertn., and *M. littoralis* Rhode cv. 'Harbinger') with annual grasses (e.g. *L. rigidum*). The major weed was *Chondrilla juncea* L, this being confined mainly to 2-course plots.

LR2: A mixture of sown (*M. trunculata*) and volunteer medics, together with volunteer annual grasses (*L. rigidum* and *H.leporinum*). The predominant weeds other than various annual grasses and some self-sown wheat were *Amsinkia* spp., *Lamium amplexicaule* L, and *Lithospermum arvense* L.

APPENDIX C4: SAMPLING PROCEDURES FOR MR2 AND LR2

The standard sampling procedure of the Victorian Department of Agriculture was used viz: 30 cores per plot were taken to a uniform depth (0-10 cm) with a split tube sampler of internal diameter 1 inch (2.54 cm) or $1^1/_8$ inch (3.18 cm). Each plot was divided into 5 (LR2) or 10 (MR2) equal portions (strata) from each of which 6 or 3 cores respectively were taken. The moist weight of each 30 core composite was recorded, and the sample carefully crumbled by hand so that no lumps larger than c. 1 cm remained. Representative subsamples were obtained by a mixing and quartering procedure. A 100 ± 1 g sample was placed in a polythene jar, preserved with 100 ml 0.08 M $CuSO_4$ and sealed for subsequent determination of its mineral N content (Appendix C6). Another subsample (c. 1 kg) was placed in a sealed polythene bag. All samples were forwarded as rapidly as possible to the laboratory for further processing, the transit time varying from 1-4 days.

A subsample of the field-moist soil was removed for moisture determination (16 hours at 105°C), and the remainder dried at 40°C in a forced draught oven and then mechanically crushed to pass a 2 mm sieve. Larger fragments of organic material (e.g. medic burrs; grass seeds; wheat grain; glumes, straw or stem butts; sheep faecal pellets, etc.) were rejected. The fine earth was then stored at room temperature in polythene bags inside sealed metal cans until required.

The apparent (bulk) density (BD) of each plot was estimated from the following relationship:

$$BD = W/V(1-M/100)$$

where BD = g oven dry soil/cc moist soil,

W = moist weight of 30 core sample,

M = g moisture/100 g OD soil in sample,

V = volume of 30 core sample, assuming no compaction

= 1520 cc for 1 inch sampler and 1930 cc for $1^1/_8$ inch sampler.

The corresponding weight of soil per unit area to the sampling depth was calculated from the relationships:

lb/acre 0-4 in = BD (0.906×10^6)

kg/ha 10 cm = BD (1.015×10^6)

APPENDIX C5: INCUBATION PROCEDURE

a) Method

The air dry, <2 mm composited samples were subsampled by taking random scoops of c. 1 g each, and 25.0 g portions carefully introduced into tared 200 cc clear polystyrene vessels containing an appropriate quantity of distilled water (i.e. 10, 6 and 5 ml for samples of the Longerenong, Urrbrae and Walpeup soil respectively). Each container was gently tapped several times to even up the surface of the sample, fitted with a snap-on lid, and incubated for 4 weeks at 35°C in the dark. A corresponding set of samples was simultaneously stored (air-dry) at -15°C. The moisture content of the air dry soils was determined by oven drying (16 hours, 105°C) separate 2-5 g subsamples.

After incubation the containers were reweighed, the soil thoroughly mixed with a spatula, and a subsample (equivalent to c. 8-10 g oven dry soil) removed, oven dried (16 hours, 70°C) and its LF separated as in Appendix A. The container was reweighed, and the remaining sample (c. 10-15 g oven dry soil) extracted with 50 ml 1 N Na_2SO_4 by shaking for 1 hour on a end-over-end shaker at c. 15 rpm. After standing overnight at 2°C, mineral N in the equilibrium extract was determined by distillation with MgO and Devarda's alloy (Appendix C6).

b) Calculations

These are reported on an oven dry (OD) basis, and incorporate corrections for the volume of water in the sample of

moist soil extracted. The concentration of mineral N (ppm, or mg/kg) was calculated from the relation

ppm mineral N in soil = 70 (J.H.)/(G.I.)

where if A = weight OD soil incubated,

B = weight empty container,

C = " " " + incubated (moist) soil,

D = " " " + soil remaining after subsampling for LF determination,

E = weight moist soil extracted for mineral N

= (D - B),

F = moisture (g/100 g OD soil) in incubated soil,

= 100 (1-(C-B)/A),

then G = g OD soil extracted,

= E(1 - F/100),

and H = total volume of Na_2SO_4 extract

= 50 + (E - G),

I = ml equilibrium extract distilled,

J = titre (ml) 0.005 N acid (corrected for blank).

APPENDIX C6: DETERMINATION OF MINERAL N

a) Extraction

1) Field samples

Immediately after sampling, 100 g subsamples were placed in c. 700 cc polythene containers and preserved with 100 ml 0.08 M $CuSO_4$. Preliminary experiments indicated that even with the heavy calcareous Longerenong soil this ensured the presence of excess soluble copper in the soil slurry for at least 36 hours. The samples were extracted in the laboratory by adding 400 ml of 1.25 N Na_2SO_4 and shaking for 1 hour on an end-over-end shaker. After standing overnight the supernatants were filtered through fluted Whatman No. 1 filter papers and stored at room temperature. Excess soluble copper was present in all cases. Some hours prior to distillation c. 100 ml portions were clarified by shaking with c. 1 g heavy MgO powder and allowed to settle. This was found to be essential, as otherwise soluble copper caused erratic (low) recoveries of NO_3^--N. No losses of mineral N could be detected from clarified standard NH_4^+-N or NO_3^--N solutions even after standing for 1-2 days at room temperature.

2) Incubated samples

Weighed subsamples were extracted by shaking with 50 ml 1 N Na_2SO_4 for 1 hour, allowed to settle overnight at 2°C, and portions of the supernatant steam distilled. Filtration or clarification were not required. No losses of mineral N could be detected from extracts stored for 1-2 days at room temperature.

b) Distillation

Suitable portions of the extracts (25 ml for NH_4^+-N; 10 ml for (NH_4^+ + NO_3^-)N estimations) were pipetted into a Markham still and rinsed in with a little distilled water. For NH_4^+-N estimations c. 0.5 g heavy MgO (B.D.H., A.R. grade) was then carefully rinsed in; for (NH_4^+ + NO_3^-)N estimations c. 0.15 g Devarda's alloy (B.D.H., L.R. grade, ground to pass a 76 μ sieve) was added from a calibrated spoon and also rinsed in. The mixtures were then steam distilled, c. 30 ml of distillate being collected over a 3-3.5 minute period in 5 ml of boric acid solution. This solution contained 1% (w/v) H_3BO_4, 20% (w/v) absolute ethanol, and 5 ml/l of an ethanolic solution of bromocresol green (0.16% w/v) and methyl red (0.08%, w/v), and had been adjusted to c. pH 5.9 with 0.1 N NaOH. The distillate was titrated with 0.005 N $KH(IO_3)_2$ (Fluka, puriss p.a. grade) from a 5 ml precision bore burette calibrated to 0.01 ml. The blank titre was c. 0.2 ml for NH_4^+-N estimations and c. 0.5 ml for (NH_4^+ + NO_3^-)N estimations. Recoveries of mixed (NH_4^+ + NO_3^-)N standards were checked with each batch of distillations, and were always better than 95%. Calculations of the mg mineral N/kg OD soil were made as for the incubation analyses (Appendix C5(b)).

APPENDIX D1: SEASONAL CHANGES IN MEAN BULK DENSITY AND MOISTURE AND MINERAL N CONTENTS OF LR2 AND MR2 PLOTS

Although the data presented and discussed in Chapter V is largely based on results obtained from only one replicate of the LR2 and MR2 trials, determinations were also made of the bulk density and moisture and mineral N content of samples collected from the other two replicates. These results have been summarised in Tables 44, 45 and 46 respectively, and are briefly discussed below.

The relatively low bulk density values obtained for the LR2 plots (Table 44) were in agreement with local experience; Rooney (1966; pers.comm.) has suggested 1.15 g/cc and 0.85 g/cc as typical values for consolidated and disturbed topsoil respectively. The generally higher values for the MR2 samples are similar to those reported by other authors e.g. Skene (1967, pers.comm.) obtained values of 1.50 g/cc for the surface samples of the profiles described in Appendix C1. As expected there was a marked tendency for the soil to progressively consolidate with time under pasture or crop. Whilst the bulk density generally decreased following cultivation, the marked variability of the results probably reflect appreciable sampling errors resulting from the difficulty of satisfactorily defining the actual sampling depth within a variable cultivated layer.

The soil moisture contents reported in Table 44 largely reflect the amount of rainfall immediately prior to sampling. The generally higher moisture content of the surface soil of fallow

TABLE 43. MEAN BULK (APPARENT) DENSITY OF SOILS FROM LR2 AND MR2 PLOTS

Plot Nos. (LR2)	Rotation	Phase (Sept. 1964)	BD (g OD soil/cc moist soil)										
			1964			1965					1966		
			18/ix	22/x	8/xii	12/v	24/vii	24/viii	4/x	23/xi	26/i	9/iii	18/iv
1,16,19**	FW	W	0.74	ND	ND	0.83	0.94	ND	0.78	1.02	0.93	1.02	0.90
7,9,24*		F	0.78	"	"	0.77	0.88	"	0.77	1.18	1.05	1.00	0.85
4,14,18	PPFW	W	0.70	"	"	0.71	0.85	"	0.77	1.06	0.94	1.00	0.84
5,12,22		P_1	0.76	"	"	0.83	0.93	"	0.89	1.20	0.95	0.96	0.84
3,15,17**		P_2	0.69	"	"	0.89	0.87	"	0.77	0.96	0.85	0.93	0.89
6,11,23*		F	0.75	"	"	0.77	0.84	"	0.75	0.99	0.96	0.98	0.84

* Plots cultivated 1964 (20/viii, 14/ix, 21/x, 27/x) and 1965 (4/i, 1/iii, 19/v, 26/v and sown 1/vii)
** Plots cultivated 1965 (4/viii, 30/viii, 10/ix, 6/x, 30/xi, 15/xii) and 1966 (14/i, 27/iii).

Plot Nos. (MR2)	Rotation	Phase (Sept. 1964)	BD (g OD soil/cc moist soil)										
			1964			1965					1966		
			23/ix	27/x	1/xii	2/vi	7/vii	12/viii	14/ix	25/x	25/xi	13/i	2/iii	21/iv
5,8,17++	FW	W	ND	ND	ND	1.24	1.52	1.22	1.52	1.61	1.44	1.46	1.58	1.55
1,10,13+		F	"	"	"	1.25	1.58	1.33	1.62	1.56	1.55	1.39	1.52	1.61
6,9,16	PPFW	W	"	"	"	1.15	1.44	1.25	1.60	1.61	1.55	1.51	1.55	1.43
2,11,15		P_1	"	"	"	1.19	1.52	1.50	1.76	1.65	1.49	1.49	1.32	1.43
4,7,18++		P_2	"	"	"	1.33	1.66	1.31	1.58	1.54	1.33	1.36	1.21	1.48
3,12,14+		F	"	"	"	1.24	1.32	1.34	1.57	1.59	1.49	1.37	1.40	1.42

\+ Plots cultivated 1964 (17/vii, 18/ix) and 1965 (27/iv and sown 14/vi)
++ Plots cultivated 1965 (23/vii, 3-4/xii).

Rotation: W=wheat; F=bare fallow; P_1= 1st year pasture; P_2= 2nd year pasture.
Same notation used in subsequent tables.

TABLE 44. MEAN MOISTURE CONTENT OF SOILS FROM LR2 AND MR2 PLOTS

Plot Nos. (LR2)	Rotation	Phase (Sept. 1964)	Moisture* (g/100 g OD soil)										
			1964			1965					1966		
			18/ix	22/x	8/xii	12/v	24/vii	24/viii	4/x	23/xi	26/i	9/iii	18/iv
1,16,19	FW	W	35.7	32.4	19.2	28.3	37.7	39.4	32.9	20.7	18.3	19.0	16.5
7,9,24		F	33.2	36.0	30.7	28.8	38.9	39.2	20.4	13.5	12.3	14.5	11.4
4,14,18	PPFW	W	36.1	31.5	17.8	30.5	40.0	41.4	25.2	16.9	11.5	14.3	11.8
5,12,22		P_1	37.0	32.7	19.6	28.3	38.7	39.2	17.9	12.4	9.4	11.2	11.2
3,15,17		P_2	37.7	32.8	18.2	28.8	40.0	41.0	32.6	21.8	18.4	19.2	17.4
6,11,23		F	36.7	36.0	32.2	29.9	40.3	40.9	20.5	13.9	13.4	15.2	12.4

Plot Nos. (MR2)	Rotation	Phase (Sept. 1964)	Moisture* (g/100 g OD soil)										
			1964			1965					1966		
			23/ix	27/x	1/xii	2/vi	7/vii	12/viii	14/ix	25/x	25/xi	13/i	2/iii	21/iv
5,8,17	FW	W	7.0	4.0	3.7	7.0	8.3	11.2	8.9	2.9	7.3	6.0	3.6	3.4
1,10,13		F	7.9	4.0	3.1	5.9	7.4	9.8	7.2	1.6	5.9	4.9	2.5	1.7
6,9,16	PPFW	W	6.4	3.6	3.9	6.9	8.1	10.6	7.7	2.7	6.7	5.4	3.3	2.6
2,11,15		P_1	6.5	2.7	3.4	5.9	7.7	9.8	6.9	2.2	6.1	4.9	2.9	2.3
4,7,18		P_2	6.0	2.7	3.1	6.4	7.8	10.3	7.6	2.8	6.3	5.5	3.5	3.0
3,12,14		F	8.1	3.5	2.6	6.0	7.1	9.5	6.2	1.6	5.7	5.4	2.7	1.8

* determined from weight loss of field-moist sample after oven drying at 105°C for 16 hours.

TABLE 45. SEASONAL FLUCTUATIONS IN MEAN MINERAL N CONTENT OF LR2 and MR2 PLOTS

Plot Nos. (LR2)	Rotation	Phase (Sept. 1964)	mg Mineral N/kg OD soil										
			1964			1965					1966		
			18/ix	22/x	8/xii	12/v	24/vii	24/viii	4/x	23/xi	26/i	9/iii	18/iv
1,16,19	FW	W	1.3(0.2)	0.3(0.2)	0.2(0)	0 (0)	0.7(0.4) *	0.1(0)	4.7(0.8)	2.9(1.0)	8.9(2.0)	21.6(0.1)	17.0(0)
7,9,24		F	2.5(0.3)	2.0(0.4)	3.3(0)	2.7(0) *	0.5(0.1)	0.1(0)	1.1(0)	1.2(0.5)	2.4(0.7)	7.1(0.7)	6.4(0)
4,14,18	PPFW	W	1.0(0.3)	1.7(1.7)	0.5(0.3) *	0.1(0)	0.7(0.6)	0 (0)	2.7(0.8)	4.3(4.0)	4.3(1.4)	3.5(0.6)	3.4(0.4)
5,12,22		P_1	9.2(4.6)	11.7(8.3)	7.6(6.2) *	3.6(0.8)	1.4(0.7)	0 (0)	0.1(0)	4.8(4.4)	7.4(3.3)	8.1(0.3)	13.5(0.2)
3,15,17		P_2	5.0(4.3)	3.2(2.7)	2.7(1.9)	1.5(0.2)	1.4(1.2) *	5.3(3.3)	18.3(1.3)	6.6(2.6)	29.8(2.0)	39.5(0.3)	47.4(0)
6,11,23		F	12.3(2.7)	7.2(5.0)	4.6(4.3)	5.3(0) *	2.3(0.1)	0.2(0.1)	3.4(0.9)	2.1(1.1)	13.4(1.8)	33.2(0.9)	29.8(0.1)

Plot Nos. (MR2)	Rotation	Phase (Sept. 1964)	mg Mineral N/kg OD soil										
			1964			1965						1966		
			23/ix	27/x	1/xii	2/vi	7/vii	12/viii	14/ix	25/x	25/xi	13/i	2/iii	21/iv
5,8,17	FW	W	0.4(0.1)	0 (0)	0.7(0.3) *	1.3(0.7)	0.5(0.2)	0.4(0)	1.1(0.2)	3.6(2.2)	0.7(0.4)	4.8(2.5)	2.7(0.9)	10.3(0.2)
1,10,13		F	0.7(0.1)	0.4(0.2)	2.1(1.6)	2.3(2.3) *	1.1(0.3)	0.7(0.3)	1.5(0.4)	0.5(0.5)	0.2(0.2)	1.6(1.3)	1.0(1.0)	4.0(0)
6,9,16	PPFW	W	1.2(0.7)	0.7(0.3)	1.5(0.4) *	3.7(1.7)	0.9(0.1)	0.4(0.2)	1.1(0.1)	1.8(1.5)	0.6(0.6)	4.9(2.1)	5.5(1.4)	7.6(0)
2,11,15		P_1	1.0(0.2)	2.7(2.3)	3.1(2.2) *	2.8(1.8)	2.0(0.9)	1.3(1.2)	1.6(1.1)	4.2(3.9)	0.8(0.8)	4.8(2.9)	3.3(1.5)	13.3(0.6)
4,7,18		P_2	1.8(1.4)	3.9(3.3)	2.0(1.2) *	3.0(1.2)	3.1(1.6)	1.3(0.5)	2.7(1.0)	5.3(2.5)	5.9(2.3)	16.5(3.0)	16.5(1.6)	27.4(0.5)
3,12,14		F	2.0(0.5)	0.5(0.3)	5.5(4.2)	2.9(1.7) *	1.5(0.6)	0.2(0.2)	0.7(0.4)	0.5(0.5)	0.1(0.1)	3.2(2.2)	4.4(2.8)	6.3(0)

* approximate period of transition to next phase of rotation (see p. 154)

The values tabulated are total mineral N (i.e. (NH_4^+ + HO_3^-)N), with the corresponding values of NH_4^+-N shown in parenthesis.

plots was most marked during the dry summer months.

The mineral N data reported in Table 45 are geometric treatment means. These values were obtained by logarithmically transforming the original data (i.e. the mineral N content of the surface 10 cm of plots at various stages of the 2-course and 4-course rotations at each site), subjecting it to standard 'Analysis of Variance' procedures, and then retransforming the resultant treatment means. The general trends in the data are discussed in Chapter V (p. 130).

APPENDIX D2: MEAN BULK DENSITY AND TOTAL SOIL N, AND LF AND LF-N CONTENTS OF SAMPLES FROM THE C1 ROTATION TRIAL

Plot No.	Rotation	Phase	Mean BD (g/cc)	Mean Org.N (lb/ac 2.5 in)	lb/ac 2.5 in*									
					2.v.66		1.vii.66		29.ix.66		8.xii.66		16.ii.67	
					LF	LF-N	LF	LF-N	LF	LF-N	LF	LF-N	LF	LF-N
17	W	W	1.32	837	NA	NA	NA	NA	17,010	179	14,850	141	15,745	154
35	WF	W	1.40	674	NA	NA	NA	NA	13,670	122	13,640	123	14,110	134
32	PPFW	P_2	1.45	1100	17,265	193	20,300	231	22,405	267	21,255	234	18,905	231
31	"	P_2 F	1.47 (1.24)+	1090	16,235	206	18,685	232	18,725	234	16,369	200	16,319	204
30	"	W	1.31	935	12,970	163	15,330	189	14,200	180	15,365	184	14,500	180
20	PPW	P_2	1.46	1075	16,310	173	16,870	177	19,350	203	17,930	197	16,985	185
19	"	W	1.29	1133	16,365	200	20,100	245	23,020	299	18,090	228	18,485	226
15	PPPPWW	P_4	1.45	1174	16,515	198	18,840	209	21,220	259	22,420	269	18,645	231
13	"	W_1	1.34	1032	15,515	171	21,860	267	24,760	287	22,970	250	22,785	278
16	"	W_2	1.25	1090	21,155	273	23,675	313	24,655	318	23,025	295	21,070	270
29	P	P	1.42	1720	NA	NA	NA	NA	33,395	500	26,700	403	27,685	429

* assuming lb/ac 2.5 in = $(0.5664)BD \times 10^6$

\+ decrease in BD following cultivation disregarded for the purposes of comparison on assumption that the LF was uniformly distributed throughout the soil volume sampled prior to fallowing between July and Sept. '66.

LF separated as in Appendix A

NA = no data available.

APPENDIX D3: ESTIMATION OF BETWEEN PLOT (FIELD) VARIABILITY IN LR2 AND MR2

Samples were collected from all replicates of both trials immediately after the opening rains ('break') of the 1965 growing season (i.e. from MR2 in May and from LR2 in June), and also some weeks after the crops had been sown (i.e. in August). These were subsequently analysed as outlined in Chapter V and the results are summarised in Table 46. The results of an Analysis of Variance of this data are presented in Table 47 (see also Appendix D4).

TABLE 46 SUMMARY OF RESULTS FROM FIELD VARIABILITY EXPERIMENT

Date Sampled		12.v.65							24.viii.65							
Plot No. (LR2)	Rotation	Phase	BD (g/cc)	lb/ac 0-4 in							Phase	BD (g/cc)	lb/ac 0-4 in					
				Total Soil N	Initial		Final LF-N	Incubation					Total Soil N	Initial		Final LF-N	Incubation	
					LF	LF-N		ΔN_i	Final N_i					LF	LF-N		ΔN_i	Final N_i
7	FW	F	0.72	463	568	9.7	5.7	10.6	20.8		W	0.81*	580	660	7.8	5.1	6.2	7.0
9			0.73	450	463	8.7	6.0	6.3	20.0			0.82*	587	535	8.5	6.3	1.3	1.3
24			0.86	608	764	14.8	6.4	16.9	25.6			0.85*	485	786	15.0	8.6	1.5	1.5
1		W	0.68	462	844	11.8	4.9	20.5	22.2		F	0.84*	563	1035	13.3	9.2	11.9	11.9
16			0.78	481	1195	14.4	6.8	13.9	13.9			0.87*	528	1167	11.9	8.3	4.3	4.3
19			1.04	697	1037	14.5	9.4	3.6	3.6			0.86*	553	1200	17.1	8.1	12.2	14.0
6	PPFW	F	0.75	775	2073	40.4	23.5	60.4	105.4		W	0.81*	852	1674	34.0	17.1	31.2	38.0
11			0.67	656	1870	38.0	20.0	50.3	92.1			0.76*	861	2514	63.6	31.1	29.4	35.5
23			0.90	1101	2708	55.8	32.2	33.7	87.4			0.80*	899	1958	41.3	25.9	48.4	54.5
4		P_1(W)	0.72	666	1194	21.4	10.1	38.8	44.4		P_1	0.75*	693	1482	26.0	19.2	45.6	46.8
14			0.72	613	1488	24.5	11.0	21.5	24.4			0.84*	815	1332	25.9	13.9	16.7	18.0
18			0.70	673	1294	22.8	13.8	18.3	21.3			0.83*	767	1354	23.8	44.2	19.3	19.3
5		$P_2(P_1)$	0.76	703	2425	52.8	25.3	67.8	93.9		P_2	0.92*	926	2018	32.9	50.4	15.3	16.8
12			0.98	1030	3393	75.0	38.8	27.4	40.2			0.90*	881	3377	87.1	32.9	43.8	45.6
22			0.76	806	2356	47.1	21.8	60.5	70.3			0.90*	914	2186	49.8	35.2	23.1	25.1
3		P_2	0.68	801	3217	61.8	30.2	62.0	72.6		F	0.82*	907	3523	79.3	55.5	36.9	47.3
15			1.01	1282	4650	88.3	56.7	55.4	68.6			0.84*	974	3624	74.7	42.6	48.9	53.3
17			0.99	1095	4235	77.1	26.3	42.7	42.7			0.80*	812	3451	65.5	15.4	36.7	42.9

(continued)

TABLE 46 (continued)

Date Sampled		2.vi.65								12.viii.65							
Plot No. (MR2)	Rotation	Phase	BD (g/cc)	lb/ac 0.4 in						Phase	BD (g/cc)	lb/ac 0-4 in					
				Total Soil N	Initial		Final LF-N	Incubation				Total Soil N	Initial		Final LF-N	Incubation	
					LF	LF-N		ΔN_i	Final N_i				LF	LF-N		ΔN_i	Final N_i
1	FW	F	1.25	385	3240	46.3	48.6	23.1	27.5	W	1.18	364	2417	32.4	33.2	17.0	18.5
10			1.22	332	3273	50.8	44.9	22.0	25.9		1.44	470	4307	52.6	60.6	12.3	18.8
13			1.27	345	3430	43.5	44.9	16.3	22.4		1.30	412	4006	56.4	76.0	16.4	16.4
5		F(W)	1.37	323	3377	54.0	28.4	20.9	24.0	F	1.20	337	2219	22.4	25.2	17.3	21.5
8			1.27	391	4098	48.3	34.6	20.3	21.9		1.17	414	3924	50.2	47.5	19.4	20.1
17			1.08	235	3328	40.2	32.3	18.8	21.2		1.30	306	3700	48.9	37.1	13.0	15.9
3	PPFW	F	1.29	456	5869	86.9	68.9	31.0	41.7	W	1.35	453	4894	75.4	81.9	36.7	40.1
12			1.19	442	5306	89.2	83.1	36.7	47.8		1.29	433	5519	87.2	84.0	27.0	33.2
24			1.24	427	4945	79.1	110.1	32.8	45.5		1.39	378	7080	122.5	50.6	30.5	36.7
6		P_1(W)	1.24	438	5102	83.2	42.7	27.5	30.8	P_1	1.07	378	2347	33.7	30.1	23.0	25.0
9			1.17	424	4411	66.2	54.7	28.8	35.6		1.32	443	4809	69.7	63.5	24.8	26.4
16			1.04	245	5015	79.3	59.1	24.3	27.0		1.37	435	6208	99.3	69.7	24.1	28.4
2		P_2(P_1)	1.36	579	5744	85.1	78.4	38.7	46.2	P_2	1.47	533	5089	80.9	80.9	51.8	57.6
11			1.18	364	5625	120.4	75.0	30.9	41.5		1.50	408	7994	110.3	95.6	39.2	41.1
15			1.04	339	4185	58.6	85.1	31.4	42.0		1.53	541	6795	101.9	110.0	40.5	45.3
4		F(P_2)	1.47	520	7807	128.8	85.9	37.6	55.4	F	1.49	473	7292	119.6	101.0	47.4	47.4
7			1.36	592	8111	148.4	139.9	51.0	61.5		1.22	608	7652	141.5	123.8	41.0	56.0
18			1.15	417	8276	147.3	122.4	49.6	57.3		1.23	502	6956	119.6	106.1	48.8	60.6

Rotation phases shown are determined as on p. 154, and phases indicated in parenthesis are those prior to the 1965 seasonal 'break'.

* estimated by averaging BD values from same plot sampled in July and October 1965.

Total Soil N = N determined by Dumas procedure (Method B11)
Initial LF = LF in unincubated sample (see Appendix A)
Initial LF-N = organic N in LF prior to incubation (Method B13)
Final LF-N = organic N in LF after incubation
ΔN_i = net release of mineral N during incubation
Final N_i = $(NH_4^+ + NO_3^-)N$ present after incubation.

Footnotes to Table 47:

i) Variates and rotation phases are as in Table 46; LF-N data refers to unincubated samples only (= initial LF-N)

ii) All LF-N and incubation data were transformed as $\log_e$(ppm N) before statistical analysis.

iii) VR = variance ratio;
NS = not significant;
* = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$.
df = degrees of freedom

iv) Block (replicate) effects were generally not significant, except for MR2 where initial LF-N levels tended to be lower in Replicate 1.

TABLE 47. SUMMARY OF ANALYSIS OF VARIANCE OF DATA FROM FIELD VARIABILITY EXPERIMENT

Trial and Date	Variate	Treatment Effects		Treatment Means for Plots in Rotations and Phases shown					
		VR	LSD (5 df) (p=0.05)	FW			PPFW		
				1,10,13 F	5,8,17 F(W)	3,12,24 F	6,9,16 P_1(W)	2,11,15 $P_2(P_1)$	4,7,18 F(P_2)
MR2 June '65	N_t (%)	*	0.010	0.031	0.028	0.039	0.035	0.039	0.042
	LF-N ($\log_e$ppm)	***	0.367	3.723	3.742	4.326	4.290	4.363	4.771
	ΔN_i (")	***	0.328	2.887	2.881	3.393	3.251	3.439	3.642
	Final N_i (")	***	0.218	3.104	2.994	3.689	3.393	3.694	3.882
				W	F	W	P_1	P_2	F
MR2 Aug.'65	N_t (%)	NS	-	0.035	0.032	0.035	0.037	0.036	0.045
	LF-N ($\log_e$ppm)	***	0.353	3.659	3.537	4.337	3.999	4.266	4.671
	ΔN_i (")	***	0.375	2.546	2.690	3.243	3.054	3.465	3.651
	Final N_i (")	***	0.351	2.717	2.844	3.403	3.159	3.554	3.826
				7,9,24 F	1,16,19 W	6,11,23 F	4,14,18 P_1(W)	5,12,22 $P_2(P_1)$	3,15,17 P_2
LR2 May '65	N_t (%)	***	0.018	0.072	0.072	0.119	0.101	0.117	0.131
	LF-N ($\log_e$ppm)	***	0.267	2.742	2.900	4.149	3.566	4.334	4.543
	ΔN_i (")	**	1.151	2.704	2.605	4.208	3.648	4.164	4.192
	Final N_i (")	**	1.034	3.453	2.631	4.913	3.786	4.414	4.316
				W	F	W	P_1	P_2	F
LR2 Aug. '65	N_t (%)	***	0.012	0.074	0.071	0.122	0.104	0.103	0.121
	LF-N ($\log_e$ppm)	***	0.626	2.588	2.889	4.134	3.542	4.152	4.587
	ΔN_i (")	***	1.203	1.121	2.402	3.902	3.513	3.413	3.998
	Final N_i (")	***	1.217	1.162	2.446	4.070	3.548	3.486	4.161

APPENDIX D4: STATISTICAL ANALYSES

1. Evaluation of errors involved in LR2 and MR2 data

Most of the data discussed in Section V from these two trials refers to single analyses of composited samples from one replicate only, as complete analysis of all the samples from both trials would have required more time than was available. Consequently it was necessary to estimate the magnitudes of appropriate errors for the evaluation of the differences found both between plots (treatment effects) and within any given plot (seasonal effects). The error estimates required were:

a) Between plot comparisons

(1) for the comparison of any two single analyses of 30-core composites from different plots i.e. for the evaluation of treatment (rotation) effects at any given sampling date;

(2) for the comparison of the means of pooled data from different plots i.e. for the evaluation of average treatment effects over either or both of the seasons concerned;

b) Within plot comparisons

(3) for the comparison of single estimates from a 30-core composite (i.e. standard error of a single determination);

(4) for comparison of any two estimates derived from sampling the same plot at different times;

(5) for the comparison of the means of pooled data (each representing the mean of several single estimates) from the

same plot whilst at different phases of a rotation e.g. for the evaluation of the mean differences resulting from the fallowing of a pasture plot.

Estimates have been made of the errors applicable to between plot comparisons of LF-N and ΔN_i, and of within plot comparisons of LF-N, these calculations involving several assumptions, viz:

1. that the errors involved in the determination of the LF present in a sample were similar to those associated with a corresponding determination after incubation, and
2. that the errors involved in either of these LF determinations were not appreciably increased by the analytical errors involved in the determination of the N content of the extracted LF.

It is then possible by evaluating the actual errors associated with the determination of the initial amount of LF in the soil (i.e. before incubation) and with ΔN_i to derive estimates of the errors involved in the comparisons listed above. Because of the number of assumptions involved, the maximum estimates were used throughout so that the conclusions drawn using these estimates would be conservative.

a) Between plot comparisons

The data summarised in Appendix D3 was used as follows for the estimation of errors appropriate to between plot (treatment) comparisons. The error mean square (MS) values from the analysis of variance of this data were:

Trial	Date	Error MS (10 df) for Variate			
		Initial LF (%)	$\log_e$(Initial LF-N) (ppm)	Incubation N_i (ppm)	
				Final	ΔN_i
MR2	June '65	*3.24×10^{-3}	*3.06×10^{-2}	1.08×10^{-2}	2.44×10^{-2}
"	Aug. '65	2.22×10^{-3}	2.87×10^{-2}	2.80×10^{-2}	*3.19×10^{-2}
LR2	May '65	4.77×10^{-4}	1.62×10^{-2}	2.42×10^{-1}	3.01×10^{-1}
"	Aug. '65	*2.25×10^{-3}	*8.89×10^{-2}	3.36×10^{-1}	*3.28×10^{-1}

* values used to estimate errors

On the assumption that the maximum error mean square so obtained was a valid, although very approximate, estimate of the variance applicable to each value of the variates to be compared (i.e. that both values were derived from populations with essentially the same variance as that above), then the difference between the two values which was required for significance at the 5% level of probability (LSD (p=0.05)) was calculated as follows:

1. For the comparison of the results of single analyses of a composite from each plot,

$$LSD = t_{10} \sqrt{2} \text{ (error MS)}$$

2. For the comparison of 2 treatment means, each representing the average of a number of single analyses of composited samples collected from a plot either throughout the period studied (1964/6) or from a single season only (i.e. 1964 or 1965/6),

$$LSD = t_{10} \sqrt{2} \ (1/n_1 + 1/n_2)(\text{error MS})$$

where the number of results from each plot pooled to calculate the means to be compared are n_1 and n_2 respectively.

For means derived from pooled data referring to a single season only, the greatest LSD for either trial was when $n_1 = n_2 = 3$, corresponding to the comparison of means based on as few observations as were available for 1964.

For means calculated by pooling all the available data, the maximum LSD values were for LR2 when $n_1 = n_2 = 11$, and for MR2 when $n_1 = n_2 = 12$.

These LSD values are summarized below.

Comparisons Between 2 Plots of Data	LSD (p = 0.05) for Comparisons of Variates							
	LF (%)		$\log_e$ LF-N* (ppm)		$\log_e \Delta N_i$ * (ppm)		LF** (kg/ha 10 cm)	
	MR2	LR2	MR2	LR2	MR2	LR2	MR2	LR2
i) from 1 determination per plot	0.18	0.15	0.55(1.74)	0.94(2.56)	0.56(1.75)	1.80(6.05)	2650	1750(1300)
ii) averaged:								
from 1 season only	0.15	0.10	0.45(1.57)	0.77(2.16)	0.46(1.58)	1.47(4.35)	2200	1170(870)
from all samples	0.08	0.07	0.24(1.27)	0.42(1.52)	0.24(1.27)	0.81(2.25)	1170	820(600)

* Values in parenthesis are retransformed values which are applicable to the ratios of the two (untransformed) values being compared. As BD within either trial is relatively constant, the ratios can also be used for comparison of data computed on kg/ha basis. Further, as the variances for determinations of total N_i and ΔN_i are closely similar, the calculated LSD values may be applied to the evaluation of data derived from either determination.

** Based on a mean BD for MR2 plots of 1.45 g/cc, and for LR2 of either 1.15 g/cc (compacted plots), or (in parenthesis) of 0.80 g/cc (cultivated plots) (cf. Table 39).

b) Within plot comparisons

Calculation of the error associated with the determination of the amount of LF in a subsample from a 30-core composite was based on the fact that this error variance comprises two major components, i.e. the error associated with field sampling and compositing procedures, and that associated with the determination of the LF in the sample (laboratory error). These components were estimated as follows:

(i) Field error

Thirty individual cores were collected in May 1967 from a 4-course plot in each trial which was in its second year of pasture but which had not yet been fallowed. The plots selected were from the same block (replicate) as that from which the seasonal data to be evaluated had been derived (i.e. plot 4, LR2 and plot 9, MR2). Each core was dried at 40°C, gently ground by hand to pass a 2 mm sieve, thoroughly mixed and then subsampled for estimation of its LF by the procedure described in Appendix A.

(ii) Laboratory error

Composited samples from similar second year pasture plots (plot 5, LR2 and plot 2, MR2) were collected in April 1966, and the LF separated from twelve individual subsamples of each composite. (see Table 23).

Analysis of the data from both the above experiments yielded the following results:

Source of Error	Trial, Plot and Date	LF (mg/5 g OD Soil)				
		$\bar{X}$	S^2	S	SE	CV
i) Field	MR2:9, May '67	30.6	141.4	11.9	2.17	0.389
	LR2:4, May '67	16.1	42.2	6.5	1.19	0.405
ii) Laboratory	MR2:2, April '66	22.1	2.9	1.7	0.49	0.077
	LR2:5, April '66	26.4	2.5	1.6	0.45	0.059

$\bar{X}$ = mean, S^2 = variance, S = standard deviation, SE = standard error of mean, CV = coefficient of variance.

The variance associated with the analysis of a single sample from a 30-core composite ($S^2_{\bar{30}}$), assuming that both variance estimates may be pooled (i.e. that they are derived from closely similar populations), for samples from MR2 was thus

$$S^2_{\bar{30}} = 2.9 + (141.4-2.9)/30 = 7.53 \text{ mg LF/5 g OD soil.}$$

The corresponding value for LR2 samples was 3.78 mg LF/5 g OD soil. On the assumptions

(i) that similar errors would be associated with corresponding analyses of composites from other plots in the same replicates, irrespective of rotation or time of sampling, and

(ii) that appropriate mean bulk density values for the two soils were for MR2, 1.45 g/cc (i.e. 1.47×10^6 kg/ha 10 cm); and for LR2, 0.85 g/cc (i.e. 0.86×10^6 kg/ha 10 cm), and that seasonal

variations from these means could be disregarded, and also (iii) that an appropriate number of degrees of freedom was 20 then the following estimates were calculated:

3) the standard error of single determination (i.e. $\sqrt{s^2_{\bar{30}}}$);

4) the LSD (p=0.05) applicable to single determinations on two different composites from the same plot (i.e. $t_{20}\sqrt{2s^2_{\bar{30}}}$); and

5) the LSD (p=0.05) applicable to the means of two sets of pooled data relating to the same plot at different phases of a rotation (i.e. $t_{20}\sqrt{2(1/n_1 + 1/n_2)s^2_{\bar{30}}}$).

The maximum values (c. 67% of the LSD calculated as in 4) were obtained for MR2 when n_1 =3, n_2=9; and for LR2 when n_1=3, n_2=8.

The standard error of a single measurement was thus:

(i) for MR2: 2.74 mg LF/5 g OD soil, i.e. 0.05% LF, or 15 kg LF-N/ha 10 cm, and

(ii) for LR2: 1.94 mg LG/5 g OD soil, i.e. 0.04% or 14 kg LF-N/ha 10 cm.

Appropriate LSD (p=0.05) values for within plot comparisons were:

Comparison of Data	MR2		LR2	
	LF (%)	LF-N (kg/ha 10 cm)	LF (%)	LF-N (kg/ha 10 cm)
i) from 2 different samples	0.16	47	0.12	20
ii) averaged from each of successive phases of rotation	0.11	32	0.08	14

As these estimates were considered to be the best available they have been used for the evaluation of the data obtained from these trials (see Table 39).

APPENDIX D4: 2. RELATIONSHIPS BETWEEN TOTAL, LF AND MINERALIZABLE N FOR LR2 AND MR2 DATA

The data from one replicate of each trial (Tables 50 and 51) was pooled as indicated in Table 49, and a series of simple linear regression analyses conducted between pairs of the variates concerned (cf. Table 42). The results are summarised below.

TABLE 48: SIMPLE CORRELATION COEFFICIENTS FROM GROUPED LR2 and MR2 DATA

1. Effect of Soil Type and Environment

Trial and Rotation	Variate[+]	MR2: Whole Trial				
		N_t	LF-N	ΔLF-N	ΔN_i	Final N_i
LR2: Whole Trial	N_t		0.80 ***	ND	0.68 ***	0.74***
	LF-N	0.87***		ND	0.72 ***	0.85***
	ΔLF-N	ND	ND		0.26 *	0.29**
	ΔN_i	0.46***	0.61***	0.62***		ND
	Final N_i	0.51***	0.66***	0.60***	ND	

(continued)

TABLE 48 (continued)

2. Effect of Rotation

a) MR2

Trial and Rotation	Variate+	MR2: 2-course				
		N_t	LF-N	ΔLF-N	ΔN_i	Final N_i
MR2 4-course	N_t		0.53**	ND	NS	NS
	LF-N	0.71***		ND	NS	0.36*
	ΔLF-N	ND	ND		NS	NS
	ΔN_i	0.53***	0.50***	NS		ND
	Final N_i	0.63***	0.72***	NS	ND	

b) LR2

Trial and Rotation	Variate+	LR2: 2-course				
		N_t	LF-N	ΔLF-N	ΔN_i	Final N_i
LR2 4-course	N_t		0.48**	ND	-0.37*	NS
	LF-N	0.73***		ND	NS	NS
	ΔLF-N	ND	ND		NS	NS
	ΔN_i	NS	NS	0.37**		ND
	Final N_i	NS	0.37**	0.34**	ND	

(continued)

TABLE 48 (continued)

3. Effect of Phase of Rotation

Trial	Rotation	Phase	Significant correlations		
			Y	X	r
MR2	2-course	F	N_t	LF-N	0.52*
"	"	W	-	-	-
"	4-course	P_1	N_t	LF-N	0.59*
	"	P_2	N_t	LF-N	0.62*
		"	"	ΔN_i	0.64**
		"	"	Final N_i	0.65**
	"	F	N_t	LF-N	0.84***
		"	"	ΔN_i	0.55*
		"	"	Final N_i	0.83***
		"	ΔN_i	LF-N	0.63***
		"	Final N_i	"	0.89***
	"	W	-	-	-
LR2	2-course	F	N_t	LF-N	0.77**
	"	W	N_t	ΔN_i	-0.53*
"	4-course	P_1	N_t	LF-N	0.52*
		"	Final N_i	"	0.61*
		"	Net N_i	ΔLF-N	0.55*
		"	Final N_i	"	0.63*
	"	P_2	N_t	LF-N	0.62*
	"	F	N_t	LF-N	0.71**
		"	"	ΔN_i	-0.71**
	"	W	N_t	LF-N	0.84***

+ variates as in Table 43.

ND = not determined
NS = not significant
- = no significant correlations found between pairs of variates examined.

APPENDIX D5: SEASONAL DATA FROM LR2 (REP.1) AND MR2 (REP.2)

TABLE 49: SUMMARY OF DATA FROM LR2, REPLICATE 1

Plot Rotation	1 FW								7 FW							
Date Sampled	Phase	BD (g/cc)	lb/ac 0-4 in						Phase	BD (g/cc)	lb/ac 0-4 in					
			Total Soil N	Initial LF	Initial LF-N	Final LF-N	Incubation ΔN_i	Incubation Final N_i			Total Soil N	Initial LF	Initial LF-N	Final LF-N	Incubation ΔN_i	Incubation Final N_i
18/ix/64	W	0.68	431	567	9.5	8.7	21.0	22.9	F	0.74	510	832	16.3	7.2	39.6	41.2
22/x/64	"	0.68*	468	938	13.1	7.0	21.4	21.4	"	0.73*	463	582	8.1	4.2	35.6	36.7
8/xii/64	"	0.68*	444	1035	15.5	5.5	14.5	16.2	"	0.73*	503	490	9.7	5.6	38.6	41.9
12/v/65	"	0.68	462	924	11.0	7.1	22.2	23.9	"	0.72	463	574	10.0	6.5	13.2	24.5
24/vii/65	"	0.82	543	1115	32.3	13.4	25.9	26.5	W	0.81	587	778	13.8	7.2	18.3	21.7
24/viii/65	F	0.84*	563	1081	14.1	9.4	16.3	17.1	"	0.81*	580	580	10.5	6.2	8.5	10.0
4/x/65	"	0.86	592	1559	18.6	8.8	9.1	16.0	"	0.80	537	725	10.3	5.0	2.4	3.0
23/xi/65	"	1.00	653	1269	21.4	18.8	20.0	28.9	"	1.15	771	1167	28.1	20.2	0	2.1
26/i/66	"	0.80	464	964	16.7	13.4	10.7	25.5	"	0.92	567	667	12.6	8.1	21.7	26.6
8/iii/66	"	1.01	613	878	18.9	11.1	0.6	22.1	"	1.01	586	696	10.3	7.5	11.9	21.9
18/iv/66	"	0.88	526	1053	15.2	9.5	18.1	38.9	"	0.79	444	680	12.5	10.5	15.2	24.1

Plot Rotation	4 PPFW							5 PPFW							
18/ix/64	W	0.66	616	1172	23.6	16.8	61.0	64.7	P_1	0.76	805	2780	46.4	12.0	59.6	78.2
22/x/64	"	0.69*	638	1188	19.8	11.0	52.6	52.6	"	0.76*	743	2037	39.1	19.9	64.3	81.5
8/xii/64	"	0.69*	644	1626	30.9	10.6	56.2	56.9	"	0.76*	736	2175	48.5	16.0	81.2	90.9
12/v/65	P_1	0.72	672	1344	24.0	11.3	50.5	55.1	P_2	0.76	702	2698	57.1	27.3	80.9	95.9
24/vii/65	"	0.75	748	1414	26.4	12.2	57.1	61.2	"	0.87	946	3091	64.0	35.8	62.9	68.0
24/viii/65	"	0.75*	693	1516	28.2	15.6	48.0	50.0	"	0.92*	926	2518	43.0	37.7	21.2	23.2
4/x/65	"	0.76	682	1405	32.6	17.4	14.9	25.1	"	0.97	958	3657	61.5	51.5	39.2	42.2
23/xi/65	"	1.06	1050	2114	40.6	24.4	24.4	28.1	"	1.22	1172	3981	80.2	47.9	80.7	87.0
26/i/66	"	0.82	706	1784	36.2	18.7	45.9	54.8	"	0.86	795	3071	56.5	35.2	54.2	62.7
8/iii/66	"	1.02	943	1978	44.1	20.2	27.7	38.1	"	0.98	862	3180	61.7	33.2	38.2	49.5
18/iv/66	"	0.79	716	1747	29.5	22.1	42.2	48.6	"	0.78	749	3344	61.9	38.3	56.9	73.0

Plot Rotation	3 PPFW							6 PPFW							
18/ix/64	P_2	0.71	824	4865	69.2	30.8	86.7	98.9	F	0.67	662	3327	59.9	21.1	90.4	108.6
22/x/64	"	0.69*	794	2927	47.4	22.4	64.7	65.4	"	0.71*	734	1750	40.0	17.8	73.2	80.2
8/xii/64	"	0.69*	838	6054	108.9	33.5	66.9	69.0	"	0.71*	779	1879	45.1	19.2	88.1	103.2
12/v/65	"	0.68	807	3759	71.6	33.5	75.6	85.9	"	0.75	775	2216	41.1	23.5	72.1	115.0
24/vii/65	"	0.82	1033	4325	77.4	44.4	107.4	115.6	W	0.81	866	1674	36.9	21.1	62.7	74.1
24/viii/65	F	0.82*	914	3434	82.5	40.8	45.8	51.3	"	0.81*	852	1622	33.6	20.3	35.8	42.6
4/x/65	"	0.83	985	3160	61.3	49.4	47.2	72.8	"	0.82	907	2497	58.7	28.5	50.2	54.7
23/xi/65	"	1.05	1171	4568	98.8	79.3	23.5	70.8	"	0.95	1092	3272	86.1	48.0	66.6	78.6
26/i/66	"	0.81	830	3186	62.8	46.3	58.1	117.1	"	0.88	909	2664	53.8	43.3	58.5	87.7
8/iii/66	"	0.91	973	4223	86.2	44.5	62.7	144.3	"	1.00	952	2329	45.7	25.6	20.0	59.5
18/iv/66	"	0.86	889	3406	66.8	32.5	50.7	123.2	"	0.86	826	2377	48.7	33.4	55.7	85.4

* estimated values (cf. p. 153).

TABLE 50. SUMMARY OF DATA FROM MR2, REPLICATE 2

Plot Rotation	8 FW								10 FW							
Date Sampled	Phase	BD (g/cc)	lb/ac 0-4 in						Phase	BD (g/cc)	lb/ac o-4 in					
			Total Soil N	Initial		Final LF-N	Incubation				Total Soil N	Initial		Final LF-N	Incubation	
				LF	LF-N		ΔN_i	Final N_i				LF	LF-N		ΔN_i	Final N_i
23/ix/64	W	1.45*	421	4547	50.5	49.4	19.5	26.5	F	1.25*	329	3795	50.5	42.7	18.2	24.7
27/x/64	"	1.45*	394	4600	58.0	55.7	22.2	23.1	"	1.25*	317	3784	48.0	45.9	23.8	24.2
1/xii/64	"	1.45*	460	4416	66.2	42.2	19.5	20.4	"	1.25*	329	3569	46.4	43.1	15.6	18.8
2/vi/65	F	1.27	391	4098	48.3	34.6	20.3	21.9	"	1.22	332	3273	50.8	44.9	22.0	25.9
7/vii/65	"	1.47	413	4556	62.5	49.8	17.6	21.9	W	1.51	356	5926	69.9	56.7	25.3	27.2
12/viii/65	"	1.17	414	3924	50.3	47.5	19.4	20.1	"	1.44	470	4307	52.6	60.6	12.3	18.8
14/ix/65	"	1.57	466	5877	62.9	42.8	28.2	28.2	"	1.78	532	7130	81.3	71.0	26.0	33.9
25/x/65	"	1.58	473	5456	66.6	55.8	22.3	25.9	"	1.50	435	5370	76.3	60.6	22.0	25.8
24/xi/65	"	1.34	352	4652	58.2	52.1	20.0	23.1	"	1.64	431	4965	63.0	82.0	17.7	24.8
13/i/66	"	1.47	333	5116	67.5	66.3	17.9	28.2	"	1.44	326	4712	63.2	48.3	15.4	24.1
2/iii/66	"	1.27	334	3971	49.3	45.4	12.1	17.0	"	1.66	376	5296	66.2	68.9	11.7	16.5
21/iv/66	"	1.42	386	5753	63.8	51.1	16.0	23.3	"	1.47	413	5836	92.2	73.8	17.2	20.9

Plot Rotation	9 PPFW								11 PPFW							
23/ix/64	W	1.45*	526	7018	94.1	82.9	34.2	36.9	P_1	1.45*	473	6440	96.6	85.8	38.0	42.7
27/x/64	"	1.45*	460	5993	86.9	76.6	28.6	30.1	"	1.45*	486	7596	122.2	102.6	44.6	47.3
1/xii/64	"	1.45*	526	6505	91.7	77.7	33.0	34.8	"	1.45*	499	6085	97.9	78.2	41.4	51.6
2/vi/65	P_1	1.17	424	4411	66.2	54.7	28.8	35.6	P_2	1.18	364	5625	120.4	75.0	30.9	41.5
7/vii/65	"	1.44	574	7361	89.8	70.2	38.4	42.5	"	1.58	558	7862	121.9	105.0	26.1	50.0
12/viii/65	"	1.32	443	4809	69.7	63.5	24.8	26.4	"	1.50	408	7994	110.3	95.6	39.2	41.1
14/ix/65	"	1.54	572	7063	93.2	67.7	33.2	39.1	"	1.77	561	8775	140.4	112.9	46.7	51.8
25/x/65	"	1.78	726	8228	127.6	92.8	24.5	30.8	"	1.78	661	8163	127.3	98.6	39.7	44.8
24/xi/65	"	1.61	554	7953	139.9	118.9	23.6	32.0	"	1.31	463	5937	92.0	76.5	22.0	28.7
13/i/66	"	1.69	536	8363	107.1	102.5	21.6	46.1	"	1.53	513	8667	128.3	113.4	37.0	57.5
2/iii/66	"	1.50	462	8361	103.7	82.9	19.8	29.0	"	1.35	416	7182	115.6	83.7	20.7	34.5
21/iv/66	"	1.39	479	8819	122.6	84.5	23.4	34.6	"	1.38	463	6116	104.6	94.3	39.5	50.2

Plot Rotation	7 PPFW								12 PPFW							
23/ix/64	P_2	1.45*	644	8398	139.4	105.0	66.2	70.4	F	1.25*	419	5936	94.9	71.3	39.1	48.7
27/x/64	"	1.45*	697	8937	150.1	151.7	56.9	59.3	"	1.25*	476	6072	94.7	80.2	29.6	36.0
1/xii/64	"	1.45*	670	9081	145.3	119.1	44.8	58.9	"	1.25*	487	5699	87.8	81.3	29.2	39.7
2/vi/65	F	1.36	592	8111	148.4	139.9	51.0	61.5	"	1.19	442	5306	89.2	83.2	36.7	47.8
7/vii/65	"	1.67	848	11700	189.5	155.0	71.1	81.3	W	1.38	475	6116	86.8	78.0	41.4	44.5
12/viii/65	"	1.22	608	7652	141.5	123.8	41.0	56.0	"	1.29	433	5519	87.2	83.9	27.0	33.2
14/ix/65	"	1.58	659	9838	167.3	139.9	56.9	77.6	"	1.60	580	8715	124.6	118.0	36.1	45.4
25/x/65	"	1.66	782	11254	186.9	157.2	47.7	70.6	"	1.48	577	7901	124.1	110.7	29.6	36.2
24/xi/65	"	1.26	594	8268	157.9	158.3	32.5	63.8	"	1.51	479	7390	113.0	106.7	28.2	37.9
13/i/66	"	1.31	617	9807	160.9	108.4	29.0	62.7	"	1.42	412	7619	109.7	92.9	30.9	48.1
2/iii/66	"	1.20	522	7526	127.1	122.8	32.2	59.3	"	1.47	453	6901	110.4	92.2	16.7	36.5
21/iv/66	"	1.32	598	7585	116.2	103.6	30.6	62.6	"	1.46	503	6219	98.3	88.0	22.0	35.6

* estimated values (cf. p.153)

APPENDIX E: PROPERTIES OF SOME QUEENSLAND SOILS AND OF THEIR LF

Group	Series	Location	History	Depth (cm)	pH*	g/100 g OD Soil				g/100 g LF	
						<2μ	C	N	LF	'Ash'	'Humus'
Black earth	Waco clay	Darling Downs, Queensland	Cultivated	0-7	8.5	78	2.0	0.14	0%'OT : 0.4 0.1%'OT': 0.6	44 35	7.8 ND
Krasnozem	Gabbinbar	Toowoomba, Queensland	Virgin	0-5	5.8	16	11	0.84	0%'OT' :13.0 0.1%'OT':17.4	46 38	9.0 ND

* 1:5 (w/v) in water ND = not determined

Methods:
i) LF separated as in Appendix A with and without surfactant
ii) LF-'Ash' determined by ignition (Method B1)
iii) LF-'Humus' determined by treatment with alkali (Method B4)

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