MICROBIAL BIOMASS AND CARBON METABOLISM IN SOILS

by

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TABLE OF CONTENTS

LIST OF TABLES

Table

LIST OF FIGURES

Figure

SUMMARY

Studies on the decomposition of organic substances in soil have been reviewed with particular emphasis on organic fractions defined biologically rather than chemically. Methods available for the determination of microbial biomass in soil were also reviewed. The fumigation technique and the determination of ATP appear to be most suitable for estimating microbial biomass, especially in studies concerning the dynamics of organic carbon in soil. However, the application of the above two methods is limited by the conditions which prevail in the field, and during sampling and handling in the laboratory.

Seven soil samples collected fresh from the field were examined after various pretreatments in terms of content of ATP and biomass carbon. The ATP extracted was markedly and rapidly reduced by air-drying. However a short wetting phase prior to freeze-drying of air-dried soils increased the ATP content significantly. The increase in the content of ATP extracted during wetting of air-dried soils occurred in the presence of dinitrophenol and therefore was not due to synthesis but to other reactions. The net effect of freeze-drying on the extraction of ATP depended on the physiological state of the organisms. However, the nature of the changes associated with freeze-drying of the soils and their influence on the extraction of ATP was not fully understood. Storage of the freeze-dried soils at 25°C and -15°C led to substantial losses of ATP.

The effects of various pretreatments on the biomass carbon content of the soils were compared based on the amounts of CO_2 evolved from the fumigated and unfumigated soils during the 0-10 day incubation period. Biomass carbon content of the soils decreased after air-drying. The concentration of ATP in the biomass of the field moist, air-dried - freeze-dried, and soils incubated with water did not change significantly and were similar to the values reported by Jenkinson and coworkers. The

wide biomass C/ATP ratios in the air-dried soils were thought to be due to incomplete extraction of ATP. By contrast the ratios of biomass C/ATP in the biomass of the field moist and the soils incubated with water after freeze-drying were much lower because more ATP was extracted.

Two soils, viz. the Urrbrae fine sandy loam and the Northfield clay were fractionated based on particle size and density after dispersions using a Spex shaker or an ultrasonic probe. The content of organic carbon, nitrogen, ATP and the monosaccharide composition of the soil fractions were determined. The recovery of ATP from the soil fractions obtained after dispersion using the Spex mixer was poor and ATP was evenly distributed amongst the soil fractions. By contrast after ultrasonic dispersion the concentration of ATP was high for fractions of diameter 5-2 μm and <1 μm in the Urrbrae fsl and silt size particles of the Northfield clay. Determination of the ratios of the galactose + mannose/ arabinose + xylose indicated relative enrichment of microbial materials but not necessarily the living organisms in these fractions. A fractionation scheme was formulated based on the amounts of ATP and the organic material contained in the soil fractions obtained by physical means only and was used to study the decomposition of ^{14}C glucose in the two soils.

After incubation of ^{14}C-glucose the proportion of ^{14}C present as biomass was much higher in the Northfield clay than the Urrbrae fine sandy loam. A range of biomass C/ATP ratios for the labelled microbial population in the soils incubated with ^{14}C glucose is reported. Most of the ^{14}C and ATP in the Urrbrae soil was located in the <0.5 μm fraction during early stages of the incubation but subsequently there appeared to be a transfer of ATP and ^{14}C, presumably as microbial biomass, to the 5.0-0.5 μm fraction. The non-biomass-^{14}C present in the <0.5 μm and the 20-5 μm fraction was largely responsible for the disappearance of ^{14}C from the Urrbrae fsl as compared to the rapid losses of biomass-^{14}C from the 5.0-0.5 μm and the 20-5 μm fractions of the Northfield clay. Although

the retention of ^{14}C in the two soils was similar, considerable differences were observed in the dynamics of biomass and non-biomass materials.

DECLARATION

This thesis contains no material which has been accepted for the award of any other degree or diploma in any University, and, to the best of my 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.

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INTRODUCTION

The role of microbial biomass in soil is vital in the transformation of organic substances and nutrient cycling in soil. The biomass is also considered as a relatively labile pool of soil organic matter. Estimation of microbial biomass in soil by direct microscopic observation is often limited by technical problems, reliability and time. Alternatively indirect methods such as the fumigation technique and the determination of ATP offer promise for the estimation of microbial biomass, although they are not without limitations.

Over the last two decades the intensive application of isotopic tracer techniques have allowed identification of artefacts arising from the use of chemical reagents for extracting and fractionating soil organic matter. These chemical fractions have proved of little value in studying the dynamics of organic matter in soil. However, fractionation of soil by physical means only has been found to separate to some extent components of organic material based on their biological origin. The chemical nature and the content of organic material in these soil fractions has provided information on bonding mechanisms and nature of organo-mineral complexes in soil. However limited information is available on the abundance of microbial biomass in various fractions obtained from soils by physical means and the range of stabilities of organic materials in soils remains unexplained.

This thesis describes attempts to measure the microbial biomass in various fractions of soil obtained using physical methods only.

1. REVIEW OF LITERATURE

1.1 Dynamics of Organic Matter in Soil

1.1.1 Decomposition of organic substrates in soils

Decomposition of an organic substrate in soil is accompanied by synthesis of microbial materials which are protected by various mechanisms, against further rapid microbial degradation (Sorensen, 1969; Legg *et al.*, 1971; Felbeck, 1971; Cheshire, 1977). Studies with pure cultures have shown that the efficiency of carbon assimilation by microbial cells is usually about 60 percent of the carbon metabolized (Payne, 1970). Efficiencies lower than 40 percent have been reported for hydrocarbon-utilizing organisms (Vary and Johnson, 1967). Theoretically the efficiency of carbon assimilation may be as high as 70 percent for the aerobic bacteria (Camp, 1963).

Simple substances like glucose or other carbohydrates decompose in soils at a rapid rate initially, with a biological half-life of a few days (Sorensen and Paul, 1971; Oades and Wagner, 1971; Ladd and Paul, 1973). For plant residues, the calculated half-lives are 3-6 months (Jenkinson, 1965; Sauerbeck and Gonzalez, 1977). More resistant components, including microbial residues formed in soils decompose in soils with half-lives in the range of 4-8 years (Jenkinson, 1965; Sorensen, 1967; Shields *et al.*, 1973; Ladd *et al.*, 1981). In the early stages of decomposition, the rates are curvilinear indicating that a heterogeneous group of materials take part in the decomposition reactions. As the decomposition process slows down, the reactions involve more resistant homogeneous products of decomposition (Sorensen, 1972).

The proportions of the added carbon retained in the soils have been similar, irrespective of the source and climatic conditions (Jenkinson, 1971). Fifteen to twenty percent of glucose carbon remained after 3 years compared to 20 percent of straw carbon after 4 years of decomposition.

Decomposition was faster in

i) a sandy soil than in a clay soil (Jenkinson, 1977; Ladd *et al.*, 1977b);

ii) a cultivated soil compared with a soil under pasture (Martel and Paul, 1974);

iii) a bare soil compared with a soil under plant cover (Fuhr and Sauerbeck, 1968; Shields and Paul, 1973; Jenkinson, 1977);

iv) a tropical soil compared with a soil under temperate climate (Ayanaba *et al.*,1976).

1.1.2 Evaluation of conventional methods to study organic matter dynamics

Most studies of organic matter dynamics have been carried out according to classical methods,i.e.fractionation into humic acid, fulvic acid and humin components (Kononova, 1961) or have involved isolation and characterization into groups of specific chemical compounds (Bremner, 1965, 1967; Felbeck, 1965).

Most of the organic matter in soil exists in combination with mineral particles. In order to study the nature and dynamics of organic matter,it is often necessary to extract and then fractionate into groups of materials based on solubilities in acids, alkalies or organic solvents. Strong alkalies have extracted organic materials most efficiently but can create conditions suitable for oxidation, polymerization, condensation and hydrolysis reactions to take place (Sauerbeck and Fuhr, 1968). Classical schemes of this kind may indicate the nature of organic matter already present in the soil but have given limited information on organic matter dynamics. Studies of transformations of organic substances were aided by the use of labelled compounds or labelled plant materials (Jansson, 1958) but the duration of these experiments has generally been short as compared to the formation of organic matter in soil in the natural state. Artefacts during extraction can create problems, where minor changes amongst the conventional humus fractions are important (Sauerbeck and

Fuhr, 1968).

Jenkinson (1971), Oades and Ladd (1977) and Persson (1968) reviewed extensively the problems in applying the classical methods of soil organic matter fractionation to studies on the transformation of organic materials in soil. According to them, the main problem was the inability to obtain biologically discrete fractions. Classical fractions did not permit discrimination between fresh, decomposing and stabilized organic material and therefore could not be justified in terms of the microfloral development and activity in the soil (Jansson, 1958). Using a mild extractant like $Na_4P_2O_7$ to isolate recently metabolized materials also proved unsuccessful (McGill, 1971).

From the work of Persson (1968), it is evident that hydrolysis of soils by acids of increasing concentrations prior to alkali extraction could produce fractions which were related to biological processes. Most of the microbial tissues were extracted by reflux in 0.28 M H_2SO_4; materials soluble in hot 6 M HCl were thought to be the products of microbial synthesis and the plant components resistant to 6 M HCl were located in the humic acid and the humin fractions (Jansson and Persson, 1968).

Short term experiments have revealed that acid hydrolyzable amino acid components contributed to a greater extent than other fractions to the available N pool (Stewart *et al.*, 1963; Chu and Knowles, 1966; Miki and Mori, 1968; Freney and Simpson, 1969). However the dominance of the amino acids on the labile pool of N was short-lived and long term field experiments did not indicate any preferential degradation of amino acids compared with other components (Keeney and Bremner, 1964, 1966; Broadbent, 1968a, 1968b). Isirimah and Keeney (1973) reported the greatest percent decline in the amino sugar-N and unidentified-N component, whereas McGill (1971) found no difference in the percent decomposition rates of the amino acid, amino sugar and the unidentified-N components of the soil acid hydrolysate. Oades and Ladd (1977) after reviewing studies on soil

organic matter dynamics concluded that neither the classical fractions nor the chemical fractions based on 6M HCl hydrolysis are especially useful in tracing the biological transformations of organic compounds in soil.

1.1.3 Biological concepts of organic matter

Persson (1968) considered soil organic matter as an ecological unit and indicated that a study of the true humic substances alone would not reveal the biological evolution of humus in soil. Jansson (1966) considered that soil organic matter was composed of three groups of materials:

i) Decomposable material - consisting of dead plants and animals. Under normal soil conditions this group constitutes only a small proportion by weight of soil organic matter and may vary widely depending on the soil management.

ii) Decomposing material - constituting the soil microflora. Percent decline in the amount of organic-^{15}N derived from microbial biomass was greater than that of the total organic-^{15}N of the soils (Ladd *et al.*, 1977c).

iii) Decomposed material - consisting of material resistant to further decomposition.

From the existing literature on radiocarbon dating of soil organic matter, Clark and Paul (1970) indicated that the soil organic component consists mainly of three types of materials:

i) Decomposing plant residues and the associated soil biomass which turn over at least once every few years.

ii) Microbial metabolites and cell wall constituents that become stabilized in soil and possess a half life of 5-25 years.

iii) Resistant fractions which in grassland soils are composed of humic components ranging in age from 250-2500 years.

It is clear from the decomposition of organic substrates in soil that recently added substrates are metabolized very rapidly. Even after 4 years of decomposition, the rate of decrease of residues was several times faster than that of the native soil organic matter (Jenkinson, 1965). Thus the average biological stability of such material is intermediate to that of the fresh plant residues and that of very old parts of soil organic matter. Jenkinson (1971) suggested that the organic matter of intermediate stability may consist of

i) Soil biomass - although under normal soil condition, biomass is reasonably stable, appreciable mineralization of the zymogenous part of the soil population occurs due to conditions very similar to partial sterilization (Jenkinson, 1966). It was also observed that the microbial polysaccharides decomposed faster than the plant polysaccharides. The stability of microbial cell components varied widely (Mayaudon and Simonart, 1963) and the accumulation of cell wall material in soil from melanic fungi has been reported (Hurst and Wagner, 1969).

ii) Material sorbed on soil colloids. These are secondarily formed substances due to microbial metabolism.

iii) Mostly lignin together with a small proportion of cellulose. These substances are relatively resistant to decomposition.

Oades and Ladd (1977) suggested that fractionation by physical methods prior to chemical analysis would separate organic components into 1) soil biomass, separated according to organism size and its association with their larger plant fragments or with inorganic components of the soil, 2) extracellular or lytic nitrogenous products separated as soluble compounds of different molecular weights or as insoluble constituents, either free or bonded to inorganic soil colloids.

1.1.4 Physically defined fractions and their characterization

It has been suggested that the partly humified organic material is a potentially mineralizable component of organic matter (Clarke and Marshall, 1947; Greenland, 1962; Saunder and Grant, 1962, Nye and Greenland, 1964). It can be separated by density techniques (Khan,1959). Greenland and Ford (1964) and subsequently Ford *et al*. (1969) developed a method of separating the partly humified material from soil using ultrasonic dispersion and floatation on a bromoform-petroleum spirit mixture of density 2.0 g cm^{-3} with an addition of a surfactant to the suspension. Oades and Ladd (1977) indicated that physical fractionation techniques involving particle size and density separation of organo-mineral complexes could give biologically defined components. Important classes of such materials are discussed under the following groups.

1.1.4.1 Light fraction

Ford (1968) made detailed investigations of the light fractions of some South Australian soils. Microscopic examination of the light fraction showed recognizable cellular structure in the size range of 100-250 µm. This fraction may constitute 0.8-8.0 percent of soil and may represent 20-85 and 11-100 percent of the carbon and nitrogen respectively. The ash content of the light fraction was approximately 50 percent and included amorphous phytoliths of various sizes and form (Greenland and Ford, 1964). The chemical nature and amounts of this fraction were governed by soil management. Sometimes lower amounts of these fractions may be attributed to higher biological activity and in clay soils, the amount of this fraction was greater in a sandy soil.

The C/N ratio for light fraction from most soils was lower than 25 and may be as low as 11 for clay soils. Khan (1959) and Monnier *et al*. (1962) however, obtained C/N ratios higher than 25. About 3-14 percent of the material in this fraction was humified. Amino sugar and

carbohydrate analysis indicated that the composition of this fraction was intermediate between that of fresh plant residues and humic acids. Glucose was the dominant sugar (45%) together with xylose and only small proportions of galactose, mannose, fucose and rhamnose (Whitehead *et al.*, 1975). Ford (1968) suggested that microorganisms may be present in the light fraction, but there was no experimental evidence in this regard.

Light fraction was correlated with the stability of aggregates (Oades, 1967) by acting as a substrate for the synthesis of polysaccharides by soil microorganisms (Allison, 1968).

Ford (1968) showed that light fraction-N was more labile and mineralizes 4-40 times faster than the remainder of the soil. He concluded that, although light fraction may provide a better measure of the available-N as compared to the chemical techniques, it did not fully account for the available-N.

1.1.4.2 Silt and clay fractions

Use of ultrasonic dispersion techniques together with fractionation of soil based on particle size and density yielded comparatively less heterogeneous organic components than the whole soil (Monnier and Turc, 1964; Greenland, 1965; Bremner, 1967; Pokotilo, 1967; Chichester, 1969; Turchenek and Oades, 1974). Turchenek (1975) reviewed the literature on the distribution of C and N in the particle size fractions and revealed two classes of soils : 1) calcareous clay soils with highest concentrations of C and N content in the coarse clay and fine silt fractions; 2) leached soils with highest concentrations of C and N in the finest particle size fractions. Enrichment of carbon and nitrogen of the order of 2-3 times that in the whole soil was reported in the fine silt and colloidal fractions (Titova , 1976). Cameron and Posner (1979) suspected that most of the microbial material was located in these size ranges. McGill *et al*(1975) demonstrated that fungal cell wall materials were concentrated

in the >0.2 μm fraction and the cytoplasmic materials were adsorbed in the <0.04 μm fraction. Materials associated with the >0.2 μm fraction were predictably more stable than the <0.04 μm fraction (Wagner, 1968).

C/N ratios were higher for low density fractions and varied widely within each particular size fraction for the acidic soils, but for the clay soils, C/N ratios did not differ amongst density fractions of a particular size fraction, indicating similarity in the composition of organic material. A relatively high concentration of carbonaceous materials was reported to be present in the fine silt fraction separated at density <1.8 g cm^{-3} (Satoh and Yamane, 1972; Turchenek, 1975). Relative enrichment of C and N occurred in coarse sand, coarse silt and fine silt fractions and at densities <2.30 g cm^{-3} within each particular size fraction (Turchenek, 1975).

Organic materials in the clay size fractions are more aliphatic (Turchenek, 1975; Travnikova and Titova, 1978), more hydrolyzable, less humified, richer in nitrogen and are more susceptible to microbial attack than organic materials in silt size fractions (Kyuma *et al.*, 1969). Organic materials in the fine silt fraction were more extractable ($Na_4P_2O_7$ followed by $Na_4P_2O_7$-NaOH) than those in the clay fraction (Turchenek, 1975) but opposite trends were observed for clay soils (Grati *et al.*, 1965; Kyuma *et al.*, 1969). Factors responsible for the differential extractability of the fractions were ascribed by Arshad and Lowe (1966) to differences in surface areas, type of clay minerals, nature of bonds, physical accessibility and the extent of humification. Stepanov and Vysotskaya (1975) indicated that fractionation based on particle size and density will give dissimilar humic materials.

Rapid microbial incorporation of ^{14}C from glucose and ^{14}C from dextran into galactose and mannose was shown by Oades and Wagner (1971). Swincer *et al.* (1968) considered mannose, arabinose, rhamnose and ribose as microbial polysaccharides. But the significance of the proportions of

galactose, mannose, arabinose and xylose as markers of microbial or plant origin is questionable (Lowe, 1978). Lowe (1978) and Morita and Montgomery (1980) concluded that the use of arabinose/xylose and rhamnose/glucose ratios provided better indication about the nature of origin and the use of mannose/galactose ratios were unsatisfactory. Cheshire *et al.*(1971), Morita and Montgomery (1980) obtained hexose/pentose ratios of 3-4 and the higher hexose content was attributable due to the synthesis of microbial polysaccharides and not due to physical protection. The amount of hexoses synthesized from the recently added organic material are very small as compared to the hexoses present in the native soil organic matter and therefore often no changes in the ratios of total polysaccharide sugars are observed (Cheshire *et al.*, 1971).

However the use of hexose:pentose ratios may not always provide information regarding microbial enrichment when determined on the soil, because of the possible presence of large quantities of free plant residues in which glucose is the dominant component. Significantly higher mannose + galactose: arabinose + xylose ratios are observed for the coarse clay and fine silt fractions. These fractions were thought to be enriched with microbial bodies and products which contributed to the stability of these microaggregates towards ultrasonic dispersion (Turchenek, 1975). From the literature discussed so far it is evident that the indirect chemical methods of biological characterization did not always indicate the nature and origin of organic materials in soil. Therefore, measurement of microbial biomass on soil fractions obtained using physical methods may be useful in understanding the nature and origin of the organic material present in the fractions.

1.1.5 Mineralization studies involving size and density fractions

Chichester (1969) showed that the mineralization capacity of the finer size particles was several times greater than that of the coarser

fractions. This difference in the nitrogen mineralization from various particle size fractions was attributed to the differences in the proportions of readily, versus difficultly, extractable chemical forms of nitrogen.

Nitrogen immobilization and mineralization studies with $^{15}NO_3^-$ after addition of glucose and straw in two calcareous soils of different texture were carried out by Ladd *et al.* (1977a, 1977b, 1977c), Ladd and Amato (1980) and Amato and Ladd (1980). The extent of decomposition of the immobilized ^{15}N was faster in sandy soil than in a clay soil, and in a glucose amended soil compared with a straw amended soil. The availability of recently immobilized ^{15}N was greater than the native soil-N. Organic materials from the soils were then fractionated into high and low molecular weight compounds extractable in $NaHCO_3$ and into different physical fractions based on particle size and density. The changes in labelled and unlabelled N are discussed in the following sections.

1.1.5.1 High and low molecular weight compounds

During the immobilization phase of $^{15}NO_3^-$ in soils amended with either straw or glucose, rapid formation of $NaHCO_3$-extractable high (>10,000) and low (<10,000) molecular weight compounds resulted. These were highly labelled extracellular compounds. The degree of labelling of the high molecular weight compounds in the clay soil was higher than in the sandy soil. This difference was thought to be due to the differential adsorption of both the labelled and unlabelled products by the clay soil and, as well as to the differences in the nature of microbial populations. The nitrogen content of these high and low molecular weight compounds was related to the specific absorbance at 260 nm and 400 nm, indicating that these molecular weight fractions are loosely adsorbed humic materials.

During the early period of immobilization (2 days), the low molecular weight compounds had the highest degree of labelling which declined rapidly

and became the least labelled fraction which appeared stable by day 8 of the incubation. Similarly the high molecular weight compounds also became strongly labelled at the beginning of the immobilization period but the decline in label commenced before the net mineralization period. This suggests that these compounds are utilized by the actively metabolizing microflora and transferred to some other fractions (Ladd and Paul, 1973; McGill *et al.*, 1975).

1.1.5.2 Light fraction

Light fraction separated at density 1.59 g cm^{-3} by Ladd *et al.*(1977a) represented about 7 percent and 3 percent of the unlabelled N for a sandy and a clay soil respectively. The weight of the fraction was highly dependent on soil type, nature of carbon addition and the period of incubation. A higher yield of this fraction was obtained if the separation was done on particle size fractions rather than the whole soil. Approximately 20 percent of the organic-^{15}N became associated with the light fraction in straw amended soils whereas in glucose amended soilsvery little (<1.2 percent) was immobilized in the light fraction. Light fraction of the straw amended soils showed a decline in the weight accompanied by significant increases in the percentage N content during the early immobilization phase. In glucose amended soils neither the weight of the fraction nor the percentage of unlabelled N varied with time. Light fraction of the soils after fumigation showed a greater decline in the ^{15}N-labelled material than the unlabelled N.

1.1.5.3 Silt and clay fractions

Distribution of immobilized ^{15}N and the pattern of changes amongst soil fractions with time were dependent on the type of carbon amendment and not on the soil type. Light fractions (sp.gr. <2.06) of the glucose amended soils at the end of the net immobilization phase represented 43-64

percent of the total ^{15}N of the silt, 1-9 percent of the coarse clay and 19-21 percent of that in the fine clay fraction. In soils incubated for two days with $^{15}NO_3^-$ and amended with glucose, the fine clay fraction of the sandy and clay soils contained 34 and 63 percent respectively of the total immobilized-^{15}N. After two days there was a significant decline in the ^{15}N of the fine clay fraction (day 8) accompanied by increases in ^{15}N of the silt and coarse clay fractions. This was partly attributable to the biomass. At the end of the net mineralization phase, the proportions of the residual ^{15}N in the fine clay and coarse clay fractions exceeded that of native ^{14}N in these fractions. There was a net loss of ^{15}N from the silt fraction in the glucose amended soils and the availabilities of ^{15}N and ^{14}N became similar. The silt fraction contained an increased proportion of more stable nitrogenous residues, resulting from either slower rate of decomposition of the nitrogenous residues or transfer of immobilized N from other fractions, or both.

The nature of the nitrogenous materials transferred from the fine clay was not known. Three kinds of possibilities may exist. Firstly extracellular and lytic products resembling high molecular weight compounds ($NaHCO_3$-extractable) were metabolized by microbial cells and were associated with larger particle size fractions. Secondly, most of the nitrogenous substances in the fine clay fraction were probably comprised of microbial cells of low viability and were subsequently metabolized by larger organisms. Thirdly, the population in the fine clay fraction on day 2, was rapidly growing and was more easily dispersed than the fairly old stable microbial cells associated with the later phase of the incubation period.

At the end of the net mineralization phase (160 days), the proportion of the labelled to unlabelled residues was higher in the fine clay fraction than the coarse clay and the silt fraction, but the amount of

^{15}N biomass was less in the fine clay fraction. In the long term as the ratio of mineralization of labelled and unlabelled residues in the whole soils decreased from approximately 5:1 to 1:1, the labelled material continued to decompose disproportionately faster in the fine clay components. Because the labelled biomass material decomposed faster than the non-biomass fraction, at the end of the incubation the silt and coarse clay fractions contained the highest proportions of biomass.

The distribution of ^{15}N in the light and heavy subfractions varied with time. There was a gain in ^{15}N in the light and heavy subfractions between day 2 and 16 and then a decline followed by fumigation at 160 days, indicating that biomass represented a significant proportion of the light fraction nitrogen in the recently immobilized organic residues.

The distribution of organic-^{15}N in the light and heavy subfractions (sp.gr. 2.06) of the silt size particles were similar (about 50 percent). During the period of net mineralization the overall decrease in the organic-^{15}N from silt size fractions was about 65 and 50 percent for the sandy and clay soils respectively. Approximately 80 percent of the decrease in the organic-^{15}N of the fine clay fraction during the net mineralization period was due to the decrease in the organic-^{15}N of the heavy subfractions, although the relative decline was less than in the complementary light fraction. It was concluded that when small proportions of soil organic N were associated with macroorganic debris amended with glucose and $^{15}NO_3^-$, densimetric fractionation at a sp.gr. as high as 2.06 yielded light and heavy subfractions with nitrogenous components similarly susceptible to biological attack. Use of lower density (e.g. 1.6 g cm^{-3}) was thought to separate relatively higher proportions of macroorganic matter and thereby enhanced metabolism of the light fraction was expected.

1.2 Microbial Biomass in Soil

1.2.1 Methods for the determination of microbial biomass

Microbial biomass in soil plays a key role in the metabolism of organic substances in soil and is involved in almost all the biochemical reactions. Biomass is involved in nutrient cycling and many aspects of nutrient availability.

It is therefore useful to be able to determine the size of this important component of the ecosystem. Studies of microbial growth and nutrient turnover by soil microorganisms could be achieved by simultaneous measurement of microbial biomass by microscopic techniques, adenosine triphosphate content (ATP), respiration and specific enzymic activity. Biomass estimation from measurements of muramic acid, diaminopimelic acid (DAP) and deoxyribonucleic acid have also been reported.

1.2.1.1. Microscopic techniques

To date available microscopic techniques for the estimation of biomass include the agar slide technique with either phenol aniline blue (Jones and Mollison, 1948) or phase contrast microscopy (Frankland, 1974). Organic dyes like acridine orange (Trolldenier, 1973), fluorescein isothiocyanate (FITC) (Babuick and Paul, 1970) or 1-amino-8-naphthalene sulphonic acid (Mayfield, 1975) are used in fluorescent microscopic techniques which can easily resolve microorganisms from the detrital material in soil. Peterson and Frederick (1979) described a simple and rapid direct microscopic counting procedure using polystyrene beads as "ratio particles" to estimate microbial numbers and biomass in soil. Similar estimates of biomass were obtained with agar film techniques and the bead ratio method, but gave 2 to 35 times greater numbers than obtained by plate count. Greaves *et al.*(1973) also observed that the bacterial counts by fluorescein isothiocyanate (FITC) method was about 10^4 or 10^5 times higher than the dilution plate method. Existing microscopic techniques for the estimation of microbial biomass such as direct microscopic counting and plate counting are extremely

unreliable and not realistic in terms of time and labour. Plate counting greatly underestimates the biomass since not all classes of organisms grow in a particular medium whereas direct microscopic examination provides only the quantitative picture of the organisms but cannot distinguish between inactive cells, spores and metabolising organisms. Babuik and Paul (1970) found no correlation between the numbers of organisms obtained by direct microscopy and plate count technique. Bacterial biomass in an agriculturally productive soil is made up of several billion cells per gram of soil (Clark and Paul, 1970; Torsvik , 1980). Clark and Paul (1970) stated that the approximate biomass ($g/m^2/30cm$) of bacteria and actinomyctes was 57 g and 138 g for fungi.

1.2.1.2 Muramic acid

Bacterial biomass in soil can be estimated from the measurement of muramic acid content, a cell wall component of bacteria. The content of muramic acid for 33 different soils ranged from 0-150 µg g^{-1} dry weight of soil. The concentration of muramic acid in the bacterial cells in soils was 100-1000 times greater than in pure cultures. However comparable concentrations of muramic acid in the bacterial cells of soils were obtained when the total number of the bacterial population was determined by some methods other than plate counts. It is therefore suggested that the measurement of muramic acid in soil may provide a reasonable estimate of bacterial biomass (Miller and Casida, 1970).

Moriarty (1975) described a method for the determination of bacterial biomass from the measurement of muramic acid. Later he (Moriarty, 1977) improved the sensitivity of the previous method in order to be able to determine the muramic acid content of marine and terrestrial sediments. Muramic acid was determined by assaying the reduced NAD (a product of the oxidation of D-lactate, derived from muramic acid), with bacterial

luciferase. In order to be able to obtain reliable estimates of bacterial biomass, it is necessary to determine the approximate proportions of the main groups of bacteria, as the level of muramic acid amongst different groups of bacteria was found to vary widely. Bacterial biomass C can be calculated using the formula:

$$C = 1000\ MA/(12n + 40p)$$

where n is the proportion of Gram negative or weakly Gram positive bacteria and p is the proportion of strongly Gram positive bacteria. For colloidal material in surface marine waters, where strongly Gram positive organisms (with a high content of muramic acid) are probably abundant, a value of 15, substituted for the denominator would accommodate some variation in population composition. Muramic acid in the cell wall fragments degraded rapidly, therefore it is unlikely that accumulation of muramic acid in soil would be the source of large errors in the estimation of bacterial biomass.

1.2.1.3 Diaminopimelic acid

Diaminopimelic acid (DAP), a constituent of bacterial cell wall mucopeptide was used to estimate the proportion of bacterial biomass in rumen contents (Shazly and Hungate, 1966). The variability in the level of DAP limited its application as a criterion for biomass measurements (Synge, 1953).

1.2.1.4 Deoxyribonucleic acid and chlorophyll

Torsvik (1980) found that the DNA content of soils ranged from 90-187 $\mu g\ g^{-1}$ soil and the bacterial biomass could be estimated from the DNA content, 8.4 fg (10^{-15} g) per bacterial cell. Holm-Hansen (1969) reported that biomass estimation of the phytoplankton and microzooplankton from measurement of chlorophyll content were in good agreement with direct observation. He considered that the use of DNA for biomass estimation

of pure cultures was reliable but for ocean waters, DNA overestimated the biomass due to the presence of DNA in the detrital fraction.

1.2.1.5 Fumigation technique

Jenkinson (1966) compared the amount of labelled and unlabelled CO_2 released after different stress treatments viz. air-drying, irradiation, $CHCl_3$ vapour, CH_3Br vapour, oven-drying at $100^{o}C$ and autoclaving, which were known to partly or completely sterilize the soil. The highest amount of CO_2 was released from soils treated with $CHCl_3$ or CH_3Br vapour, prior to inoculation and incubation. He examined different theories for the flush of decomposition and considered that the flush largely, if not completely, was due to the decomposition of the organisms killed during fumigation. Fumigation followed by incubation rendered a small heavily labelled fraction of soil organic matter decomposable. He also showed that the C/N ratio of this fraction was low compared with the rest of the organic matter fraction and considered that this fraction was the soil biomass. Labelled C in the biomass declined relatively faster than the non-biomass C. He considered that the labelled part of the biomass as zymogenic (with a half life of approximately 1.5 years) and the unlabelled biomass was largely autochthonous, stable part of the soil population.

Jenkinson (1966) suggested that the size of the soil biomass could be estimated from the flush of decomposition which was defined as $F = (h' + b') - (h + b)$ where b' and b are the amounts of C in the biomass mineralized in time Δt at the time of fumigation from the fumigated and unfumigated soils respectively; h' and h are the corresponding amounts of non-biomass C mineralized in time Δt from the fumigated and unfumigated soils. Jenkinson and Powlson (1976a) showed that the flush was caused by the killed or damaged organisms and the fumigation did not accelerate the decomposition of non-biomass C. Shields *et al.*(1974) suggested that over-

estimation of biomass C could occur due to the accelerated decomposition of non-biomass C after fumigation. Subsequently Jenkinson and Powlson (1976a) applied a k value of 0.5 instead of 0.3 to the results of Shields *et al.*(1974) and the calculated biomass was 44% of the labelled residual C. Similar estimates of biomass were reported by Jenkinson (1966) and therefore Jenkinson and Powlson (1976a) disagreed with the possibility of accelerated non-biomass C decomposition resulting from fumigation and on this basis h'= h. Jenkinson (1976) also showed that the incubation period was short as compared to the turnover time of soil biomass, then b'>>b and therefore F = b'. If k is the fraction of biomass C mineralized to CO_2 in time Δt and B is the total amount of C in the biomass, then approximately B = F/k.

Ayanaba *et al.*(1976) showed that the amount of CO_2 evolved was closely correlated (r = 0.96) with amount of the nitrogen mineralized after fumigation and suggested that a rough estimate of biomass also could be obtained by multiplying the flush of mineral N by 8.

1.2.1.5.1 Measurement of CO_2 from unfumigated soil

Determination of the size of the flush (F) involves the measurement of CO_2 evolved from the fumigated soil less that evolved from the unfumigated soil, incubated over the same period of time, under the same conditions. Jenkinson (1966) suggested that the amount of CO_2 evolved from fresh unfumigated soils should be measured during the 0-10 day incubation period. Increased respiration was observed for soils stored frozen (Ivarson and Sowden, 1970; Mack, 1963; Soulides and Allison, 1961; Ross, 1972; Jenkinson and Powlson, 1976a) but the effects were short lived. Jenkinson and Powlson (1976a) and Ayanaba *et al.*(1976) suggested that the amount of CO_2 released from unfumigated soils which was treated in some other way, should be measured over a 10-20 day incubation period in order to overcome any sudden flush due to handling and storage.

1.2.1.5.2 The k factor

Jenkinson (1966) showed that 28 percent of the labelled C in Nitrosomonas cells were released as CO_2 after fumigation, inoculation and incubation for 10 days. He suggested that a provisional k value of 0.3 could be used roughly to estimate soil biomass until a better value was established, based on the decomposition of a wide range of organisms. Subsequently Jenkinson (1976) found the k value (the fraction of C in the killed organisms respired as CO_2) as 0.5, based on the decomposition of a range of organisms. He considered that the new value (k = 0.5) was a little large probably for two reasons, firstly the indigenous soil population had greater proportion of C in the cell walls and secondly the fumigation of the undried organisms renders less biomass C decomposable than fumigation of the freeze-dried organisms. Considering the existing literature on the decomposition of microbial cell components (Hurst and Wagner, 1968; Meyer, 1970), Jenkinson (1976) suggested that a k value of 0.45 was the closest approximation.

Anderson and Domsch (1978a) reported that the mineralization of 15 fungal and 12 bacterial species after fumigation and incubation for 10 days at $22^{o}C$ was 43.7 ± 5.3 and 33.3 ± 9.9 percent respectively. It is interesting to note that these two groups of organisms differed considerably in the extent of decomposition in contrast to the finding of Jenkinson (1976) who observed that the extent of decomposition of the bacteria and fungi after fumigation was similar. However, Anderson and Domsch (1978a) suggested that this difference was due to the differences in the chemical composition of the cell walls as well as in the proportion of cell wall and cytoplasmic materials in bacteria and fungi. Since cell walls were generally more resistant to degradation than cytoplasm, fungi with higher proportions of cytoplasmic material were considered to be mineralized more than bacteria.

Anderson and Domsch (1975) showed that the biomass accounted for as

bacteria and fungi was 25% and 75% respectively of the soil biomass. Anderson and Domsch (1978a) therefore suggested that instead of using the mean value of the percent mineralization from bacteria and fungi, the proportion of bacteria and fungi present in soil should be taken into consideration when calculating the k value. On that basis bacterial contribution to the k value was (0.25 x 33.3) 8.3 percent, for fungi (0.75 x 43.7) 32.8 percent and the common value of 0.411 at 22°C.

1.2.1.5.3 Validity of the fumigation technique

Jenkinson and Powlson (1976b) indicated that the correctness of the measure of biomass was influenced by difficulties associated with the determination of respiration from the unfumigated soil, apart from the use of an arbitrary k value (Jenkinson, 1976). Jenkinson and Powlson (1976b) also stressed that soils should not be air-dried before fumigation. Air-drying resulted in the death of a fraction of the organisms and rendered a portion of the non-biomass C decomposable. Analagous effects due to freezing and thawing and mechanical disturbance were expected but the effects were thought to be much less than for air-drying.

Jenkinson and Powlson (1976b) suggested that measurement of respiration from the unfumigated soils during 0 -10 or 10-20 day period would lead either to underestimation or overestimation of the biomass of soils that had some kind of pretreatments. Also this method would not provide accurate estimates of biomass of the soils with recent additions of substrate. Additional errors also could arise from the decomposition of bicarbonate in highly calcareous soils.

Jenkinson and Powlson (1976b) suggested that almost all of the artefacts could be avoided if the fumigation method was applied to a soil that had 10 days preincubation period in the presence of alkali. However, he indicated that the preincubation treatments may not be suitable under all circumstances.

1.2.1.6 Physiological methods

Anderson and Domsch (1978b) developed a simple, rapid and objective estimation of the biomass carbon of non-resting living microbial population in soil. The method is based on the initial respiratory response of microbial populations to amendment with an excess of carbon or energy source.

As a preliminary step, four substrates were tested to determine the substrate induced maximal rate of soil respiration. Of them glucose and casamino acid produced the highest respiration rate. Because of its wide application in microbiological studies, glucose was considered the most suitable substrate. Measurement of the rate of release of CO_2 one hour after glucose addition yielded zero slope on the respiration rate curve and was considered to be representative of the original population.

Quantification of the values of the substrate induced maximal initial respiration were done from the measurement of biomass carbon by a slight modification of the fumigation technique (Jenkinson and Powlson, 1976b) where CO_2 for the fumigated soils was measured for periods starting at a time when respiration was greater than the control soil to the period approaching unity. A significant correlation ($r = 0.96$) existed between the values of the initial maximal respiration rate induced by the substrate and the biomass C. At $22^{o}C$ 1 ml of CO_2 h^{-1} represented 40 mg biomass C. It was suggested that the method could be successfully applied to find out the biomass of different groups of organisms by selective inhibition techniques.

1.2.1.7 Adenosine triphosphate

The ubiquitous nature of ATP (Lehinger, 1965; Atkinson, 1971), its absence in dead cells (Holm-Hansen and Booth, 1966), rapid disappearance of extracellular ATP (Conklin and MacGregor, 1972), relative constancy of concentration under most endogenous conditions and sensitivity towards

metabolic activity (Patterson *et al.*,1970) along with the analytical simplicity and rapidity of determination make it a very useful tool for estimating microbial biomass (Bancroft *et al.*,1976).

Determination of ATP involves the measurement of light emitted from the interaction of ATP with Luciferin (LH_2), Luciferase (E) and atmospheric oxygen. The amount of light is proportional to the concentration of the ATP present, as long as other constituents of the reaction are present in excess. Representation of the bioluminescence reaction are described as follows (McElroy *et al.*,1969).

$$ATP + LH_2 + E \xrightarrow{Mg^{++}} E - LH_2 - AMP + P - P$$

$$E - LH_2 - AMP + O_2 \xrightarrow{\text{neutral pH}} E + \text{product} + CO_2 + AMP + \text{light}$$

The amount of light emitted is usually measured with liquid scintillation spectrometers (Stanley and Williams, 1969; Jenkinson and Oades, 1979).

1.2.1.7.1 Factors affecting the determination of ATP

The reliability of the determination of ATP depends on the completeness of extraction which is dependent on the retention of ATP, or destructive hydrolysis during extraction, or the presence of higher concentrations of inhibitory solutes in the extract (Bancroft *et al.*,1976). For marine water samples, the yield of ATP was lowered by 10-50 percent due to the adsorption phenomena associated with the sediments and the yield was considerably improved by extracting small sample volumes (Bancroft *et al*,1976).

Lee *et al.*(1971a) explained the poor recovery of added ATP due to the adsorption of ATP on the positively charged sites of the Fe and Al hydroxides at neutral pH or to the adsorption of ATP to the negatively charged sites after protonation of the $-NH_2$ group on the adenine part of the molecule.

The yield of ATP was lower for the soils with higher clay content (Anderson and Davies, 1973; Ausmus, 1973), but Lee *et al.*(1971a) reported

that ATP recovery was not related to any sediment property and was a characteristic reproducible property of a given sediment.

1.2.1.7.2 Efficiency of various extractants on the recovery of ATP

Jenkinson and Oades (1979) considered that cold 0.3M H_2SO_4, butanol-octanol, boiling Tris buffer and cold 0.5M $NaHCO_3$-$CHCl_3$ were more satisfactory reagents for the extraction of ATP. Karl and La Rock (1975) showed that ATP extraction with Tris buffer yielded very low (3-6%) recovery of added ATP as compared to the H_2SO_4-EDTA extraction procedure. EDTA complexed the Ca^{++} ions from solution and reduced ionic effects and interferences by the bacterial enzymes present in the extract and improved light emission (Lundin and Thore, 1975).

Paul and Johnson (1977) pointed out that the use of acidic reagents could create problems like neutralization by the carbonates, reactions with organic matter and coextracting large concentrations of sesquioxides. Their extraction procedure included vortexing with $CHCl_3$ which lysed the cells and the inclusion of $NaHCO_3$ to the extractant to compete with ATP for the adsorption sites. Dhople and Hanks (1973) also used $CHCl_3$ to lyse the cells followed by the removal of $CHCl_3$ by heat and evacuation and then reconstituted the cell material in 0.05M N-tris(hydroxymethyl)-methyl-2-aminoethane (TES) buffer immediately before assay.

Jenkinson and Oades (1979) devised a method for the extraction and measurement of ATP from soil. They showed that the recovery was greater using an extractant containing trichloroacetic acid, orthophosphate and paraquat than trichloroacetic acid alone or with sulphuric acid. Soil extractant ratio of 1:10 and ultrasonification were recommended. Wider soil:extractant ratios were shown to improve the recovery of ATP added from highly calcareous soils. The assay procedure included a buffer solution (0.1M arsenate, 0.001M Mg^{2+} and 0.002M EDTA and pH 7.4) and an addition of 12 µg of luciferin per vial. Light emission was monitored in

the Liquid Scintillation Spectrometer with the gain set at 100 percent and with a narrow window setting (50-90). The vials were counted for 0.1 minute one hour after addition of the enzyme.

Jenkinson, Davidson and Powlson (1979) demonstrated that the recovery of added ATP was greater by the $NaHCO_3$-$CHCl_3$ method (Paul and Johnson, 1977) but the extraction of native ATP was only a third of that by TCA-phosphate paraquat method.

Eiland (1979) showed that extraction with H_2SO_4 or dimethylsulphoxide (DMSO) followed by clean up step with Na^+ resin and then adjusting to pH 9.8 with ethanolamine improved the recovery of added ATP and were sensitive enough to detect as low as 5 ng ATP g^{-1} soil.

1.2.2 ATP content of soils

Jenkinson and Oades (1979) reported that the ATP content of 13 different Australian soils ranged from 0.64 to 9.03 µg g^{-1} soil and subsequently Jenkinson *et al.*(1979) reported the ATP content of 6 English soils, ranged from 1.22 to 8.74 µg g^{-1} soil. ATP content of the soils were highly correlated with the organic carbon and nitrogen content of the soils. It was also indicated that the management practices that improved the level of organic matter gave higher ATP contents. Grassland and forest soils had the highest ATP contents and arable soils least.

1.2.3 Concentration of ATP in the biomass

Lee *et al.*(1971b) stated that under most growth conditions ATP represented 1 to 4 µg mg^{-1} biomass (dry wt basis). Ausmus (1973) studied the ATP concentration of bacteria, actinomycetes, fungi and algae at different growth phases, using H_2SO_4 as an extractant. Mean ATP levels of the six species of the four different groups at log growth phase were: bacteria 1.81 µmoles, fungi 3.98 µmoles, actinomycetes 4.14 µmoles and algae 6.34 µmoles g^{-1} dry wt of biomass. The concentration of ATP in

these organisms fell to 35 percent at the lag phase. He indicated that the biomass estimated from ATP data was significantly higher than the biomass estimated from dilution plating and direct counting. He indicated that 73-95 percent of the ATP content of the soil and litter ATP could be predicted from the regression equation between ATP and CO_2 evolution from soil.

In spite of the above variations in the level of ATP, D'Eustachio and Johnson (1968) suggested that bacterial biomass could be estimated from the mean value of 4.7×10^{-10} µg ATP/cell.

Bancroft *et al.*(1976) reported that the biomass C/ATP ratios at the stationary phase of the Aerobacter Aerogenes and a marine isolate were 132:1 and 292:1 respectively. Nannipieri *et al.*(1978) observed that the biomass/ATP ratios (biomass determined by microscopic techniques) ranged from 313 for the soils after prolonged storage and could be as low as 49 for soils after 30 hours of C + N + P treatment and stabilized at 82 during 12-22 days incubation period.

Cavari (1976) reported that the biomass C/ATP ratios determined for lake water ranged from 200:1 to 5000:1 and the variation was ascribed to P deficiency. However he regarded the biomass estimation from ATP measurements as more reliable than counting procedures. He stressed that under most conditions the ATP content of the biomass would not be expected to vary more than 10 times as compared to the colony counting in which case the variability could rise up to 10^4.

Oades and Jenkinson (1979) observed a linear relationship between the ATP content and the biomass C content, as measured by the fumigation technique. The ATP content of the biomass (based on k value as 0.45) was 7.49 mg ATP g^{-1} biomass C or biomass C/ATP ratio of 133. In glucose incubated soils, ATP content of the biomass was a little higher than the native soil biomass.

Subsequently Jenkinson *et al.*,(1979) reported the biomass C/ATP ratio as 138 or 7.25 mg ATP g^{-1} biomass C, based on the combined data on Australian and English soils under different climatic conditions. They observed that the ATP content of the biomass was unaffected by P status ($NaHCO_3$ extractable P) which is contradictory to the findings of Nannipieri *et al.*(1978). They considered that under the conditions of Nannipieri's *et al.*(1978), the organisms were fresh and rapidly changing and therefore were more susceptible to environmental stress in contrast to the population normally existing in the soil.

1.2.4. Implications of ATP data

Metabolic states of the organisms could be assessed from the calculation of energy charge (Atkinson and Walton, 1967), which is defined by the following equation:

$$\text{Energy charge (EC)} = \frac{(ATP) + (\frac{1}{2}ADP)}{(ATP) + (ADP) + (AMP)}$$

Chapman *et al.*,(1971) observed that organisms in their active phase of growth had EC greater than 0.8, at stationary phase 0.8-0.5 and below 0.5 either death occurs or the organisms survive in dormant forms of life.

The amount of different nutrient elements immobilized in the microbial tissues could be estimated from the ATP data and hence would be useful in terrestrial decomposition and immobilization studies (Ausmus, 1973). By applying the ATP/C/N/P/S ratios of 1:250:40:8:2.6, Bautista (1973) estimated that microbial biomass in the Pawnee grassland soil contained (g/m^2) 2.8-15 carbon , 0.47-2.6 nitrogen, 0.10-0.53 phosphorus and 0.03-0.16 sulphur.

1.2.5 Effect of storage on the ATP content of soils

The ATP content of the biomass was altered in the soils stored at $10^{\circ}C$ (Oades and Jenkinson, 1979). Bautista (1973) showed that soils stored

at 5°C for 5 days did not show any change in the ATP content,and then the ATP content declined to 77 percent after 16 days,and to 8.8 percent of the value for freshly collected soils after 29 days of storage. ATP in the soil extracts was stable up to 30 days when stored at -20°C then the ATP declined very rapidly. Holms *et al.*(1972) stated that ATP in the perchloric acid extract was not stable even at -10°C and decayed with a half-life of 30 days but ATP in the aqueous solution when stored frozen was fairly stable.

Oades and Jenkinson (1979) suggested that their value of biomass C/ATP ratios may not be valid for the soils receiving large recent additions of fresh substrate or subjected to any form of stress treatments like freezing and thawing, drying etc.

1.2.6 Biomass content of soils

Biomass in soil can range from 170-1180 μg C g^{-1} soil and constitute 1.1 to 3.7 percent of soil organic carbon (Jenkinson and Powlson, 1976b; Lynch and Panting 1980; Sorensen, 1979). These biomass estimates in general are much higher than the values reported by Clarke and Paul (1970). The amount of biomass present in soil is greatly influenced by the soil management and is related to the cropping history (Powlson and Jenkinson, 1976). More biomass exists in uncultivated soils than arable soils (Jenkinson and Powlson, 1976b; Lynch and Panting, 1980) and soils with a standing crop contained more biomass than fallow soils (Powlson and Jenkinson, 1976). Jenkinson and Powlson (1976b) considered that the biomass in the fallowed soil comprised three groups of organisms: (a) organisms decomposing the older and more stable parts of organic matter, (b) new biomass synthesized during the decomposition of small quantities of plant material left from the previous years crop and (c) the dormant organisms.

The amount of biomass synthesized in an acid soil was only half that in a comparable neutral soil, although the amount of ^{14}C retained in both the soils did not differ greatly. Liming increased the concentration of the biomass in acid soils (Powlson and Jenkinson, 1976).

Biomass in soil is subject to seasonal fluctuations. During the winter season more biomass was sustained by the decomposing roots and crop residues whereas in drier seasons, the amounts of nutrients supplied as root exudates failed to meet the energy requirements of the organisms for maintenance (Lynch and Panting, 1980).

Clarholm and Rosswall (1980) observed that the bacterial biomass in forest soil doubled after each rainfall occurrence and after two days, the biomass declined with subsequent grazing by the amoeba and protozoa. Higher biomass after the rainfall was probably associated with the additional supply of nutrients washed down from the forest canopy (Tukey, 1970).

Jenkinson (1966) reported that the biomass (calculated using k value of 0.3) in the soil incubated for 1 year in the field with labelled plant material represented 10-12 percent of the labelled C originally added to the soil and 31-39 percent of the residual C which then declined to 19 percent after 4 years.

Ladd *et al.*(1981) showed that biomass ^{14}C accounted for 4-7 percent of the plant ^{14}C input at 4 to 8 weeks from the incorporation of the plant residues and the amount of labelled C in the biomass was higher for the clay soils (18% of the residual C) than the sandy soils (8% of the residual C). These estimates are comparatively lower than the value reported by Jenkinson (1966).

Sorensen (1979) showed that the amount of biomass was higher for soils incubated in the field than soil incubated under laboratory conditions, probably due to the higher temperature in the laboratory. The amount of the biomass was greatly increased (2.6% of the soil organic

C) after two additions of straw but was reduced (1.4% of the soil organic C) with 4 successive additions. Biomass in the soils receiving successive additions of straw, experienced unfavourable conditions or stress due to the increase in the proportion of biostable compounds derived from the lignin fraction of the straw and consequently resting organisms died. Resting organisms are believed to make up the bulk of the soil biomass.

1.2.7 Effect of storage on the microbial population

Storage of soil samples altered the microbiology of soil (McLaren and Skujins, 1967) and spore forming organisms were present in greater quantities than in a fresh soil (Stotzky *et al.*,1962). Sparling and Cheshire (1979) showed that air-drying decreased the microbial counts. At the initial stage of air-drying numbers of yeasts declined relatively faster than bacteria and fungi. However with water contents below 1.5 percent, the yeast population remained fairly stable but the population of bacteria and fungi continued to decline. So after a certain period of storage, the soil population contained dominantly yeasts. Although the total microbial population increased several fold, the proportion of the different microbial groups remained unaffected, probably due to the conditions created by air-drying which prevented bacterial recolonization.

Freezing at low temperatures reduced the number of viable fungi but the number of bacteria was increased (Mack, 1963). Drying was more harmful to the young population in the incubated soil (Soulides and Allison, 1961).

Soderstrom (1979) reported that the fluorescein diacetate (FDA)-active fungal biomass dropped to 40% immediately after storage at -20^{o}C and declined to 20% after storage at -20^{o}C for 3 weeks. The reduction

in the FDA active biomass was due to the destruction of fungal hyphae resulting from freezing. However the FDA active fungal biomass was stable for up to a week at either 12°C or 5°C.

2. AIMS AND OBJECTIVES

It is evident from the literature that physical fractionation schemes based on the separation of biological entities have provided some information on the mineralizable pool of soil organic matter. The optimum input of energy for dispersing the soil before fractionation is not known. The main aims of the present study were:

1) To determine a method for dispersing soils efficiently with minimum damage to the microbial cells followed by fractionation based on biological criteria.
2) To determine the microbial biomass in soil using a fumigation technique and determination of ATP. The use of ATP to determine microbial biomass has the advantage that it can be used on fractions of soils to which the fumigation technique is difficult to apply. Because the extraction of ATP from soils is a relatively new procedure, considerable effort was expended in studying the influence of soil pretreatments on the amount of ATP extracted.
3) To follow the synthesis and stability of microbial biomass in soils incubated with ^{14}C-glucose using the techniques developed in 1 and 2 above.

3. THE EFFECT OF SOIL PRETREATMENTS ON THE DETERMINATION OF MICROBIAL BIOMASS

3.1 Introduction

Among the indirect methods for determining microbial biomass, the fumigation technique (Jenkinson, 1966) has received a great deal of attention (Shields *et al*, 1973; Jenkinson, 1976; Jenkinson and Powlson, 1976a,b; Jenkinson *et al*., 1976; Powlson and Jenkinson, 1976; Anderson and Domsch, 1978a,b). It is based on the flush of decomposition as the tissue of the organisms killed by the fumigation is utilized by a new population.

The amount of adenosine triphosphate (ATP) has been used as a measure of biomass in marine sediments (Holm-Hansen, 1969), and Strickland *et al*., (1969) and Spector (1956) proposed that the biomass C/ATP ratio was 250. However the ATP concentration may vary in the microbial population by a factor of 7 depending on the availability of nutrients and energy source (Nannipieri *et al*. 1978) and a range of biomass C/ATP ratios has been reported.

Jenkinson and Oades (1979) introduced a method for the determination of ATP in soils using trichloroacetic acid-phosphate-paraquat as an extractant. The biomass C (determined by the fumigation technique) to ATP ratio was 120 for a range of 11 Australian soils based on a regression equation. Subsequently Jenkinson, Davidson and Powlson (1979) obtained a ratio of 126 for 6 English soils based on a regression through the origin. The ratio obtained from the combined data (11 Australian and 6 English soils) was 124.

To obtain reproducible values for the ATP contents of soils by the method of Jenkinson and Oades (1979) it is necessary to sample the soils moist, and to incubate the soils for several days at about 50% of the water holding capacity. The aim of the work reported here was to determine the effects of different storage conditions and pretreatments of soil on the amount of ATP extracted and the resultant biomass C/ATP ratio.

3.2 Materials and Methods

3.2.1 Soils

The seven soil samples used in the present study (Table 1) were collected in August (winter) from the same sites as described by Jenkinson and Oades (1979). Soils were moist at the time of sampling. Roots, earthworms and soil animals were removed from the soils by hand before passing through a 5 mm sieve. These soils are referred to as field moist soils. The content of organic carbon, inorganic carbon and total nitrogen of the Urrbrae fine sandy loam and the Northfield clay were determined. The properties of other soils are quoted from Jenkinson and Oades (1979).

3.2.2 Pretreatment and incubation of the soils

Portions of the field moist soils were allowed to dry in air at 25°C for 3 days. Freeze-drying was done by suspending the required amount of soil in distilled water for up to 30 minutes in a 1 dm^3 round bottom flask and rapidly freezing in liquid N_2 before connecting to the freeze-drying assembly.

Soils which had been air-dried for one year were preincubated with distilled water, or distilled water plus glucose, at 25°C for four days in 600 cm^3 jars containing 5 cm^3 of distilled water. Soils, air-dried for one year (equivalent of 2.5 g oven dry soil) were placed in 40 cm^3 centrifuge tubes for ATP determination. The equivalent of 20 g oven dry soil was placed in a 40 cm^3 vial for the determination of biomass.

Appropriate volumes of water were added to bring the final water content of the soils to 55% water holding capacity (WHC). In the case of the glucose pretreatment, glucose solution was added to the soil to give a final glucose C content of 0.25%. Additional volumes of water were added to bring the final water content of the soil to 55% WHC. The

Table 1 Properties of soils

Series or association	Great soil group	Soil Key	Vegetational cover or land use	pH (H_2O)	Water holding capacity (g 100 g^{-1} soil)	Clay <2μm (%)	Organic C (%)	CO_3-C (%)	Total N (%)
Penola	Terra-rossa	Uf 5.31	Permanent pasture	7.2	49	21	2.21	0	0.217
Urrbrae fine sandy loam	Red-brown earth	Dr 2.23	Permanent pasture	5.0	44	17	1.85	0	0.200
Shepparton fine sandy loam	Red-brown earth	Dr 2.33	Peaches with straw mulch	4.8	40	17	1.83	0	0.164
Mount Schank	Andosol	-	Rough pasture with bracken	7.1	72	12	5.95	0	0.515
Longerenong clay	Grey clay	Ug 5.2	Alternate wheat fallow rotation	8.4	71	57	0.73	0.16	0.083
Furner clay	Ground water rendzina	Ug 5.11	Wheat	8.2	88	48	3.42	1.89	0.465
Northfield clay	Black earth	Ug 5.16	Pasture	8.4	76	54	1.50	0.61	0.170

centrifuge tubes or the vials were placed inside the incubation jars which were stoppered with rubber bungs. Occasionally the incubation jars were opened temporarily to ensure optimum oxygen supply.

Northfield clay, air-dried for 3 days, was used to study (a) the effect of wetting under $CHCl_3$, (b) incubation of chloroform treated soil samples and (c) freezing and thawing, on the ATP content of the soil. Wetting under $CHCl_3$ was performed in a desiccator lined with moist filter paper and a vial containing 20 cm^3 of pure, redistilled chloroform. ATP was extracted from soil (the equivalent of 2.5 g oven dry soil) after wetting (with water to bring the moisture content of the soil to 55% water holding capacity) with or without chloroform treatment. Freezing of the soil samples was carried out by placing the centrifuge tubes in liquid N_2 and then immediately transferring to the deep freeze (-15°C) until required. For all the treatments ATP was extracted immediately except for the frozen samples where a maximum period of one hour was allowed for thawing.

Northfield clay stored at -20°C for about 2 years and then air-dried for 3 days was used to study the effect of an ATP-uncoupling agent on the ATP content of soil. An aliquot of 0.4 ml of 0.01 M dinitrophenol was added to duplicate samples (2.5 g oven dry equivalent soil) and then appropriate amounts of water were added to bring the soil to 55% water holding capacity. Water only was added to control soils. ATP was extracted from soils sampled after 0, 10, 20, 40, 60 minutes, 2, 4 hours, 1, 2, 3 and 4 days of incubation at 20°C. The ATP assay was performed within one week of extraction.

3.2.3 Analytical methods

ATP was determined on duplicate samples of soil according to the method of Jenkinson and Oades (1979) using TCA-phosphate-paraquat as an extractant. Biomass C was determined on the soils after appropriate pretreatments of the soil by the fumigation technique of Jenkinson and

and Powlson (1976b).

3.3 Results and Discussion

3.3.1 Effect of air-drying

The ATP contents of the field moist soils ranged from 1.43 to 6.44 $\mu g\ g^{-1}$ oven dry soil (Table 2). Nine determinations of the ATP content of the Urrbrae and Northfield soils showed that the standard deviations for the ATP contents were 9.3 and 7.6% respectively. This confirms the variability quoted for the method by Jenkinson and Oades (1979), i.e. a standard deviation of $< 10\%$.

Jenkinson and Oades (1979) stressed that during the pretreatment of the soils "no part of the soil ever became air dry". The importance of air-drying on the ATP content is illustrated in Table 2. Air-drying for 3 days at 25°C resulted in a marked drop in the apparent ATP content of soils; to less than 30% and about 10% of the values obtained for field moist soils in the case of loams and clays respectively.

The speed with which air-drying affected the extraction of ATP is shown in Fig.1. Within 6 hours the apparent ATP content dropped to an almost constant level which then changed very little for up to one year (Table 2).

3.3.2 Effect of freeze-drying

The ATP content of the field moist - freeze-dried soil samples (Table 2) showed a slight decline for most soil samples except Urrbrae fine sandy loam. The apparent drop in ATP content of the field moist - freeze-dried soil samples may be attributed to the rapid decay of extracellular ATP from roots, soil animals and the soil microflora. In contrast, there were marked increases in the amounts of ATP extracted from freeze-dried soils which had been air-dried, and even soils air-dried for one year, yielded ATP contents approaching those of field moist soils. The small differences

Table 2 Effect of air-drying and freeze-drying on the ATP content ($\mu g\ g^{-1}$) of soils

Soil Samples	ATP Content ($\mu g\ g^{-1}$)					
	Field moist soil	Field moist soil AD** for 3 days	Field moist soil FD	Field moist soil AD for 3 days + FD	One year AD	One year AD + FD
Penola loam	1.68	0.48	1.58	1.54	0.65	1.65
Urrbrae fine sandy loam	1.43	0.54	1.94	1.69	n.d.	1.94
Shepparton fine sandy loam	1.98	0.29	1.74	1.65	0.25	1.93
Mount Schank loam	3.18	0.78	2.80	1.84	0.19	3.08
Longerenong clay	2.94	0.35	2.06	1.27	0.47	1.28
Furner clay	6.44	0.77	6.06	4.75	0.76	4.30
Northfield clay	6.05	0.48	5.82	4.20	0.48	4.34

* FD Freeze-dried

** AD Air-dried

n.d. not determined

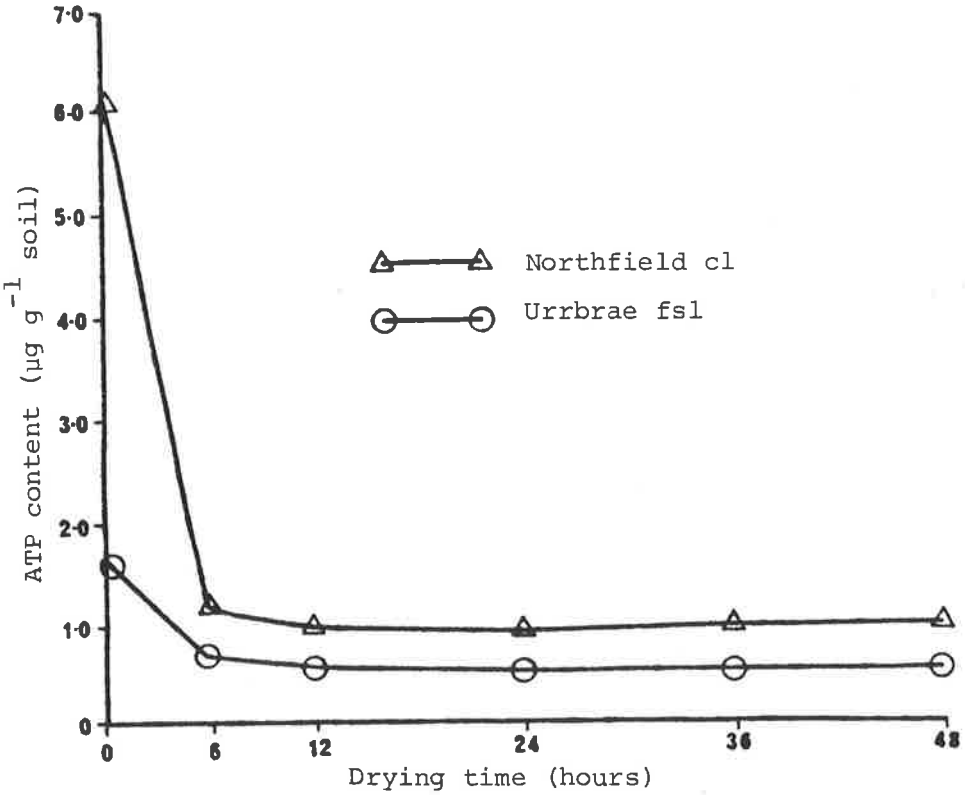

Fig.1 Changes in the content of ATP in soils with period of air-drying

in the ATP content between air-dried - freeze-dried soils and field moist - freeze-dried soils is probably due to the death of a small fraction of the biomass and resultant enzymatic degradation of ATP.

It was considered that the additional ATP extracted from air-dried soils after freeze-drying might be due to better extraction of organic materials caused by the increased dispersion of the soil by some part of the freeze-drying procedure. However particle size analysis did not show any significant increase in fine particles due to freeze-drying and the extraction of saccharides as determined by anthrone was not increased by freeze-drying.

It is suggested that at the onset of air-drying most of the soil organisms prepare themselves for dormancy (Stotzky *et al.*, 1962) and that the ATP extracted from air dry soil comes from active organisms or those not protected by encapsulation.

The most important part of the freeze-drying procedure is in fact the wetting of the air-dry soil prior to freezing in liquid nitrogen, although this alone is not sufficient to explain all of the increase in the ATP extracted from soils wetted just prior to freeze-drying. The ATP extracted from an air-dried soil incubated after water was added increased rapidly with time (Fig. 2), but even after one day was still marginally less than the ATP extracted after 2 to 21 days incubation and less than the value obtained after freeze-drying.

3.3.3 Effect of wetting and addition of substrate

The results in Fig. 2 show that soils which have been air-dried must be wetted and preincubated for at least two days before the amount of ATP extracted becomes reasonably constant and similar to the amounts obtained from field moist samples. For the two soils examined it appeared that ATP extracted after wetting and 14 days incubation was significantly more

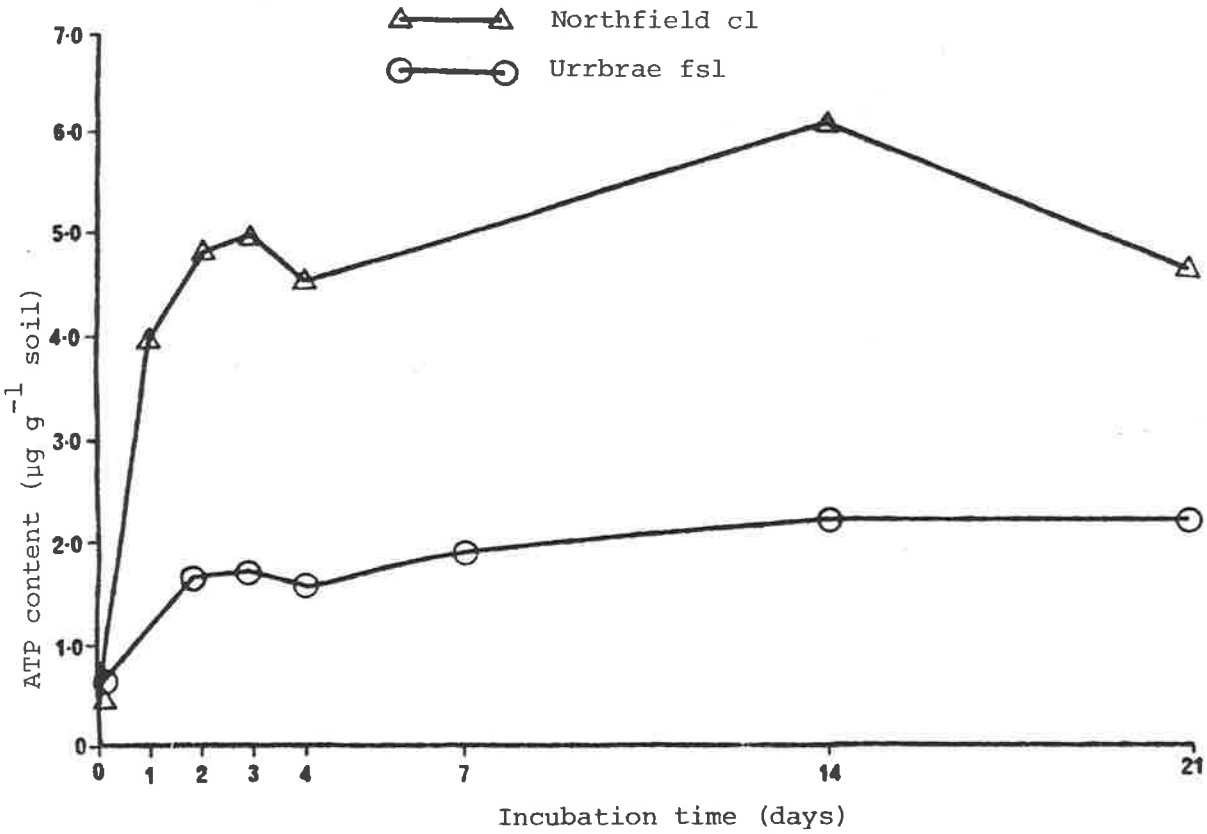

Fig. 2 Effect of pre-incubation on the ATP content of soils

than that obtained from field moist soils. Biomass C/ATP ratios of the air-dried Northfield clay and Urrbrae fine sandy loam incubated with water are illustrated in Fig.3. Biomass C was calculated using CO_2 released from the unfumigated soil during 10-20 day incubation period. It appears that the two soils had similar biomass C/ATP ratios to start with, then the ratio declined initially and at the end of two days, the biomass C/ATP ratio of the Northfield clay soil tended to be reasonably constant whereas the ratio for the Urrbrae soil dropped further for two days. At the end of 4 days the two soils behaved almost similarly. The rise and fall of the ratios for the soils may be due to a succession of microbial populations.

It was observed that the Northfield clay soil had always a higher biomass C/ATP ratio than Urrbrae fine sandy loam. Analysis of the data of Oades and Jenkinson (1979), revealed that the soils with high clay contents had a mean ratio of 142 whereas in the case of the soils with lower amounts of clay the ratio was 100. Therefore the value of biomass C/ATP ratio for the Northfield clay of 162 and 105 for the Urrbrae fine sandy loam are in reasonable agreement with the values of Oades and Jenkinson (1979). The use of a single value for the biomass C/ATP ratio for soils of different texture appears questionable and needs further attention.

The ATP content of soils air-dried for one year and incubated for 4 days with water or with glucose was high (Table 3). The ATP content of the air-dried samples after 4 days incubation with water increased 5 to 10 fold for six soil samples and 20 fold for the soil from Mount Schank. The ATP content of the samples incubated and then freeze-dried was almost twice (Table 3) that of air-dried and freeze-dried samples (Table 2) and the values are higher than those obtained for field moist soils. The additional ATP released from the incubated soils after freeze-drying may indicate that a longer incubation period is required before all organisms become "active".

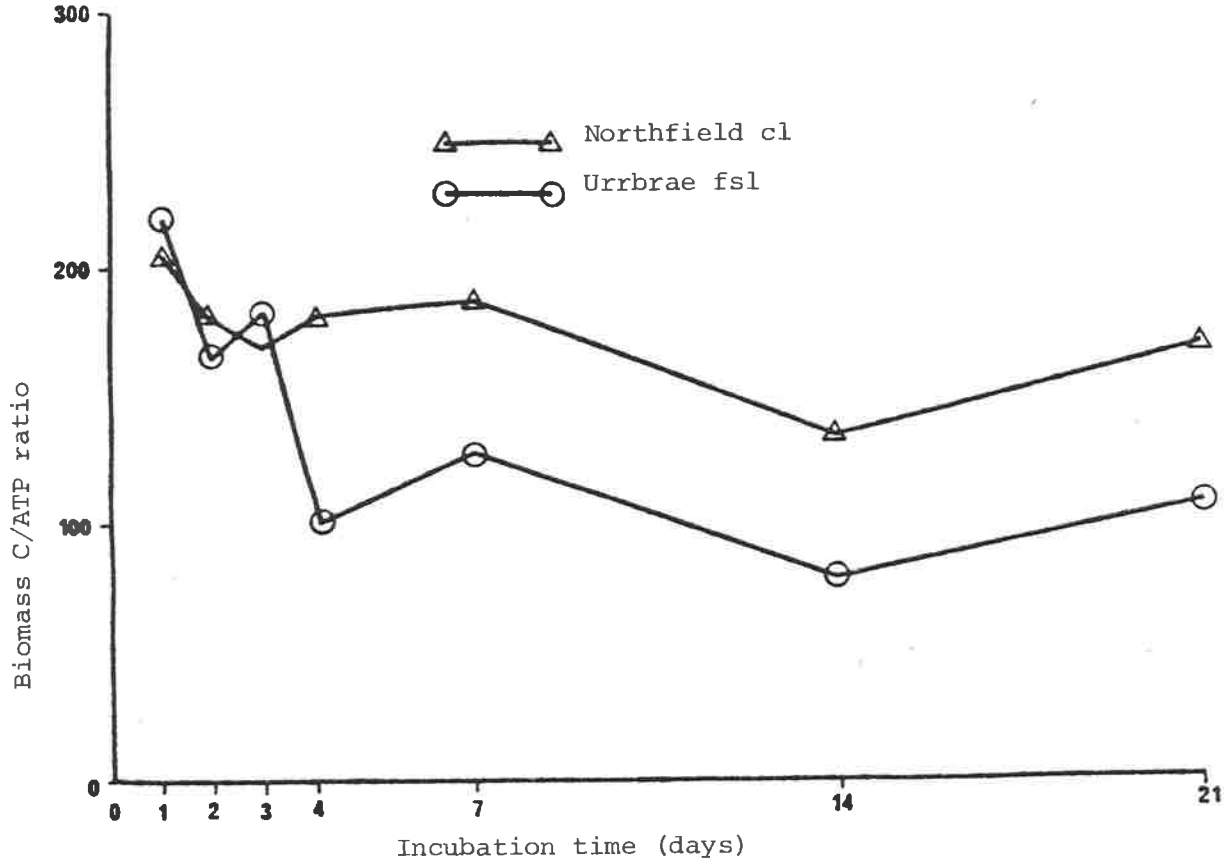

Fig. 3 Effect of pre-incubation on the biomass C/ATP ratios of soils

Table 3 ATP content of the soils after incubation with water and glucose

Soil Samples	ATP content of soil ($\mu g\ g^{-1}$)				
	One year air dried	Incubated with water at 55% WHC for 4 days	Incubated with water at 55% WHC for 4 days and then FD	Incubated with glucose at 55% WHC for 4 days	Incubated with glucose at 55% WHC for 4 days and then FD
Penola loam	0.25	3.45	3.74	5.47	4.59
Urrbrae fine sandy loam	n.d.	1.69	2.35	3.17	3.74
Shepparton fine sandy loam	0.25	2.32	2.52	4.03	5.73
Mount Schank loam	0.19	7.06	5.02	3.41	5.51
Longerenong clay	0.47	3.32	4.62	4.54	5.50
Furner clay	0.76	4.95	7.01	8.81	8.19
Northfield clay	0.48	4.59	6.20	6.40	9.45

WHC = water holding capacity

FD = freeze-dried

Incubation of the air-dried soils with water (Table 3) increased the ATP content by a factor of 3 and freeze-drying of the samples released further ATP. The increase in the ATP content of the samples incubated with water may be due to the release of more labile constituents of organic matter from the air-dried soil by wetting of the soil which enhances the growth and activity of the microbial population (Birch 1960; Stevenson, 1956; Soulides and Allison, 1961). The ATP content of the soils was even greater when glucose was added to the soil samples (Nannipieri *et al.*, 1978; Oades and Jenkinson,1979) and high values were obtained after freeze-drying.

3.3.4 Understanding the freeze-drying process

During the freeze-drying procedure, it was necessary to freeze the soil suspension in liquid nitrogen before attaching to the freeze-dryer. The soil samples usually remained frozen under vacuum for a period of 12-48 hours depending on the amount of water to be removed. It is evident from Table 4 that the ATP content of the air-dried soils, moistened, quickly frozen in liquid nitrogen and stored at $-15^{o}C$ for 12 hours resulted in a loss of half the ATP content and thereafter storage up to 48 hours did not cause any appreciable change in the ATP content. Freezing of the moist soils probably resulted in the death of cells and a thawing period of up to one hour was sufficient for enzymic degradation of ATP in the killed organisms (Conklin and Macgregor,1972; Jenkinson and Oades, 1979). Two cycles of freezing and thawing presumably resulted in the death of more cells and lowered the ATP content almost to that in the air-dried soil.

It was hoped that wetting of the soil under chloroform would kill and lyse the organisms and that ATP-ase in the soil would be inactivated; thus the effect of wetting not including neosynthesis of ATP could be isolated. Chloroform certainly killed the majority of the organisms (Jenkinson and Powlson, 1976 a),and yet the ATP content of the wetted soils under

Table 4 Effect of freezing and thawing and storing of soil on the ATP content

Treatment no.	Treatment details	ATP ($\mu g\ g^{-1}$)
1	AD soil	0.72
2	Wetted for 10 minutes	2.42
3	Wetted for 10 minutes and immediately frozen in liquid N and then stored at -15°C for 12 hours	1.31
4	Wetted for 10 minutes, immediately frozen in liquid N and stored at -15°C for 48 hours	1.22
5	Wetted for 10 minutes, frozen in liquid N and stored at -15°C for 48 hours, then thawed, frozen in liquid N and then freeze-dried	0.78

chloroform was very low (Table 5), indicating that the ATP-ase enzyme was active in the presence of chloroform, so no further insight into the freeze-drying procedure was obtained.

Addition of an uncoupling agent like dinitrophenol (DNP) has been found to be effective in inhibiting the synthesis of ATP for most pure cultures. However it has been pointed out that these uncoupling agents have very little effect on the synthesis of ATP by growing cells but were most effective for resting cells (Brodie and Gutnick, 1972). The effect of wetting with water and dinitrophenol on the ATP content of the Northfield clay are reported in Table 6. Addition of DNP to the soil and subsequent measurements of its concentration indicated that adsorption was negligible. Therefore it may be assumed that the DNP concentration was in excess of that needed to prevent ATP synthesis, and the ATP extracted from the soils immediately after wetting with water must be due to other processes. The increase in the ATP content of the air-dried samples treated with dinitrophenol was entirely due to the effects of wetting (Δ_1ATP). The extra ATP in the samples wetted with water with respect to the samples treated with dinitrophenol is accounted for by synthesis of ATP (Δ_2ATP). It is evident that wetting the soils with dinitrophenol resulted in a linear increase in the ATP (Δ_1ATP) content of the air-dried soil for a period of up to one hour and thereafter there was no further increase in ATP extracted. Wetting with water for up to one hour resulted in little synthesis of ATP (Δ_2ATP) but thereafter there was a gradual increase in the ATP synthesised for a period of up to 24 hours before the content became stable. Thus it appeared that during the initial phase of wetting, chemical or physical processes were largely responsible for the improved extraction of ATP in the air-dried soils, but synthesis also became important during extended periods of incubation. The ATP content of soils incubated with water and with DNP declined after several days.

The ATP content of soils in which the soil population developed after fumigation and incubation (Table 5) was about one fifth of the field moist

Table 5 Effect of chloroform on the ATPase activity and various pretreatments on the newly developed soil population

Treatments	ATP ($\mu g\ g^{-1}$)
Wetted for 6 hours under chloroform	0.15
Wetted for 12 hours under chloroform	0.07
Wetted for 48 hours under chloroform	0.07
Wetted for 48 hours under chloroform, inoculated with fresh soil and incubated for 10 days	1.23
Wetted for 48 hours under chloroform, inoculated, incubated for 10 days and then freeze-dried	0.98
Wetted for 48 hours under chloroform, inoculated, incubated for 10 days and then air-dried for 3 days	0.33
Wetted for 48 hours under chloroform, inoculated, incubated for 10 days and then air-dried for 3 days followed by freeze-drying	0.29

Table 6 Effect of dinitrophenol and water on the ATP content of soil

Time of incubation	ATP content ($\mu g\ g^{-1}$)			
	Wetted with water	Wetted with dinitrophenol	Δ_1ATP	Δ_2ATP
0	0.97	0.96	0.00	0.01
10 min.	2.99	2.51	1.55	0.48
20 min.	4.06	3.70	2.74	0.36
40 min.	5.37	4.78	3.82	0.59
60 min.	5.01	4.73	3.77	0.28
2 hours	5.73	3.94	2.98	1.79
4 hours	5.01	4.06	3.10	0.95
24 hours	6.21	4.54	3.58	1.67
2 days	6.21	4.30	3.34	1.91
3 days	5.01	3.10	2.14	1.91
4 days	4.66	3.58	2.62	1.08

Δ_1ATP = Increase in the content of ATP in the air-dried soil after addition of dinitrophenol

Δ_2ATP = ATP content of the air-dried soil after wetting with water minus the ATP content of the air-dried soil after wetting with dinitrophenol

soil. (Oades and Jenkinson, 1979) and the organisms were sensitive to air-drying (Table 5). Freeze-drying of the fumigated and incubated soil caused a decline in the ATP content and even in the fumigated, incubated, air-dried soils (Table 5) the population which developed after fumigation and incubation was sensitive to air-drying which caused the death of a large proportion of cells, probably because they were in a youthful active phase. By contrast, a large majority of the organisms normally present in the soil exist as spores or survive in a dormant form. Apparently in the freeze-drying procedure two conflicting processes operate simultaneously; one, the positive wetting effect, which results in greater extraction of ATP and two, a negative process resulting from the death of some cells followed by enzymatic degradation and an absolute loss of ATP.

3.3.5 Stability of ATP in freeze-dried soils

It was hoped that the ATP would be stable in freeze-dried soils particularly if these were stored at low temperatures. If the ATP content remained constant it would enable samples to be prepared and then stored for extraction and determination of ATP. However, the results (Table 7) show that freeze-dried soils lost almost half of their ATP contents on storage at 25^{o}C for 45 days. Nash *et al.*(1963) observed 55% death of Aerobacter aerogenes after freeze-drying. The causes of the disappearance of ATP in killed organisms have already been discussed (section 3.3.4). Storage of freeze-dried soils at -15^{o}C for 100 days also led to substantial losses of ATP (Table 8).

3.3.6 Measurement of biomass carbon

The determination of biomass carbon requires the measurement of CO_2 released from the fumigated and unfumigated soils. Evolution of CO_2 from the fumigated soil is usually measured over a period of 0-10 days. The measurement of the amount of CO_2 released during 0-10 day incubation

Table 7 Stability of ATP in freeze-dried soils at 25°C

Soil samples	ATP content of soil ($\mu g\ g^{-1}$)			
	One year AD + FD	One year AD + FD stored at 25°C for 45 days	4 days glucose incubation + FD	4 days glucose incubation + FD stored at 25°C for 45 days
Penola loam	1.65	1.15	4.59	2.99
Urrbrae fine sandy loam	1.94	1.07	3.74	2.47
Shepparton fine sandy loam	1.93	0.73	5.73	5.16
Mount Schank loam	3.08	1.31	5.51	1.80
Longerenong clay	1.28	0.79	5.50	4.66
Furner clay	4.30	n.d.	8.19	4.50
Northfield clay	4.37	1.65	9.45	5.34

AD = air-dried

FD = freeze-dried

Table 8 Stability of ATP in freeze-dried soils stored at -15°C

Soil samples	ATP content (μg g^{-1})			
	Field moist soil FD	Field moist soil FD stored at -15°C for 100 days	Field moist soil AD for 3 days FD	Field moist soil AD for 3 days and stored at 15°C for 100 days
Penola loam	1.58	0.86	1.54	n.d.
Urrbrae fine sandy loam	1.94	1.06	1.69	0.78
Shepparton fine sandy loam	1.74	1.08	1.65	0.73
Mount Schank loam	2.80	1.95	1.84	1.28
Longerenong clay	2.06	1.12	1.27	0.72
Furner clay	6.06	3.38	4.75	2.20
Northfield clay	5.82	3.17	4.20	n.d.

AD = air-dried

FD = freeze-dried

period is recommended for fresh unstored and unfumigated soil (Ayanaba *et al.*, 1976). For soils pretreated in some way, the measurement of CO_2 from the unfumigated soil is a problem (Jenkinson and Powlson, 1976b).

The mean values of the amount of CO_2 released from all the soils for each pretreatment and incubation period (0-10 or 10-20 day) are presented in Table 9. The amounts of CO_2 released during the 0-10 day incubation period were much higher than the 10-20 day incubation period for the pretreatments that included the freeze-drying procedure. By contrast for pretreatments which did not include freeze-drying, the amounts of CO_2 released from the unfumigated soil either during 0-10 day or 10-20 day incubation period were similar.

Jenkinson and Powlson (1976b) suggested that for soils having similar kinds of pretreatments, the measurement of CO_2 from the unfumigated soil during 10-20 day incubation period may compensate for the lower values of biomass carbon that may result due to the death of some organisms. However in order to be able to assess the extent of damage of these pretreatments on the soil biomass, it seems more appropriate to measure the evolution of CO_2 from the unfumigated soil during 0-10 day incubation period.

Biomass carbon of the soils after various pretreatments (calculated using the amount of CO_2 released from the unfumigated soils during 0-10 day incubation period) are presented in Table 10. Biomass carbon in the field moist soils ranged from 267 to 713 $\mu g\ g^{-1}$ soil. Biomass carbon decreased significantly after freeze-drying and air-drying and the greatest decline occurred in the field moist soils followed by freeze-drying (Table 11). These declines in the biomass carbon may be due to the rapid formation of intracellular ice crystals in the cells causing death of a portion of the soil population. However the possibility of underestimation of biomass C to some extent due to the increased decomposition of non-biomass C resulting from these pretreatments is also possible (Jenkinson and Powlson, 1976b).

Table 9 Mean values of the amount of CO_2 (μg C g^{-1} soil) released from all the unfumigated soils after each pretreatment

Incubation time (days)	Pretreatments						
	FD	FM+FD	AD	AD+FD	4d.W.Inc.	4d.W.Inc.+FD	LSD at 5% level
0-10	125	276	134	245	121	196	27
10-20	84	110	103	123	89	114	27

FM = freshly collected soils from the field

FM+FD = freshly collected soils from the field and then freeze-dried

AD = freshly collected soils air-dried for 3 days

AD+FD = freshly collected soils air-dried for 3 days and then freeze-dried

4d.W.Inc. = freshly collected soils air-dried for 3 days and then incubated with water for 4 days

4d.W.Inc.+FD = freshly collected soils air-dried for 3 days and then incubated with water for 4 days and then freeze-dried

Table 10 Biomass carbon* content (μg C g^{-1} soil) of the soils

Soil samples	Pretreatments					
	FM	FM+FD	AD	AD+FD	4d.W.Inc.	4d.W.Inc.+FD
Penola loam	267	164	252	330	435	372
Urrbrae fine sandy loam	205	169	171	233	213	149
Shepparton fine sandy loam	220	153	184	246	288	264
Mount Schank loam	310	303	287	296	461	358
Longerenong clay	264	211	241	229	437	375
Furner clay	705	422	490	548	573	491
Northfield clay	713	623	301	469	612	458

*Biomass carbon was calculated using the amount of CO_2 released from the unfumigated soils during 0-10 day incubation period.

Table 11 Mean values of the amount of biomass carbon (μg C g^{-1} soil) for all the soils after each pretreatment

Incubation time used for the measurement of CO_2 from the unfumigated soils	Pretreatments						
	FM	FM+FD	AD	AD+FD	4d.W.Inc.	4d.W.Inc.+FD	LSD at 5% level
Biomass carbon calculated using the amount of CO_2 released from the unfumigated soils during 0-10 day incubation period	392	292	275	336	431	352	34
Biomass carbon calculated using the amount of CO_2 released from the unfumigated soils during 10-20 day incubation period	473	609	342	582	496	544	34
Biomass carbon calculated using mean value of CO_2 released from the unfumigated soils during 0-10 and 10-20 day incubation periods	433	451	308	459	464	448	22

Alternatively the use of the value of the amount of CO_2 released from the unfumigated soil during 10-20 day incubation period will overestimate the biomass carbon (Table 11). Biomass carbon calculated using the mean value of the CO_2 released from the unfumigated soils during 0-10 and 10-20 day may offer a compromise. Estimation of biomass carbon using the mean value resulted in similar amounts of biomass C for the field moist, field moist - freeze-dried and the soils incubated for 4 days with water. Biomass carbon in the air-dried soils decreased significantly, whereas the biomass carbon increased for the air-dried - freeze-dried and the soils incubated for 4 days with water, but the increases were only marginally significant ($P<0.05$).

None of the above suggestions can be fully justified. However it is necessary to obtain estimates of biomass carbon in soil after these pretreatments in order to be able to convert the ATP data to biomass carbon. It appeared from the above results that the biomass carbon estimation using the amount of CO_2 released from the unfumigated soils during 0-10 day incubation period seems more appropriate until a more reliable period for the measurements of CO_2 from the unfumigated soils can be established.

3.3.7 ATP content of the biomass

Biomass C/ATP ratios of the soils were obtained from linear regressions through the origin (Table 12). The relationship between biomass carbon and ATP were good for all the pretreatments except air-drying. The concentrations of ATP in the biomass of the field-moist soils air-dried, freeze-dried and the soils incubated with water for 4 days were similar. However the concentrations of ATP in the biomass increased after freeze-drying of the field moist soils and the soils incubated for 4 days with water. This increase in the ATP concentration of the biomass in the soils after freeze-drying may be due to i) underestimation of the biomass carbon; ii) the death of some cells but very little degradation of ATP due to low temperature.

Table 12 Relationship between ATP and biomass

Pretreatments	Regression equation forcing through the origin (taking k as 0.50)	mg ATP g^{-1} biomass C (taking k as 0.45)
FM	y*= (112.34±4.52)X**; r = 0.96	8.01
FM+FD	y = (90.58±5.06)X ; r = 0.92	9.94
AD	y = (505.70±39.56)X ; r = 0.66	1.78
AD+FD	y = (126.35±7.27)X ; r = 0.95	7.12
4d.W.Inc	y = (124.49±1.95)X ; r = 0.99	7.26
4d.W.Inc.+FD	y = (76.37±3.37)X ; r = 0.89	11.78

*Biomass carbon (µg C g^{-1} soil) was calculated using the value of CO_2 released from the unfumigated soils during 0-10 day incubation period.

**ATP content (µg g^{-1} soil)

4. CHARACTERIZATION OF SOIL PHYSICAL FRACTIONS AND DEVELOPMENT OF A FRACTIONATION SCHEME

4.1 Introduction

Chemical fractionation techniques are commonly used to study soil organic matter and considerable progress has been made in understanding the nature and properties of the humus fractions (Schnitzer and Khan, 1972). However application of chemical fractionation procedures have failed to provide information on the mineralizable pool of soil organic matter (Section 1.2; Oades and Ladd, 1977). It was suggested that a fractionation procedure involving direct analysis of the recognizable biological entities may define the available pool of organic materials in soil. Appropriate components may include intact plant, animal and microbial cells together with extracellular and lytic compounds. These components may either be present in a free state or combined with the mineral part of the soil with various degrees of tenacity (Jansson and Persson, 1968). Densimetric fractionation techniques separated a more readily available component of soil organic matter, called the "light fraction" comprised mostly of intact plant, animal and microbial cells (Ford, 1968). Ultrasonic dispersion followed by fractionation based on particle size separated relatively unaltered and more homogeneous groups of organic material (Chichester, 1969; Cameron and Posner, 1979).

Particle size fractions have been studied extensively in terms of their chemical nature (Kyuma *et al.*,1969; Chichester, 1969; Turchenek and Oades, 1974) but so far there has been no report on the direct measurement of microbial biomass in the fractions. The main objectives of this section were to (i) compare the effectiveness of different dispersion treatments; (ii) characterize the particle size fractions chemically by determining the amounts of carbon, nitrogen and monosaccharide composition; (iii) characterize the fractions biologically by measuring the ATP content in these particle size fractions and (iv) eventually to formulate a suitable

fractionation scheme based on particle size for the subsequent studies on the decomposition of ^{14}C-glucose in soils.

4.2 Materials and Methods

4.2.1 Soils

Soils used in this study were the 0-15 cm layers of the Urrbrae fine sandy loam (fsl) under an old pasture and Northfield clay (cl). About 50-60 cores were collected from each soil and then thoroughly mixed. Roots and soil animals were picked out by hand. Soils were then passed through a 5 mm sieve and stored frozen (-15 ^{o}C) until required.

4.2.2 Dispersion treatments

To obtain adequate material for the subsequent characterization of each of the particle size fractions, 500 g (oven dry equivalent) of each of the soils were processed. Dispersion of the soils included shaking in a Spex mixer, ultrasonic vibration and a standard chemical treatment.

4.2.2.1 Spex shaker

Soil (20 g) was shaken as a thick paste (soil : water ratio of 1:1) in a plastic vial (100 cm^3) for 10 minutes in a Spex mixer.

4.2.2.2 Ultrasonic vibration

50 g of soil was weighed in a tall (400 cm^3) beaker and then 150 cm^3 of distilled water was added. The beaker with its contents was placed inside a large plastic beaker containing crushed ice. The soil suspension was then stirred magnetically using a Teflon coated flea. At the same time soil was ultrasonically vibrated for 5 minutes by a Branson B12 (150 watts, 20 kHz) with the meter reading at 80 amps. The probe tip was 1.5 cm below the surface of the suspension and was halfway from the centre and the side of the beaker in order to avoid the vortex caused by the magnetic stirrer.

4.2.2.3 Chemical treatment

Soil samples (100 g) were treated with hydrogen peroxide (30 percent w/v) to destroy organic matter. The carbonate in the Northfield clay was removed by addition of 2M HCl. The soil suspension was then centrifuged at 2000 rpm in 1.25 dm^3 plastic bottles for one hour in a MSE Major centrifuge and the supernatant was discarded. Soil residue was then transferred to a 400 cm^3 capacity beaker and 250 cm^3 water and 10 cm^3 of 1M NH_4OH were added. The soil was then ultrasonically vibrated for 5 minutes in a manner similar to that described in section 4.2.2.2.

4.2.3 Fractionation

After the appropriate dispersion treatments, soil suspension was passed through a 300 mesh sieve (53 μm) and was agitated in a tray of distilled water. The sieve with its contents was then placed on a large funnel and washed with a fine jet of distilled water until the suspension passing through the sieve was clear.

4.2.3.1 Separation of light and heavy subfractions >53 μm

The materials on the sieve after drying at 30°C were shaken in a centrifuge tube with a solution of $ZnBr_2$ (density 1.6 g cm^{-3}) and allowed to stand for 15 minutes and centrifuged at 1000 rpm for 5 minutes. The suspended material was carefully decanted on a funnel fitted with a Millipore-filter and washed several times with distilled water. The whole process was repeated three times to recover most of the light fraction. The Millipore prefilter was dried at 30°C and the materials were collected from the prefilter using a camel hair brush. The weight of the light fraction was calculated from the difference in the initial and final weight of the prefilter after drying at 30°C.

The heavy material (>53 μm) remaining in the centrifuge tube was washed several times with distilled water and weighed after freeze-drying.

4.2.3.2 Separation and fractionation of <2 µm size fractions

Separation of the <2 µm particles was done by centrifuging (320 g) the <53 µm suspension collected after sieving in four 1.25 dm^3 plastic bottles in a MSE Major centrifuge, For complete separation, centrifugation up to 15 times was necessary and approximately 100 dm^3 of the <2 µm suspension was collected for each soil sample. Further sub-fractionation into smaller sized fractions was done by centrifuging the <2 µm suspension in a MSE High Speed 18 centrifuge, fitted with a continuous action rotor. Flow rates and centrifuge speeds for various size fractions were calculated according to the formula used by Turchenek (1975). Centrifugation was repeated up to four times to obtain complete separation of the individual size fractions. A schematic representation of the particle size fractionation is described in Fig.4.

4.2.3.3 Fractionation of the 2-53 µm material

Fractionation of the 2-53 µm materials was done by gravity sedimentation (sedimentation time for each size fraction was calculated using Stokes Law) in 10 dm^3 glass beakers. The sediment was collected by siphoning at 10 cm depth of the suspension. Simultaneous separation from six beakers was conveniently handled by using two Venturi water pumps. During the whole part of the fractionation procedure, all the samples were stored at $2^{o}C$.

4.2.4 Removal of water from the suspended material

Each of the particle size fractions obtained as a suspension either by gravity sedimentation or centrifugation, was again centrifuged to collect most of the materials in each of the size fractions. The supernatant was concentrated by cyclone and rotary evaporation under vacuum to 300 ml and was mixed with the sediment and then freeze-dried.Freeze-dried size fractions were weighed and expressed as a percent of the whole soil. Freeze-dried soil fractions were stored at $-15^{o}C$ in sealed glass vials.

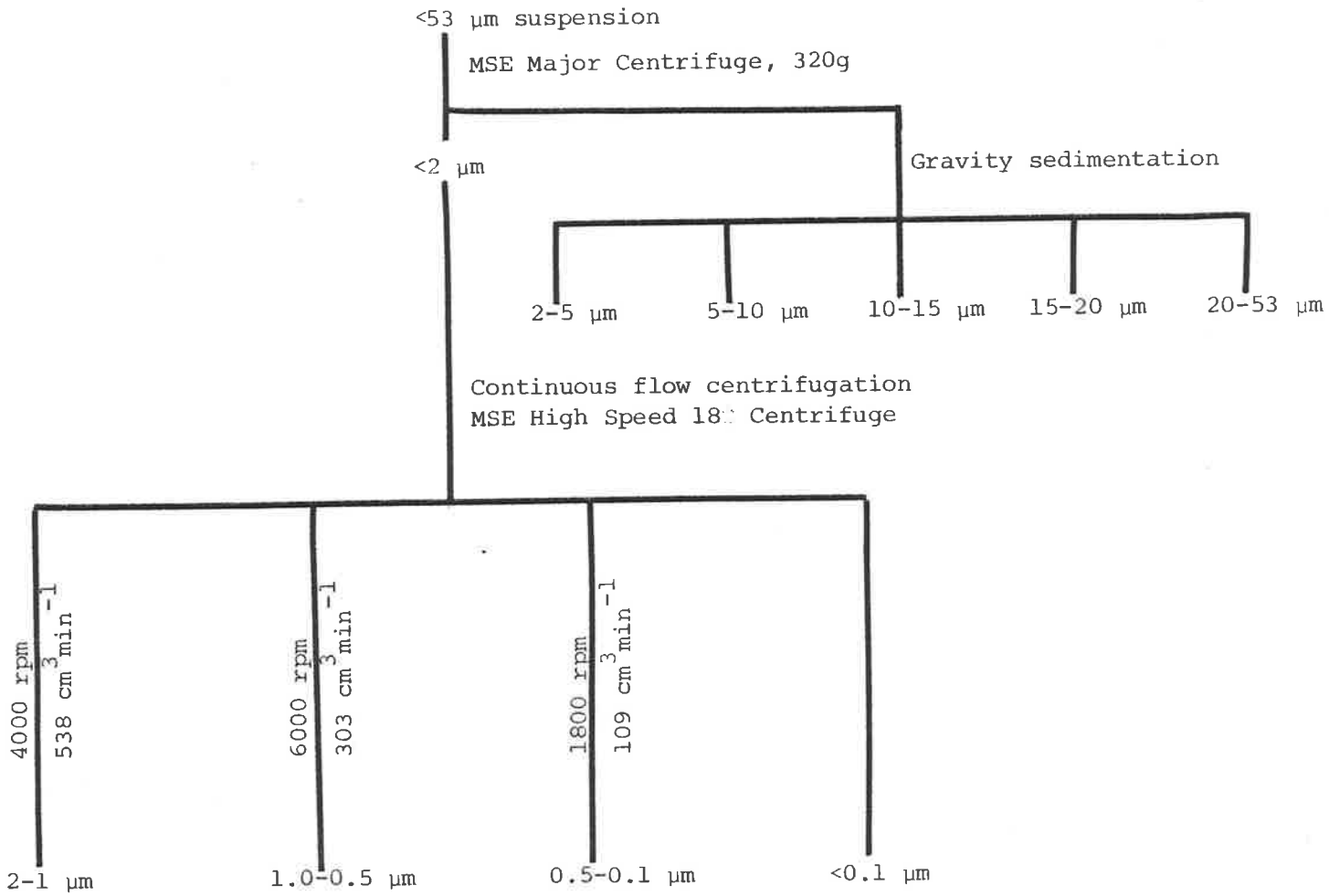

Fig. 4 Diagrammatic representation of the fractionation of <53 µm material

4.2.5 Carbon and nitrogen

Total carbon was determined by dry combustion using a Fisher carbon induction apparatus (Young and Lindbeck,1964). Inorganic carbon was determined by a volumetric calcimetric method (Allison and Moodie,1965). Organic carbon was calculated from the difference between total and inorganic carbon.

4.2.6 Determination of ATP

Extraction of ATP was done immediately after freeze-drying of the particle size fractions using TCA-phosphate-paraquat as an extractant. The extracts were stored at -15^{o}C for a maximum period of two weeks before the ATP assay was performed according to the procedure outlined by Jenkinson and Oades (1979).

4.2.7 Monosaccharide composition

Monosaccharide composition of the particle size fractions were determined by gas-liquid chromatography of the alditol acetates derived from sugars in hydrolysates obtained by refluxing in 0.25 M H_2SO_4 for 20 minutes (Oades *et al.*,1970).

4.3 Results and Discussion

4.3.1 Effectiveness of different dispersion treatments

The effects of ultrasonic vibration and shaking as a paste in a Spex shaker on the stability of different size fractions were compared with respect to a standard chemical treatment, i.e. HCl, H_2O_2 and NH_4OH (Fig. 5 and 6). It is apparent that the pattern of particle size distribution by the three different methods of dispersion was very similar for particles up to 20 µm for the Urrbrae fsl. In the case of the Northfield cl particles >5 µm were not stable to disruption by the action of the Spex mixer and ultrasonic vibration. Turchenek and Oades (1978) showed that microaggregates >250 µm were destroyed by ultrasonic vibration.

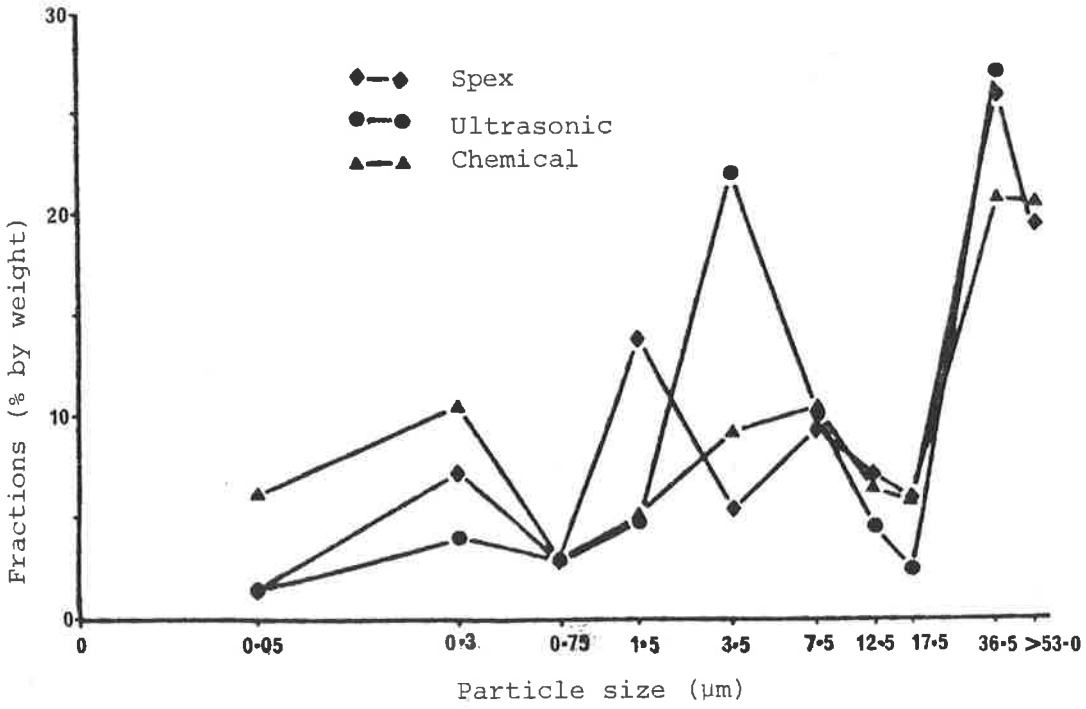

Fig. 5 Distribution of particle size fractions in the Urrbrae fsl after various dispersion treatments

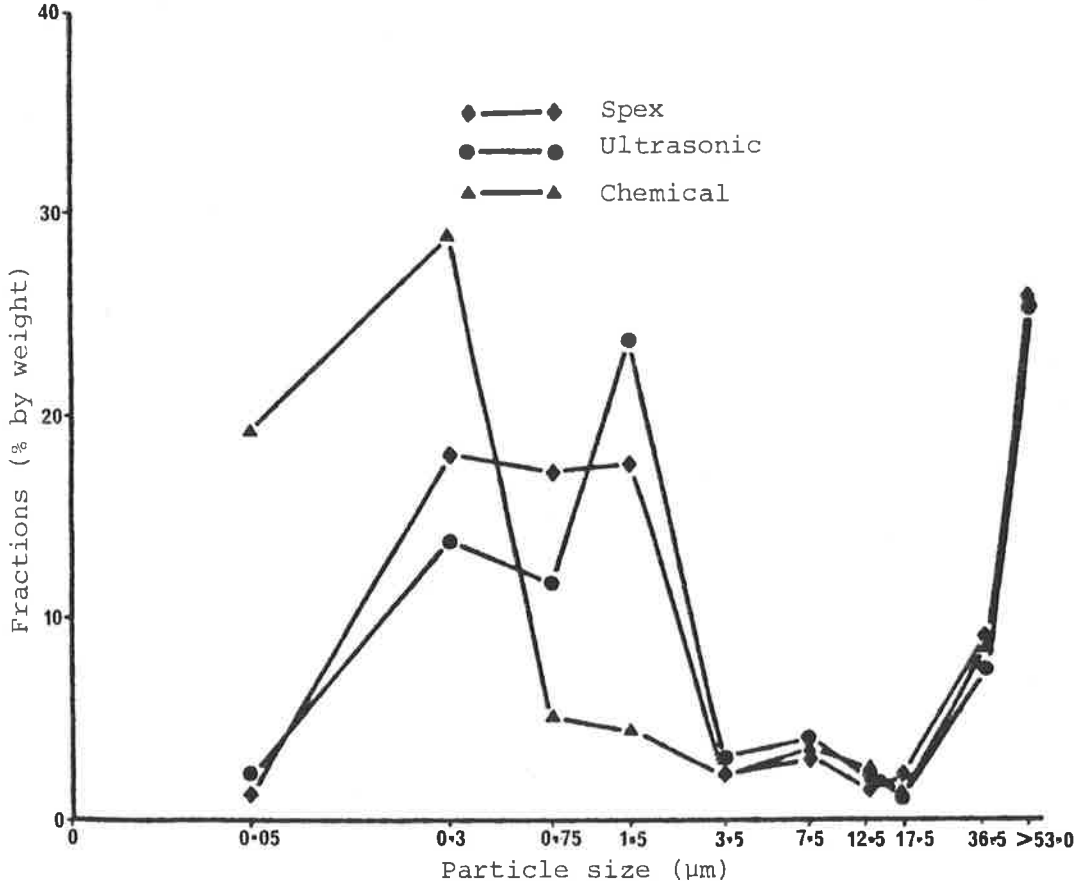

Fig. 6 Distribution of particle size fractions in the Northfield cl after various dispersion treatments

Particle size fractions with diameters 1-5 μm showed high stability and were comprised of considerable amounts of particles <0.5 μm.

4.3.2 Carbon and nitrogen

The contents of organic carbon and nitrogen in the particle size fractions of the Urrbrae and Northfield soils obtained after Spex shaking and ultrasonic dispersion treatments are plotted against the mean diameter of the particle size fractions on a log scale (Fig.7 and 8). The pattern of distribution of nitrogen in the particle size fractions obtained by both methods of dispersion was similar, but considerable variation in the content of organic carbon was observed. In the Urrbrae fsl the smaller particles contained the highest concentrations of carbon and nitrogen (Turchenek, 1975) but the size fractions with diameter 5.0-0.5 μm obtained after ultrasonic dispersion were relatively enriched in organic carbon. This confirms the proposal of Turchenek and Oades (1979) that micro-aggregates with diameter 5.0-0.5 μm were comprised of a substantial proportions of fine clay particles with high contents of organic matter, which accounts for the greater stability and relative enrichment of organic matter in the microaggregates of the Urrbrae soil.

The contents of organic carbon and nitrogen were highest in the particle size fractions with diameter 15-5 μm in the Northfield cl. Finer particles contained less organic carbon and nitrogen. The contents of organic carbon and nitrogen in the <0.1 μm particle size fraction obtained after treatment in the Spex shaker was high.

The inorganic carbon contents (Table 13) of the particle size fractions of the Northfield cl with diameter 10.0-0.5 μm were high and their cementing effect together with organic matter probably contributed to the stability of these microaggregates.

The C/N ratios of the particle size fractions in both the soils (Table 14) were high for the particles >20 μm and showed a decline from

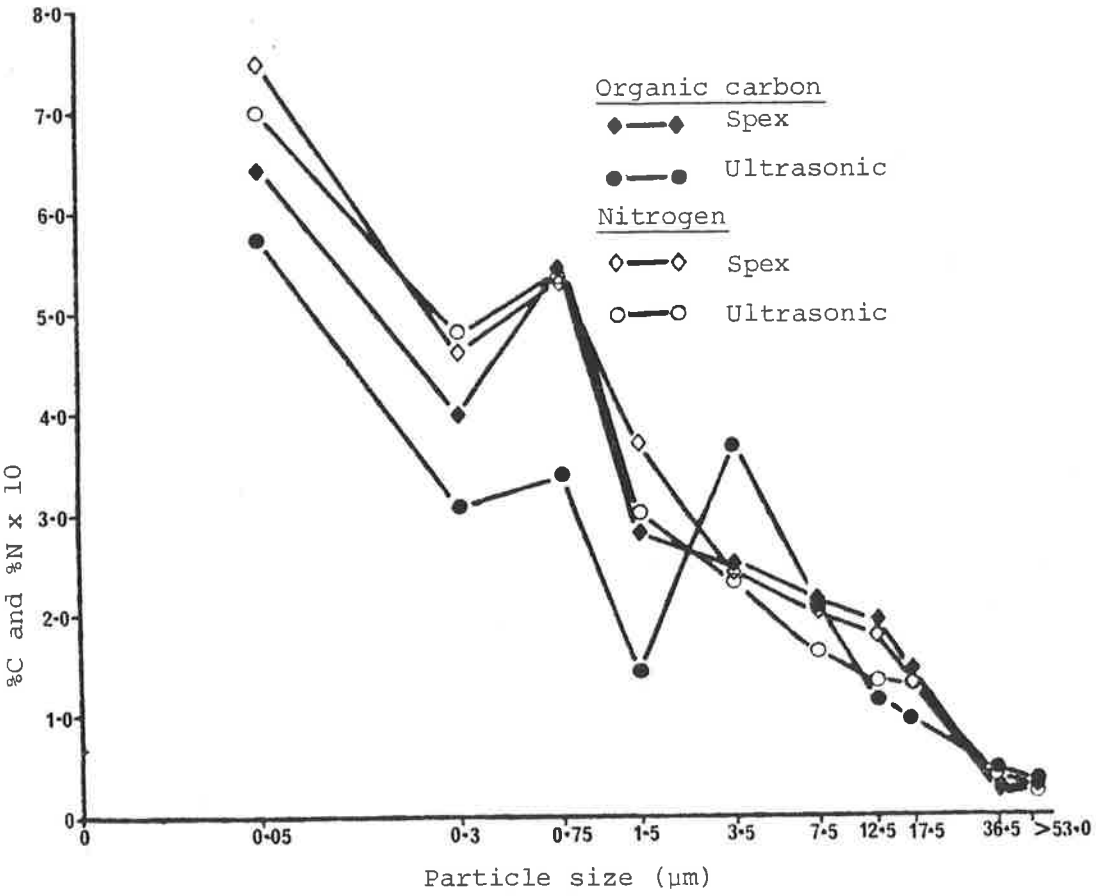

Fig. 7 Organic carbon and nitrogen content in the particle size fractions of the Urrbrae fsl

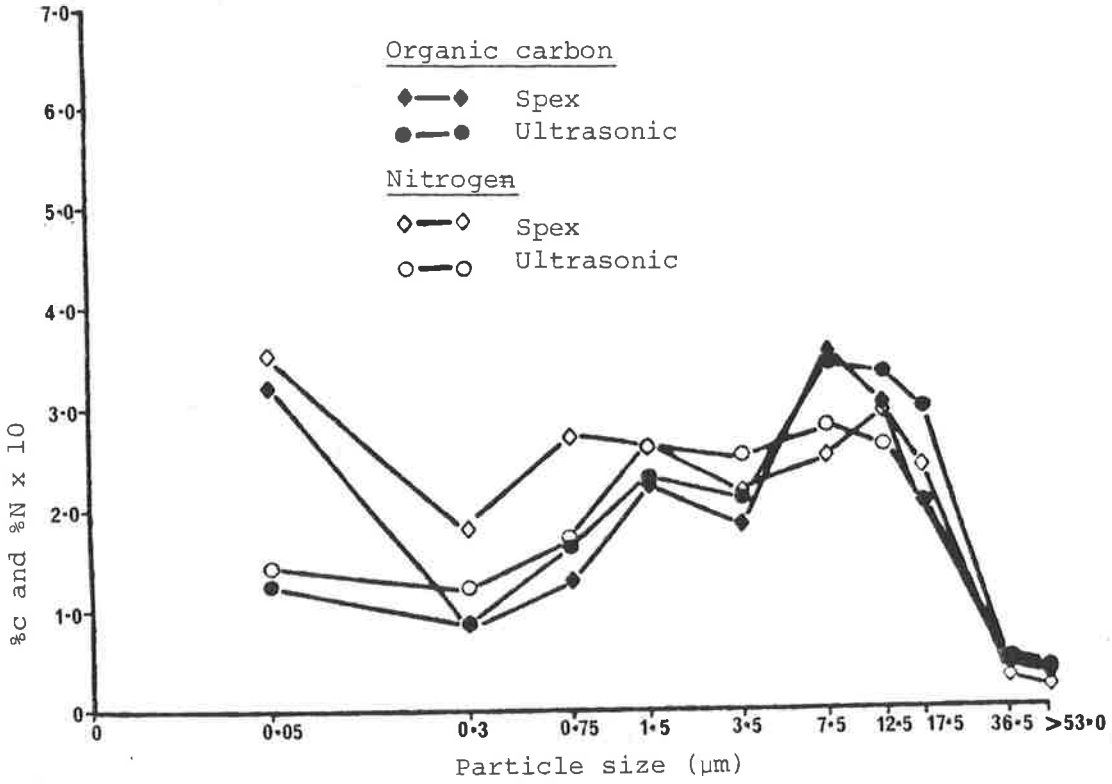

Fig. 8 Organic carbon and nitrogen content in the particle size fractions of the Northfield cl

Table 13 Inorganic carbon content in the particle size fractions of the Northfield cl

Fractions (μm)	Spex	Ultrasonic
>53	0.04	0.06
53-20	0.08	0.09
20-15	0.23	0.18
15-10	0.02	0.02
10-5	0.62	0.45
5-2	1.85	2.01
2-1	1.85	1.75
1-0.5	0.62	0.54
0.5-0.1	0.07	0.07
<0.1	2.24	0.08

Table 14 C/N ratios of the particle size fractions in the Urrbrae fsl and the Northfield cl

Fractions (μm)	Urrbrae fsl		Northfield cl	
	Spex	Ultrasonic	Spex	Ultrasonic
Light Fraction (LF)	22.9	22.5	22.0	22.1
>53	11.0	10.8	14.5	15.5
53-20	11.0	11.0	14.0	11.2
20-15	9.6	7.4	9.0	15.0
15-10	10.5	9.2	10.0	12.8
10-5	10.6	12.6	14.0	12.1
5-2	10.5	16.0	7.7	8.6
2-1	7.6	6.4	8.7	8.9
1.0-0.5	10.3	6.3	4.7	9.6
0.5-0.1	8.6	6.4	4.6	7.0
<0.1	8.6	8.2	9.2	9.2

light fraction to the 15-20 μm fraction. Turchenek (1975) indicated that the wide C/N ratios in the coarser size fractions may originate from the contamination by the plant fragments. The decrease in the C/N ratios from the coarser to finer fractions may be assumed due to the increased degradation of plant material. In case of Urrbrae fsl, the C/N ratios were wide for the fractions with diameter 2-15 μm. In the Northfield cl although the C/N ratios were wide for the fractions with diameter 5-15 μm, however, the trends were less pronounced. Such wide ratios may be due to the presence of extracellular carbon rich materials and perhaps of fungal hyphae. The C/N ratios of the fractions <2 μm were relatively low possibly because of the contribution of protein rich materials from the cytoplasmic materials released and adsorbed by the fine colloids and also due to the concentration of a large proportion of bacterial cells because of their cell size.

4.3.3 ATP content

The significance and reliability of the ATP data as an indicator of microbial biomass is well recognized (Section 1.2.3, Holm-Hansen, 1969; Jenkinson and Oades, 1979). The ATP content of the particle size fractions of the Urrbrae and Northfield soils after Spex and ultrasonic dispersion treatments are presented in Fig. 9. The ATP content in the particle size fractions obtained after Spex dispersion of the Urrbrae soil were evenly distributed. Dispersion of soil by the Spex mill involves the process of wet grinding (trituration) which presumably damaged or killed high proportions of the microorganisms present in the soil and consequently loss of ATP from the dead cells occurred (Conklin and MacGregor, 1972). The ATP was presumably smeared across many soil fractions in the same way as chemical extraction. The content of ATP in the particle size fractions of the Urrbrae soil obtained after ultrasonic dispersion was very high for the fractions with diameter <1 μm and the fraction 2-5 μm.

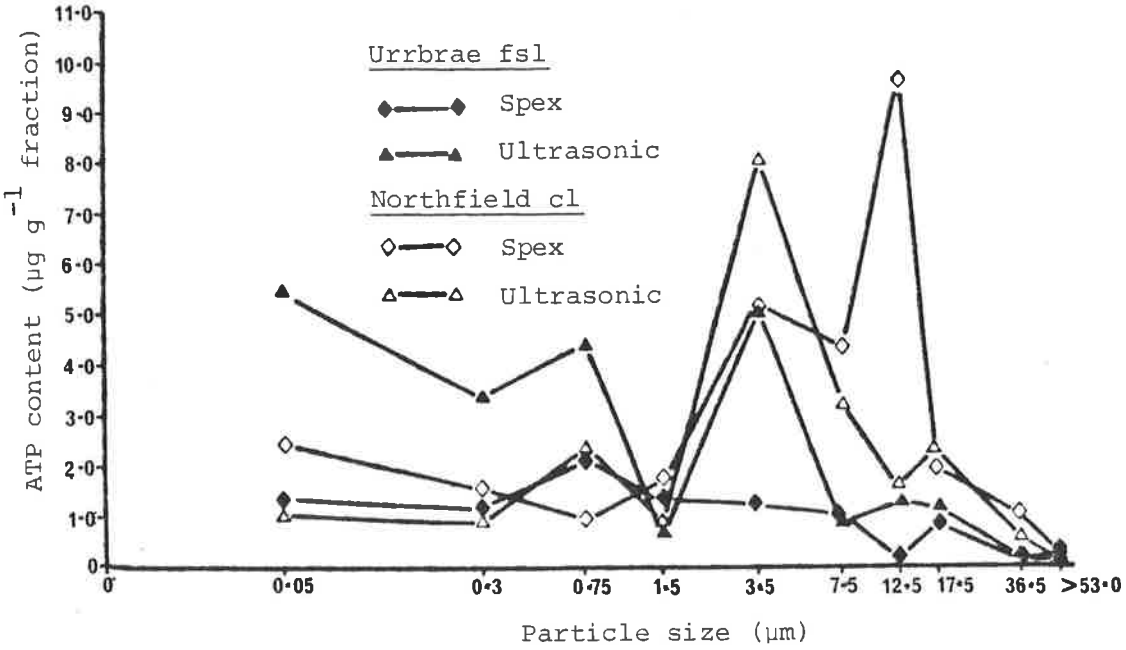

Fig. 9 Distribution of ATP in the particle size fractions of the Urrbrae fsl and the Northfield cl

In the case of the Northfield cl particle size fractions in the silt size range contained the highest quantities of ATP, irrespective of the dispersion treatments used.

The concentrations of microbial biomass (proportion of organic C present as microbial biomass, expressed as percent organic C) present in the particle size fractions was calculated from the ATP data, using the biomass C/ATP ratio of 90.6 (see section 3.3.7; Table 12) and are presented in Table 15. The concentration of biomass in the light fraction was very low in both the soils but was slightly higher in the Northfield cl than the Urrbrae fsl. The proportions of organic carbon present as biomass in the particle size fractions obtained after Spex dispersion treatment were low and the reason for this has already been discussed. Although no consistent trend could be isolated in the proportion of organic carbon in the biomass amongst the particle size fractions, it appeared that 5-2 μm fraction contained the largest concentration of biomass. Nevertheless it was observed that the mean value of the concentrations of biomass in the silt size fractions was consistently higher than either the clay or sand size fractions.

4.3.4 Monosaccharide composition

The monosaccharide composition of the particle size fractions of the Urrbrae and Northfield soils are presented in Tables 16 and 17. Glucose constituted 20-78 percent of the polysaccharide material and the proportion was higher in the silt size fractions than in the clay size fractions of the Urrbrae soil, but in the case of Northfield soil, no definite trend was observed. The relative abundance of glucose in both plant and microbial materials does not allow any observations on the nature and origin of the organic materials associated with these particle size fractions (Cheshire *et al.*,1969).

Table 15 The concentration of microbial biomass (proportion of organic carbon present as microbial biomass, expressed as percent of organic carbon) in the particle size fractions of Urrbrae fsl and Northfield cl

Fractions (μm)	Urrbrae fsl		Northfield cl	
	Spex	Ultrasonic	Spex	Ultrasonic
LF	0.02	0.02	0.10	0.03
>53	0.24	0.64	0.76	0.56
	} 0.38	} 0.48	} 1.60	} 0.95
53-20	0.52	0.32	2.41	1.33
20-15	0.71	1.28	0.92	0.80
15-10	0.09 } 0.44	1.08 } 0.93	3.19 } 1.79	0.48 } 0.75
10-5	0.52	0.44	1.25	0.96
5-2	0.52	1.39	3.41	3.82
2-1	0.48 } 0.47	0.33 } 1.01	0.79 } 1.66	0.41 } 1.90
1.0-0.5	0.40	1.31	0.78	1.47
0.5-0.1	0.30	1.12	1.88	1.09
	} 0.26	} 1.04	} 1.33	} 0.95
<0.1	0.21	0.96	0.77	0.80

*Microbial biomass carbon was calculated from ATP data

Table 16 Monosaccharide composition (expressed as $\mu g\ C\ g^{-1}$ fraction) of the particle size fractions of the Urrbrae fsl obtained after dispersion using a Spex shaker or an ultrasonic probe

Fractions (μm)	Rhamnose	Fucose	Ribose	Arabinose	Xylose	Mannose	Galactose	Glucose	Total	Carbohydrate C as % of organic C
					SPEX					
LF	415	892	1976	1727	2382	1063	1099	17251	26805	10.47
20-15	45	37	13	95	63	104	60	850	1267	10.13
15-10	223	161	86	451	365	514	382	3167	5349	28.15
10-5	98	44	24	212	178	170	273	735	1734	8.14
5-2	315	149	63	433	342	422	556	1899	4179	16.58
2-1	-	69	27	45	28	80	138	314	701	2.50
1.0-0.5	124	123	-	52	86	105	265	328	1083	1.99
0.5-0.1	250	81	54	212	81	200	213	388	1479	3.73
<0.1	1295	482	319	1329	721	719	657	1772	7294	11.34
					ULTRASONIC					
LF	478	527	1309	473	541	305	381	14218	18232	7.29
20-15	68	29	18	117	78	119	152	713	1294	13.48
15-10	156	73	46	257	158	250	169	2107	3216	27.02
10-5	77	35	21	197	128	163	254	1176	2051	10.15
5-2	506	230	118	686	500	555	1121	3293	7009	18.99
2-1	128	64	49	174	140	148	258	1600	2561	13.27
1.0-0.5	76	61	64	150	89	211	269	522	1442	4.25
0.5-0.1	369	190	192	516	235	416	609	805	3332	10.85
<0.1	1187	474	234	775	420	489	1300	3247	8216	14.21

- traces

Table 17 Monosaccharide composition (expressed as μg C g^{-1} fraction) of the particle size fractions of the Northfield cl obtained after dispersion using a Spex shaker or an ultrasonic probe.

Fractions (μm)	Rhamnose	Fucose	Ribose	Arabinose	Xylose	Mannose	Galactose	Glucose	Total	Carbohydrate as % of organic C
SPEX										
LF	395	866	1089	1043	2021	1288	5334	15382	27418	10.71
20-15	112	88	88	185	183	309	397	707	2069	10.35
15-10	87	73	84	119	116	273	241	542	1535	5.12
10-5	225	396	394	541	562	1044	1324	1553	6039	17.25
5-2	179	71	38	220	196	251	345	700	2000	10.81
2-1	178	206	205	223	182	528	693	982	3179	14.21
1.0-0.5	436	124	81	297	211	379	432	729	2689	20.68
0.5-0.1	43	36	28	47	28	59	82	133	456	5.36
<0.1	54	26	22	56	35	78	42	145	458	1.43
ULTRASONIC										
LF	295	570	958	294	530	608	536	12780	16571	6.76
20-15	78	93	102	172	161	269	346	1502	2723	13.61
15-10	93	84	75	131	127	216	256	1655	2637	8.79
10-5	370	193	142	494	464	830	777	1708	4978	14.64
5-2	200	185	125	140	115	150	200	380	1495	7.12
2-1	47	45	33	100	70	146	223	460	1124	4.89
1.0-0.5	62	32	35	78	43	72	62	96	480	3.00
0.5-0.1	45	20	21	48	37	88	58	101	418	4.92
<0.1	268	157	110	269	242	420	239	625	2330	18.64

Significant amounts of galactose, mannose, xylose, arabinose and rhamnose were also present in the particle size fractions. Experiments with ^{14}C-labelled plant materials have indicated that microorganisms synthesize greater proportions of the hexoses, galactose and mannose than the pentoses, arabinose and xylose (Cheshire *et al.*,1969; Oades, 1974). Thus the particle size fractions with diameter 2.0-0.5 µm fractions obtained after Spex dispersion and the fractions with 1.0-0.5 µm obtained after ultrasonic dispersion had the highest galactose + mannose/arabinose + xylose ratios in the Urrbrae fsl (Table 18).

In the case of Northfield cl these ratios were higher in the particle size fractions greater than 1 µm. Turchenek (1975) reported high ratios for the fine silt and coarse clay fractions. However the correlation between these ratios and the ATP content (r^2 = 0.05) or the concentration of biomass (r^2 = 0.01) in the particle size fractions was found to be very poor. The reason for the poor correlation may be due to the fact that the living organisms may be separated into a particular size and the microbially produced sugars were separated into some other fraction.

4.3.5 Relationship between chemical and biological properties of the soil fractions

It has been established that the ATP content was highly dependent on the content of organic carbon and nitrogen status of the soils (Jenkinson and Oades, 1979). The pattern of distribution of ATP, organic carbon and nitrogen of the fractions up to <5 µm in the Urrbrae fsl obtained after ultrasonic dispersion are very similar. Also the similarity between the galactose + mannose/arabinose + xylose ratios and the ATP of these fractions confirmed the usefulness of these ratios to locate the relative abundance of microbial materials. On the other hand the ATP content of the particle size fractions obtained after Spex dispersion did not follow either organic carbon or nitrogen. However the galactose + mannose/arab-

Table 18 Ratios of galactose + mannose/arabinose + xylose in the particle size fractions

Fractions (μm)	Urrbrae fsl		Northfield cl	
	Spex	Ultrasonic	Spex	Ultrasonic
LF	0.53	0.68	2.16	1.39
20-15	1.04	1.39	1.92	1.85
15-10	1.10	1.01	2.19	1.83
10-5	1.14	1.28	2.15	1.68
5-2	1.26	1.41	1.43	1.37
2-1	2.98	1.29	3.01	2.17
1.0-0.5	2.68	2.01	1.59	1.11
0.5-0.1	1.41	1.44	1.88	1.72
<0.1	0.67	1.50	1.32	1.29

inose + xylose ratios and the C/N ratios of the fractions with diameter 2.0-0.5 μm indicated that these fractions were relatively enriched with microbial materials. It may thus be assumed that the energy utilized during the process of dispersion by Spex shaker, probably killed a large proportion of the organisms but the microbial debris was still present in these fractions as shown by the galactose + mannose/arabinose + xylose ratios.

The results presented in Fig. 7 and 8 reveal that the fractions with diameter 20-1 μm obtained by both methods of dispersion in the Northfield cl contained the highest contents of organic carbon and nitrogen and were relatively rich in ATP. The galactose + mannose/arabinose + xylose ratios were also high for the particle size fractions >1 μm. The observed inconsistency between these ratios and the content of ATP in some of these particle size fractions may be attributed to the large proportion of microbial and extracellular materials partitioned into different fractions.

4.3.6 A fractionation scheme

The development of a sensible fractionation scheme, based on particle size, requires an understanding of the chemical and biological nature of the organic compounds present in these size fractions. Such a physical fractionation technique should be able to identify the major pools of carbon and nitrogen and the biological entities normally present in association with the mineral part of the soil in a natural state. It was evident that dispersion by Spex shaking was more detrimental to the biomass than the ultrasonic treatment, although dispersion was more complete by Spex shaking than the ultrasonic vibration. Thus it appeared that fractionation following dispersion with the Spex shaker yielded physical fractions which suffered from similar artefacts of cross contamination as fractionations by chemical extraction. Large scale separations of particle size fractions after ultrasonic dispersion of the

soil with very little alteration in the chemical nature were reported by Genrich (1972) and Kyuma *et al.*,(1969). It is clear from the preceding sections that the application of the ultrasonic treatment followed by particle size separation yielded dissimilar soil organic components in terms of their chemical and biological composition (Turchenek, 1975). A particle size fractionation scheme involving so many different fractions is too demanding in an investigation of the transformation of organic substances in soil. It was therefore decided to combine some of these size fractions based on the contents of organic carbon, nitrogen and the biomass. The amounts of organic carbon and nitrogen in the combined size fractions are presented in Table 19. Light fraction separated at the density of 1.6 g cm^{-3} represented 10.4-11.8 percent of the soil organic carbon and 4.2-4.7 percent nitrogen. These estimates of the light fraction carbon and nitrogen are much lower than the values reported by Ford (1968) for fractions <2.0 g cm^{-3}. The term "light fraction" as used in this study applies to fractions <1.6 g cm^{-3} whereas other workers have used a density of 2.0 g cm^{-3} (Khan, 1959; Monnier *et al.*,1962; Whitehead *et al.*,1975). Another procedural difference in the current work is that the light fraction was separated from >53 µm material obtained by sieving the soil after the appropriate dispersion treatments. Visual observation indicated that the light fraction consisted mainly of coarse plant fragments. Separation of the light fraction was not performed on the whole soil because the effect of $ZnBr_2$ on the soil organisms and the estimation of ATP is not known.

It was observed that the highest contents of organic carbon (37-54%) and nitrogen (40-58%) were located in the 5.0-0.5 µm size fraction. Contributions of other particle size fractions towards soil organic carbon and nitrogen were relatively small with each size fraction containing similar quantities of organic carbon and nitrogen.

Table 19 The amounts of organic carbon and nitrogen (expressed as percent of whole soil) in the Urrbrae fsl and the Northfield cl

Fractions (μm)	Urrbrae fsl				Northfield cl			
	Spex		Ultrasonic		Spex		Ultrasonic	
	Org.C	N	Org.C	N	Org.C.	N	Org.C.	N
LF	10.38	4.30	11.08	4.55	10.40	4.16	11.76	4.70
>53	3.46	3.90	2.78	3.00	5.01	7.61	5.26	5.99
53-20	3.09	6.50	6.48	10.90	2.55	2.68	2.99	4.11
20-15	4.24	3.90	1.23	1.55	3.11	3.05	2.15	1.27
15-10	7.38	6.50	2.95	3.00	3.47	3.02	4.45	3.07
10-5	10.69	9.30	11.36	8.20	8.19	5.18	8.95	6.51
5-2	7.39	6.50	44.08	25.04	2.57	2.96	4.31	4.44
2-1	20.99	25.65	4.92	7.10	26.37	26.89	36.43	36.34
1.0-0.5	8.49	7.65	5.50	8.10	14.38	27.19	12.73	11.71
0.5-0.1	15.32	16.40	6.64	9.60	9.93	19.22	8.30	10.46
<0.1	5.18	5.60	4.45	5.05	2.67	2.57	1.91	4.18

The amounts of ATP in the particle size fractions are presented in Table 20. It is evident that 5.0-0.5 m size fractions obtained after ultrasonic dispersion of soils contained more than 60 percent of soil ATP. On the other hand this size fraction represented only about 42-50 percent of the soil obtained after dispersion using the Spex shaker. The amount of ATP recovered in these particle size fractions dispersed by ultrasonic treatment for the Urrbrae soil was almost complete (90 percent) but was only a third using Spex dispersion. The recovery of soil ATP from the particle size fractions of Northfield clay was similar by both methods of dispersion and was only 30 percent.

Although the recoveries of soil ATP from the particle size fractions was not complete, it was clearly established that ultrasonic treatment followed by particle size fractionation identified some components of organic matter with high proportions of microbial biomass and organic compounds.

Thus it seemed appropriate to investigate the decomposition of ^{14}C-glucose in soils after ultrasonic dispersion and fractionation into the following fractions:

i) Light fraction (>53 μm) separated at density 1.6 g cm^{-3}

ii) > 20 μm

iii) 20-5 μm

iv) 5-0.5 μm

v) <0.5 μm.

Table 20 Amounts of ATP (expressed as percent of the total amount of ATP recovered from the fractions) in the particle size fractions of the Urrbrae fsl and the Northfield cl

Fractions (μm)	Urrbrae fsl		Northfield cl	
	Spex	Ultrasonic	Spex	Ultrasonic
LF	0.63	0.22	1.10	0.40
>53	2.44	1.86	3.95	3.64
	6.94	4.05	10.29	8.59
53-20	4.50	2.19	6.34	4.95
20-15	8.28	1.64	2.94	2.16
15-10	1.83 25.88	3.35 10.31	11.38 24.90	2.64 15.49
10-5	15.77	5.32	10.58	10.69
5-2	11.01	64.13	9.04	20.47
2-1	29.25 50.09	1.71 73.44	21.48 42.17	18.56 62.44
1.0-0.5	9.83	7.60	11.65	23.41
0.5-0.1	13.27	7.82	19.17	11.21
	16.46	12.30	21.28	13.09
<0.1	3.19	4.48	2.11	1.88

5. BIOMASS IN RELATION TO THE DECOMPOSITION OF ^{14}C-GLUCOSE IN SOILS

5.1 Introduction

In recent years, physical fractionation of soil has been applied in studies of the decomposition of organic materials. Such procedures have provided a better understanding of the nature of organic materials in terms of their biological origin. In this chapter the decomposition of ^{14}C-glucose in the Urrbrae fsl and the Northfield cl was studied in terms of the stability of biomass and non-biomass materials. Biomass C/ATP ratios of the newly developed population of the two soils were also considered. Soils sampled at 2, 30 and 120 days after addition of glucose were fractionated according to the scheme as outlined in section 4.3.6. Carbon-14 and ATP were determined on the soil fractions obtained physically to follow the flux of ^{14}C and the labelled microbial biomass in soils. Also an evaluation of the biomass C/ATP ratios was made to study the changes in the proportions of biomass in the soil fractions obtained using physical methods only.

5.2 Materials and Methods

5.2.1 Solutions added to the soils

A series of three experiments were conducted after addition of ^{14}C-glucose and water to the Urrbrae fsl and the Northfield cl. The solutions added to the soils were:

A. An aliquot of 300 µl of uniformly labelled ^{14}C-glucose with a specific activity of 37 MBq/cm^3 (supplied by the Radio Chemical Centre, Amersham, England) was diluted in 100 cm^3 water containing 12 g of unlabelled glucose. So the resulting solution had 111 kBq of ^{14}C and 120 mg glucose per cm^3 of the solution.

B. An aliquot of 300 µl of ^{14}C glucose, having a specific activity of 37 MBq/cm^3 was diluted with 45 g unlabelled glucose and made up to 200 cm^3. The resulting solution had 55.5 kBq ^{14}C and 225 mg of glucose per cm^3 of the solution.

C. 8.2g of $NaNO_3$ and 3.5 g of KH_2PO_4 were dissolved in water and made up to 100 cm^3. The resulting solution had 82 mg of $NaNO_3$ and 35 mg KH_2PO_4 per cm^3.

5.2.2 Soil pretreatments

Field moist soils, after passing through a 5mm sieve, were air-dried for 3 days. Eleven lots of 80 g and 24 lots of 120 g (oven-dry equivalent) of each of the soils were weighed into 200 cm^3 capacity glass vials and wetted to 40% water holding capacity and incubated for 14 days. Soils receiving the above pretreatments, henceforth are described as being at day zero of the incubation period.

5.2.3 Experiment I: Decomposition of ^{14}C-glucose in soils

To two of the vials containing 80 g (oven-dry equivalent) of each of the soils, 5 cm^3 of solution A (555 kBq of ^{14}C and 240 mg glucose C) and 1 cm^3 of solution C were added. Appropriate amounts of distilled water were added to bring the soils to 55% water holding capacity and then the contents of the vials were mixed thoroughly with a spatula. Each vial was placed in a 5 dm^3 confectionary jar provided with screw top sealed lid. Ten cubic centimetres of distilled water was placed in the confectionary jar to maintain humidity. Released CO_2 was absorbed in 20 cm^3 of 1M NaOH which was replaced every 12 hours for a period of up to 3 days. The absorbing vials were then replaced daily up to two weeks and then at the 3rd, 4th, 9th and 17th week of incubation.

5.2.4 Experiment II: Determination of biomass and ATP after addition of glucose and water

To sixteen lots of 120g (O.D. equivalent) of each of the soils 4 cm^3 of solution B (222 kBq of ^{14}C and 360 mg of glucose C) and 1.5 cm^3 of solution C were added. To another 8 lots of 120 g of each of the soils 5.5 cm^3 of distilled water was added. In both cases, appropriate amounts of distilled water were added to bring the soil to 55% water holding capacity and the contents were mixed thoroughly with a spatula. The vials were placed inside large desiccators which were connected to each other by plastic tubing. The lids of the desiccators were greased and clamps were used to ensure maximum sealing. From one end of the desiccators, CO_2 free moist air was continuously passed from a compressed air cylinder and finally bubbled through a solution of 1M NaOH to collect all the $^{14}CO_2$. Determinations included in this experiment were ATP, labelled (^{14}C) and total unlabelled ($^{12+14}C$) biomass carbon for the soils incubated with ^{14}C-glucose; and ATP only for the soils incubated with water, after 2, 4, 8, 12, 24, 72, 92 and 120 days of incubation.

The procedure for determination of ATP and biomass has been described in section 3.2.3. Biomass was calculated from the amount of CO_2 released from the fumigated and incubated soils minus the amount of CO_2 evolved from the unfumigated soils during the 0-10 day incubation period. Similarly the labelled biomass was calculated by counting 0.1 cm^3 of the NaOH solution.

5.2.4.1 Determination of total unlabelled CO_2-C

The amount of CO_2-C absorbed in NaOH was calculated by titrating 2 cm^3 of the absorbant (1M NaOH) against 1M HCl to bring the pH from 8.30 to 3.70 less the amount required for the blank. Titrations were done with an automatic titrator (Radiometer, Copenhagen, PHM62 Standard pH meter, TTT 60 Titrator and Autoburette ABU12).

5.2.4.2 Determination of $^{14}CO_2$

An aliquot of 0.1cm^3 of the absorbant was added to 10 cm^3 of the Triton X-100 scintillant in vials of known background counts and then 0.9 cm^3 of distilled water was added. The contents of the vials were shaken vigorously until a clear solution was obtained. Vials were then kept in the scintillation counter (Hewlett Packard Model 3375) and equilibrated for 24 hours to eliminate any effects resulting from chemiluminescence. Samples were counted at 6% gain with 20-100 and 20-1000 window settings (Adu and Oades, 1974). 10 µl of ^{14}C benzoic acid of known counts were added as the internal standards to six vials and the average recovery of the ^{14}C-internal standard was used to calculate the amount of $^{14}CO_2$.

5.2.5 Experiment III: Incubation of the soils before fractionation

To six vials containing 80 g (oven-dry equivalent) of each of the soils, 5 cm^3 of solution A (555 kBq of ^{14}C and 240 mg glucose-C) and 1 cm^3 of solution C were added. Simultaneously to another 3 vials containing the same amount of each of the soils 6 cm^3 of water was added. To all these samples appropriate amounts of distilled water were added to bring the soils to 55% water holding capacity. The soil samples were then incubated in a manner similar to that described in section 5.2.4.

At 2, 30 and 120 days of incubation, duplicate samples incubated with glucose and single replicate of the samples incubated with water were dispersed in water by ultrasonic vibration and fractionated according to particle size and density. The procedure and the basis for obtaining these physical fractions were discussed in detail in the preceding chapter. Therefore the fractions used in this study are described only briefly.

1) Light fraction (>53 µm), separated at density 1.6 g cm^{-3} with a solution of $ZnBr_2$.

2) >20 µm fraction, obtained after combining the >53 µm mineral particles (having densities greater than 1.6 g cm^{-3}) with the >20 µm particles

left after the separation of the <20 μm particles by gravity sedimentation.

3) 20-5 μm, separated by gravity sedimentation.

4) 5.0-0.5 μm, also separated by gravity sedimentation.

5) <0.5 μm, separated from the <5 μm fraction by centrifuging in a MSE High Speed 18 centrifuge, fitted with a continuous action rotor. The flow rate was 134 cm^3 min^{-1} at 4000 rpm.

Fractions thus separated were obtained as suspensions which were concentrated at low temperatures and finally freeze-dried. The following determinations were made on the fractions.

5.2.5.1 ATP

Extractions of ATP were performed immediately after freeze-drying of the fractions and assayed within a maximum of two weeks.

5.2.5.2 Organic carbon

Organic carbon was determined by dry combustion using a high frequency Fisher Carbon Induction Apparatus (Young and Lindbeck, 1964).

5.2.5.3 ^{14}C

^{14}C in the soil and soil fractions was determined by suspension counting (Adu and Oades, 1974). Soil and soil fractions were dried at 40°C for 24 hours and then ground in a Siebteknik mill and passed through 53 μm sieve. An aliquot of 500±10 mg of CAB-O-SIL was weighed into scintillation vials and 10 cm^3 of toluene-PPO-dimethyl POPOP scintillant (0.5% w/v PPO and 0.03% w/v dimethyl POPOP in toluene) was added. The vials were closed with caps and shaken for 2 minutes on a vortex mixer. Vials were equilibrated at 4°C for 24 hours and then equilibrated with the temperature of the counter for one hour before counting. Window settings were 20-1000 in the wider channel (red) and 20-100 in the narrow channel (green) and the gain settings were 20% for both the channels.

The machine was operating at 92.7% efficiency. After counting the samples, 10 µl of ^{14}C-benzoic acid having 39,887 dpm was added to each vial which was counted again for quenching correction. The recovery of ^{14}C for each of the samples was calculated.

5.3 Results and Discussion

5.3.1 Decomposition of ^{14}C-glucose

The results of experiment I, dealing with the decomposition of ^{14}C glucose and experiment II, concerning the dynamics of ^{14}C-biomass in the Urrbrae fsl and the Northfield cl are presented in Figs. 10 and 11. The pattern of decomposition of glucose in both the soils was similar. The curves showing the residual ^{14}C with time, were thought to consist mainly of three phases (Sorensen and Paul, 1971), (i) rapid phase (less than 3 days) involving the oxidation of ^{14}C-glucose, (ii) relatively slow phase (3-50 days), consisting of heterogeneous metabolic products decaying at different rates and (iii) the slowest phase (more than 50 days), with decomposition of more or less homogeneous and stable products of decomposition. The rapid phase in the Northfield cl was shorter (2 days) than for the Urrbrae fsl (3 days) probably because the latter soil supported a relatively smaller microbial population (as shown by the measurement of ATP in these two soils; section 3.3.1, Table 2) and therefore a much longer time was necessary to metabolize the ^{14}C-glucose. During the rapid mineralization phase the decomposition of the added ^{14}C-glucose stimulated the disappearence of the native soil organic carbon in both the soils but the effects were more pronounced and long lived in the light textured than the heavy clay soil (Table 21). Ladd and Paul (1973) have also reported that for a Canadian loam in the early part of the incubation, there was positive priming effect for a period of up to 5 days and then at the later part of the incubation a strong negative priming effect was observed.

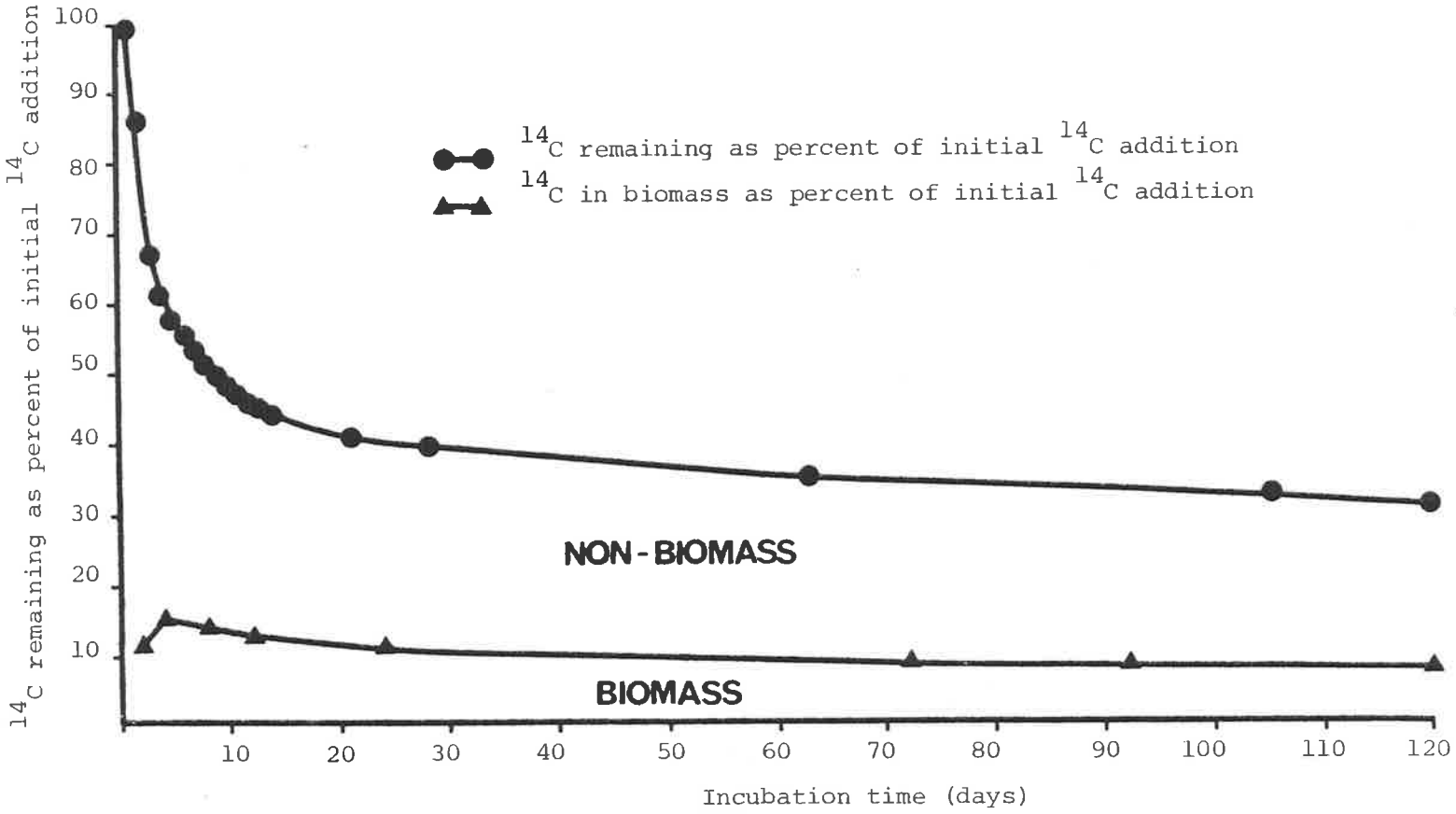

Fig. 10 Decomposition of ^{14}C-glucose in the Urrbrae fsl

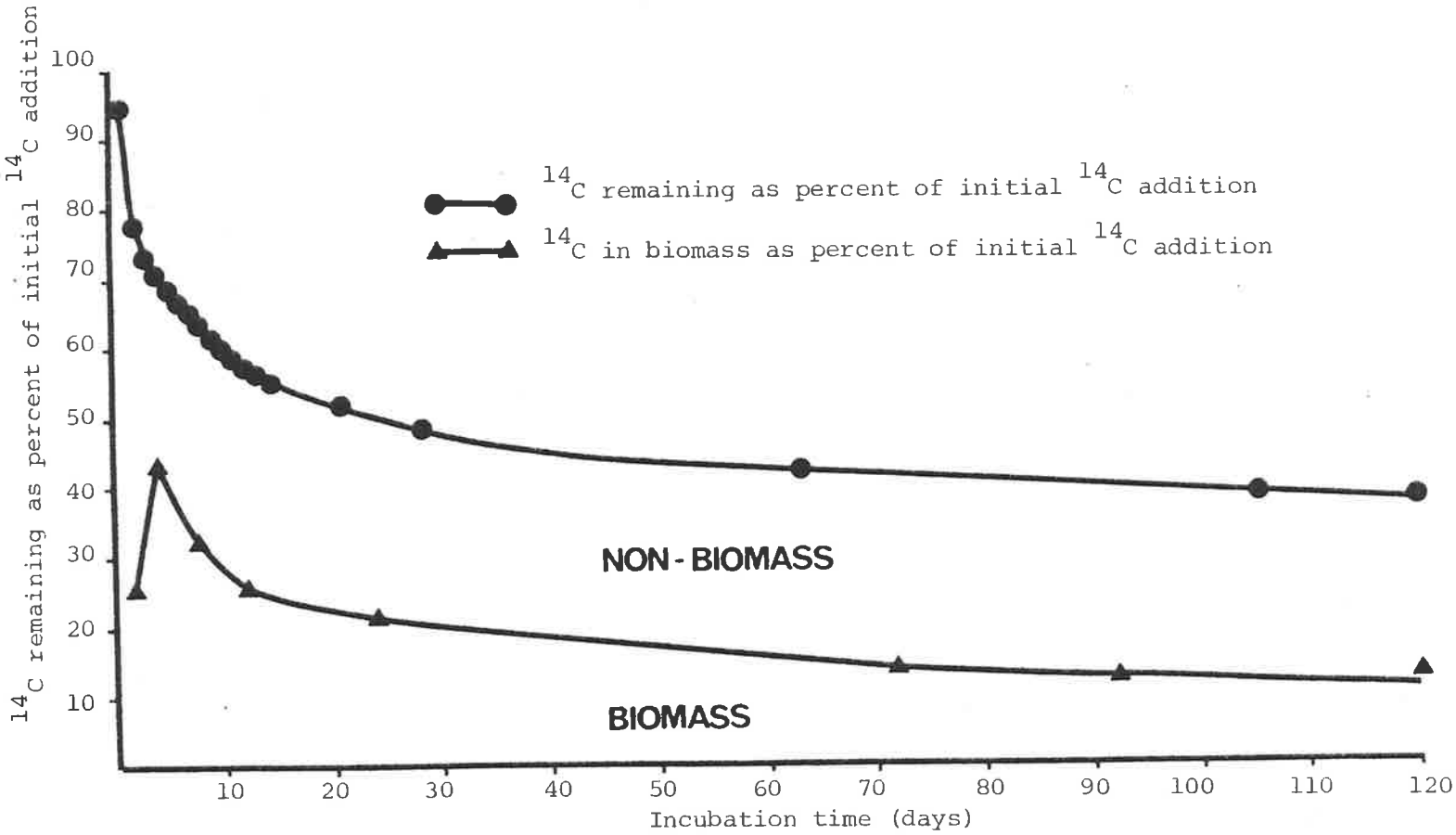

Fig. 11 Decomposition of ^{14}C-glucose in the Northfield cl

Table 21 Rate of release of unlabelled (^{12}C) CO_2 (mg C/day/80g soil) from the Urrbrae fsl and Northfield cl after addition of ^{14}C-glucose

Incubation period (days)	Urrbrae fsl	Northfield cl
1	-9.63	14.30
2	1.08	6.39
3	26.45	3.51
4	7.23	3.08
5	0.54	1.16
6	1.04	-0.03
7	3.07	-0.98
8	1.65	-0.26
9	1.41	-1.58
10	0.33	-0.86
11	1.06	-0.62
12	0.94	0.10
13	2.49	1.06
14	1.42	2.02
21	0.56	1.03
28	0.52	0.73
63	0.35	0.63
105	0.27	0.25
120	0.30	0.23

After 120 days of incubation, only 30 and 38 percent of the ^{14}C added initially could be located in the Urrbrae fsl and Northfield cl respectively. Sorensen (1975) also demonstrated increased stability of organic materials in soils due to high clay content.

The residual ^{14}C retained in the soils was thought to exist as microbial biomass and metabolic products. In the Urrbrae fsl the ^{14}C content in the microbial biomass (Fig.10) was maximal after 4 days (15.5% of ^{14}C input), biomass-^{14}C then decreased relatively quickly up to 50 days of incubation and then became relatively stable. Similarly in the clay soil the proportion of the initially added ^{14}C in the biomass attained a maximum value of 43 percent at 4 day and then declined until the 80th day of incubation before the newly synthesized biomass became fairly stable.

It was also evident that the non-biomass ^{14}C in the Urrbrae fsl during the slower phase of decomposition was decomposing at a faster rate than the biomass. On the other hand the decay of biomass accounted for most of the disappearance of ^{14}C from Northfield cl. However 120 days after ^{14}C-glucose addition, ^{14}C in the biomass of Urrbrae fsl and Northfield cl represented 7.5 and 10.0 percent respectively of the original addition. Jenkinson (1976) showed that 10% of the originally added C was present in the soil biomass after 52 days incubation.

Of the residual ^{14}C retained after 120 days in the soils, the proportion of the ^{14}C in the biomass was higher (33%) in the Northfield cl than the Urrbrae fsl (24%). These results are in good agreement with the findings of Ladd *et al.*(1981) who reported that the residual carbon present in the non-biomass as microbial metabolites was three times higher than the biomass. They also pointed out that although the proportion of ^{14}C-labelled plant material remaining in a soil was not influenced by soil texture and climate, the proportion of the ^{14}C remaining as biomass was higher in a clay than a sandy soil. On the

other hand Sorensen (1975) showed that the proportion of residual ^{14}C remaining in the soil as biomass was not correlated with the silt plus clay content of the soils.

5.3.2 Efficiencies of carbon utilization

Payne (1970) studied the efficiency of utilization of glucose for pure cultures of bacteria under aerobic conditions and concluded that at the exponential phase, the carbon assimilation efficiency was 60 percent. Consideration of the results in Fig.10 and 11, in terms of the efficiency of carbon utilization (taking efficiency as 60 percent) show that in the Urrbrae fsl at day 4, 43% and 2.5% of the initial ^{14}C-glucose added were present as metabolic products and unmetabolized ^{14}C-glucose respectively. In the Northfield cl, such a calculation yielded a negligible proportion of the initially added ^{14}C as metabolic products and a high proportion (26.25%) as unmetabolized ^{14}C-glucose. Although no determination or original ^{14}C-glucose was made after each incubation period, very little of the original ^{14}C-glucose was expected to be present in soils after a few days (Ladd and Paul, 1973; Persson, 1968, Cheshire *et al.*,1969). Therefore it seems unlikely that such a high proportion of the original glucose would remain after 4 days and the negligible proportion of the metabolic products leads to the proposal that the efficiency factor was probably higher than 60% for the Northfield cl. Taking the proportion of ^{14}C retained as the efficiency of carbon utilization, i.e. 70.5%, then in the Northfield cl the glucose carbon was completely metabolized and the metabolites constituted 27% of the residual ^{14}C. Camp (1963) has shown that theoretically carbon assimilation efficiencies of up to 70% are possible. It is likely that in a complex system such as soil, an array of products may be synthesized requiring low energy and therefore higher efficiency of carbon utilization may occur.

5.3.3 Assessment of microbial activities in soils

It has been established that a sequential change in microbial population occurs after addition of glucose (Adu 1975; McGill, 1971) and therefore any estimate of biomass based on a single criterion may lead to serious errors (Nannipieri *et al.*,1978). It was decided to compare some of the indices of microbial activity such as ATP, biomass carbon and the $^{14}CO_2$ evolution rate in soils incubated with ^{14}C-glucose. The results obtained are presented in Fig.12 and 13. Both biomass and the ATP contents increased to maximum values two days after glucose addition. The values were almost twice as large in the Northfield cl compared with the Urrbrae fsl. The rate of decline of the biomass-C and the ATP was slower in the Northfield cl than in the Urrbrae fsl (Ladd *et al.*,1977c). It was also evident that although the amounts of both biomass and ATP were reduced considerably, nevertheless at the end of 120 days, the values were still higher than at the beginning of the incubation.

The rate of release of $^{14}CO_2$ predictably increased at the beginning of the incubation and the pattern of changes in ATP, biomass carbon and $^{14}CO_2$ were very similar for the Urrbrae fsl. However in the case of Northfield cl, the $^{14}CO_2$ evolution rates were in good agreement with ATP and the biomass carbon contents until day 2 and thereafter the respiration rate declined disproportionately faster than either ATP or biomass carbon contents. The ATP content of the Urrbrae fsl reached a maximum value 24 hours earlier than the $^{14}CO_2$ evolution rate reached its maximum rate, but in the case of Northfield cl all three indices reached maximum values simultaneously at the end of two days. By contrast Nannipieri *et al.* (1978) showed that the ATP and biomass contents reached maximum values 24 hours later than the maximum CO_2 evolution. In spite of the poor correlation between CO_2 and ATP or biomass carbon during the early part of the incubation period it became apparent that with the concurrent stabilization of the easily metabolized substances, both ATP and the

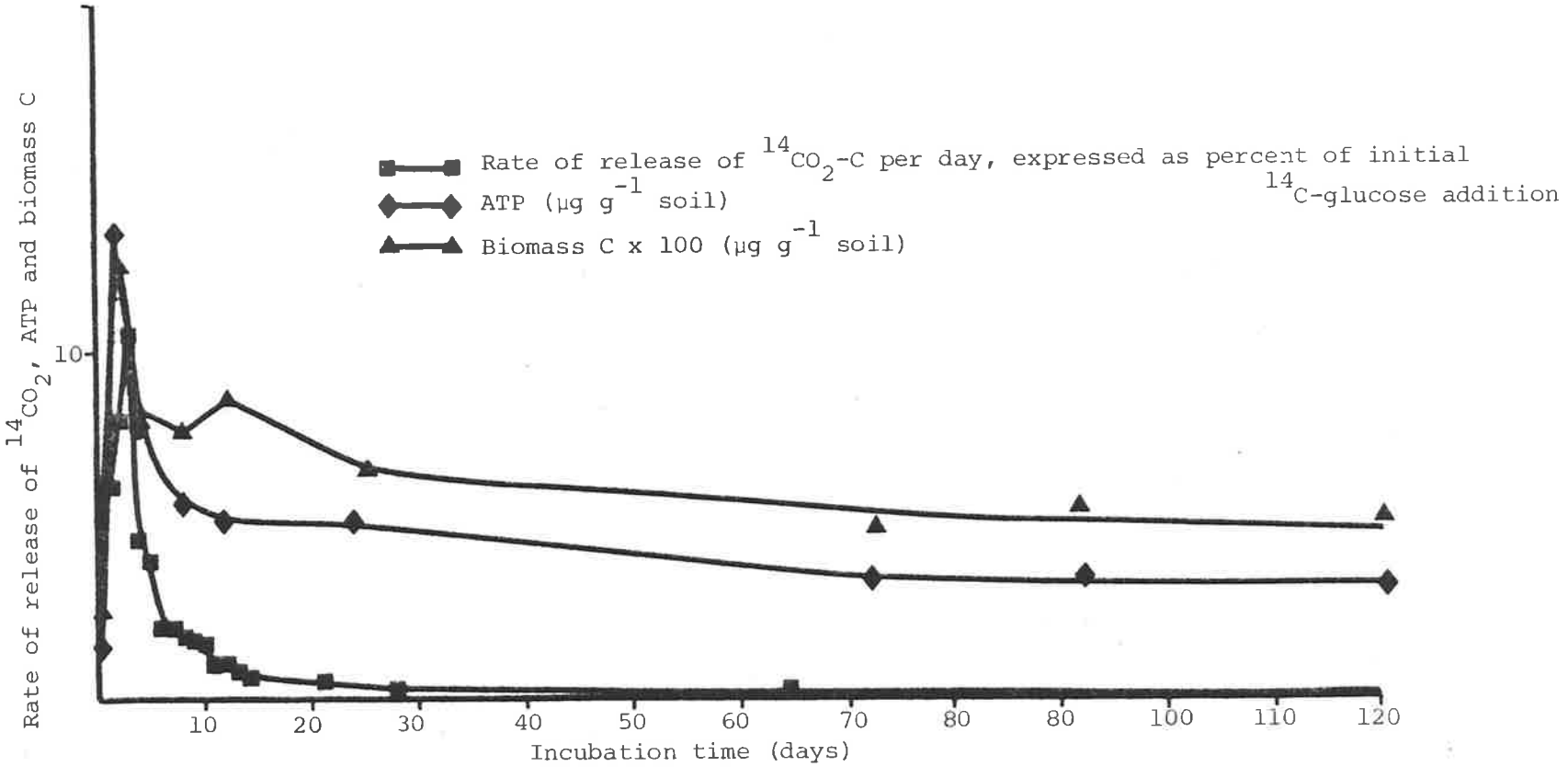

Fig. 12 Microbial activities in the Urrbrae fsl

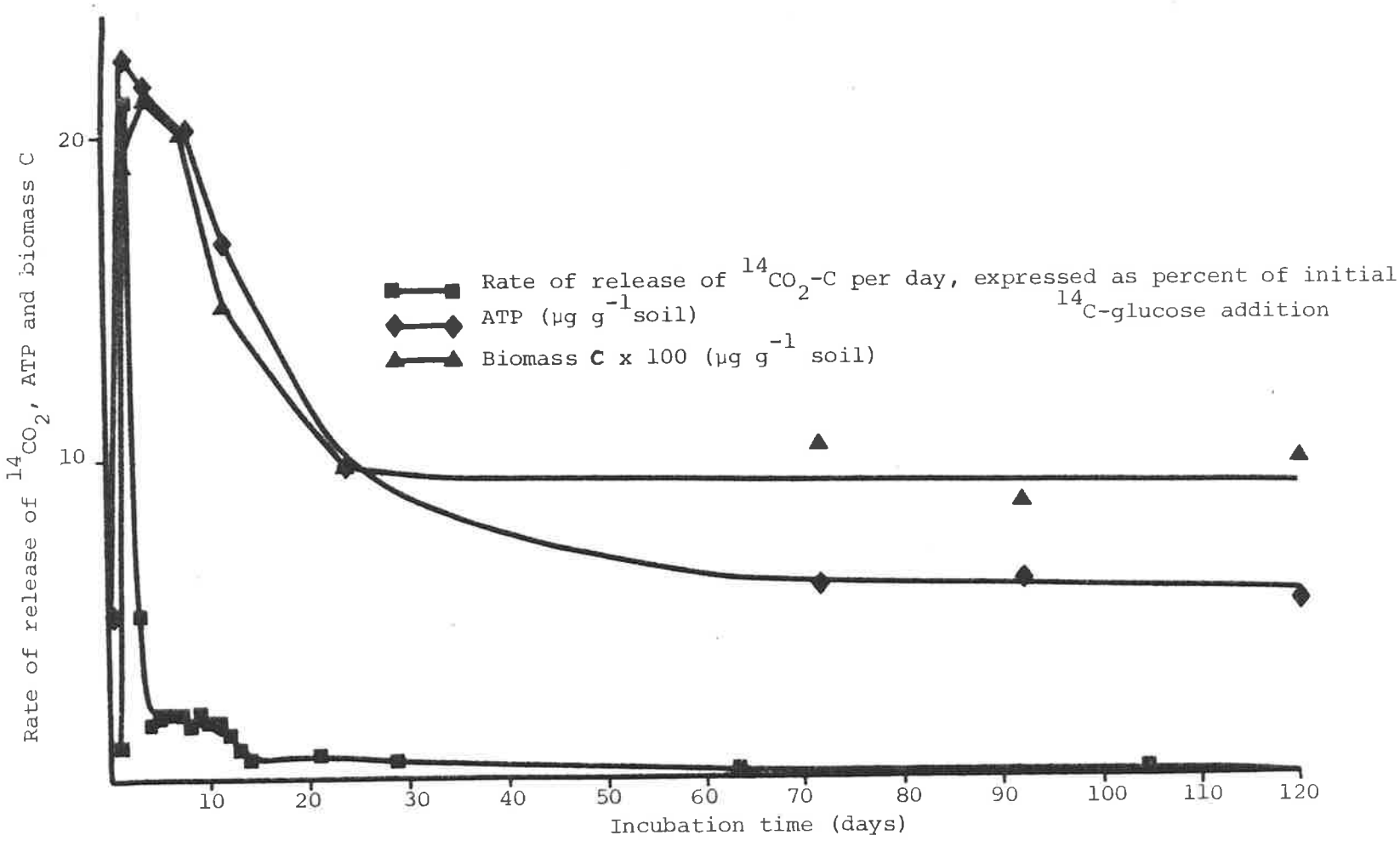

Fig. 13 Microbial activities in the Northfield cl

biomass also became reasonably stable. The observed similarity in the pattern of changes in the respiration rate, ATP and the biomass carbon indicated that ATP could be used to provide a measure of biomass in the glucose amended soils.

5.3.4 Biomass C/ATP ratios of the soils after addition of ^{14}C-glucose

The effect of glucose addition on the concentration of ATP in the newly formed microbial population was determined by calculating the amount of labelled carbon in the biomass divided by the increase in the content of ATP after glucose addition (Table 22). Biomass C/ATP ratios were very low for both the soils at day 2, although the reliability of the biomass carbon data was questionable (Jenkinson and Powlson, 1976b). Some errors were inevitable in the determination of biomass by the fumigation technique, especially during the early phase of incubation due to the use of the amount of $^{14}CO_2$ released during the 0-10 day period of the incubation of unfumigated soils. However at day 4 the amount of $^{14}CO_2$ released after fumigation was much higher in the Northfield cl than the Urrbrae fsl, indicating that a higher proportion of the ^{14}C material became susceptible after fumigation, i.e. Northfield cl supported a much larger labelled microbial population than the Urrbrae fsl.

Biomass C/ATP ratios of the two soils increased more than two fold at the end of 2 days. In the case of Urrbrae fsl there was a gradual increase at the end of 2 days but in the Northfield cl the ratios remained fairly stable for a period of up to 12 days. Two different trends could be observed for the two soils, (i) in the early phase of incubation the ratios in the Urrbrae fsl were wider than the Northfield cl, and (ii) at the later part of the incubation the trend was reversed. In spite of these differences, the striking similarity was that the ratio became very wide 24 days after addition of glucose. The biomass C/ATP ratios

Table 22 Biomass C/ATP ratios of the newly developed labelled microbial population in the Urrbrae fsl and the Northfield cl after addition of glucose

Incubation period (days)	Urrbrae fsl			Northfield cl		
	Calculated labelled biomass C ($\mu g\ C\ g^{-1}$ soil)	ΔATP*	Biomass C / ATP ratio	Calculated labelled biomass C ($\mu g\ C\ g^{-1}$ soil)	ΔATP*	Biomass C / ATP ratio
2	336	11.68	28.8	762	17.38	43.8
4	456	5.98	76.3	1302	16.75	77.7
8	420	3.69	113.8	948	13.36	71.0
12	378	3.17	119.8	762	11.60	65.7
24	324	3.27	99.2	624	4.68	133.3
72	246	1.32	186.2	402	1.09	368.8
92	246	1.48	166.2	360	0.96	359.0
120	228	1.18	193.2	378	0.96	393.8

*Increase in the value of ATP as compared to control with no ^{14}C glucose addition

of the soil population (Table 23) as a whole also reflected the influence of the biomass C/ATP ratios of the labelled population.

The biomass C/ATP ratios of the labelled biomass could be compared with the results of Nannipieri *et al.*(1978) who reported that the ratio was 49, 30 hours after addition of glucose and then increased and finally stabilized at 82 from 12-22 days of the incubation period. It may be assumed that the wider biomass C/ATP ratios after 24 days of glucose addition were due to the fact that the organisms were faced with conditions similar to starvation and were preparing for dormant forms of life. Such wide biomass C/ATP ratios may result from either the concentration of ATP in the cells dropping considerably during incubation (Lee *et al*1971b) or inefficient extraction of ATP from the soil population (see section 3.3.2) or both.

5.3.5 Fractionation of the soils

5.3.5.1 Fraction weights

The distribution of the weight of the fractions (% by weight) in the Urrbrae fsl and the Northfield cl incubated with glucose and water are presented in Fig. 14 and 15. The recovery of the weight of the fractions varied between 95.2 and 99.2%. It was evident that there was no significant change in the distribution of the weight of the fractions in both the soils, irrespective of the additions and the period of incubation. Although in the soils incubated with glucose some degree of aggregation was expected due to the synthesis of microbial polysaccharides the effects would have been transient or temporary (Tisdall and Oades, 1981). In addition the energy of dispersion of the ultrasonic probe is high (Edwards and Bremner, 1967) enough to result in efficient dispersion.

5.3.5.2 ATP content

Although the deleterious effects of freezing and thawing were recognized (section 3.3.4), soils after appropriate periods of incubation

Table 23 Biomass C/ATP ratios of the soil population of the Urrbrae fsl and the Northfield cl after addition of ^{14}C-glucose

Incubation period	Urrbrae fsl				Northfield cl			
	Biomass carbon calculated using the value of CO_2-C released from unfumigated soil during 0-10 day period		Biomass carbon calculated using the value of CO_2-C released from unfumigated soil during 10-20 day period		Biomass carbon calculated using the value of CO_2-C released from unfumigated soil during 0-10 day period		Biomass carbon calculated using the value of CO_2-C released from unfumigated soil during 10-20 day period	
(days)	Biomass C ($\mu g\ g^{-1}$ soil)	Biomass C / ATP ratio	Biomass C ($\mu g\ g^{-1}$ soil)	Biomass C / ATP ratio	Biomass C ($\mu g\ g^{-1}$ soil)	Biomass C / ATP ratio	Biomass C ($\mu g\ g^{-1}$ soil)	Biomass C / ATP ratio
2	1247	94	2037	153	1902	85	2582	115
4	821	104	1247	156	2217	104	2528	116
8	769	135	1021	179	2011	106	2311	121
12	861	170	997	193	1801	109	2087	126
24	562	108	601	114	1464	152	1687	174
72	500	151	556	167	1036	176	1156	196
92	538	155	584	168	838	162	974	162
120	508	161	516	163	944	175	943	175

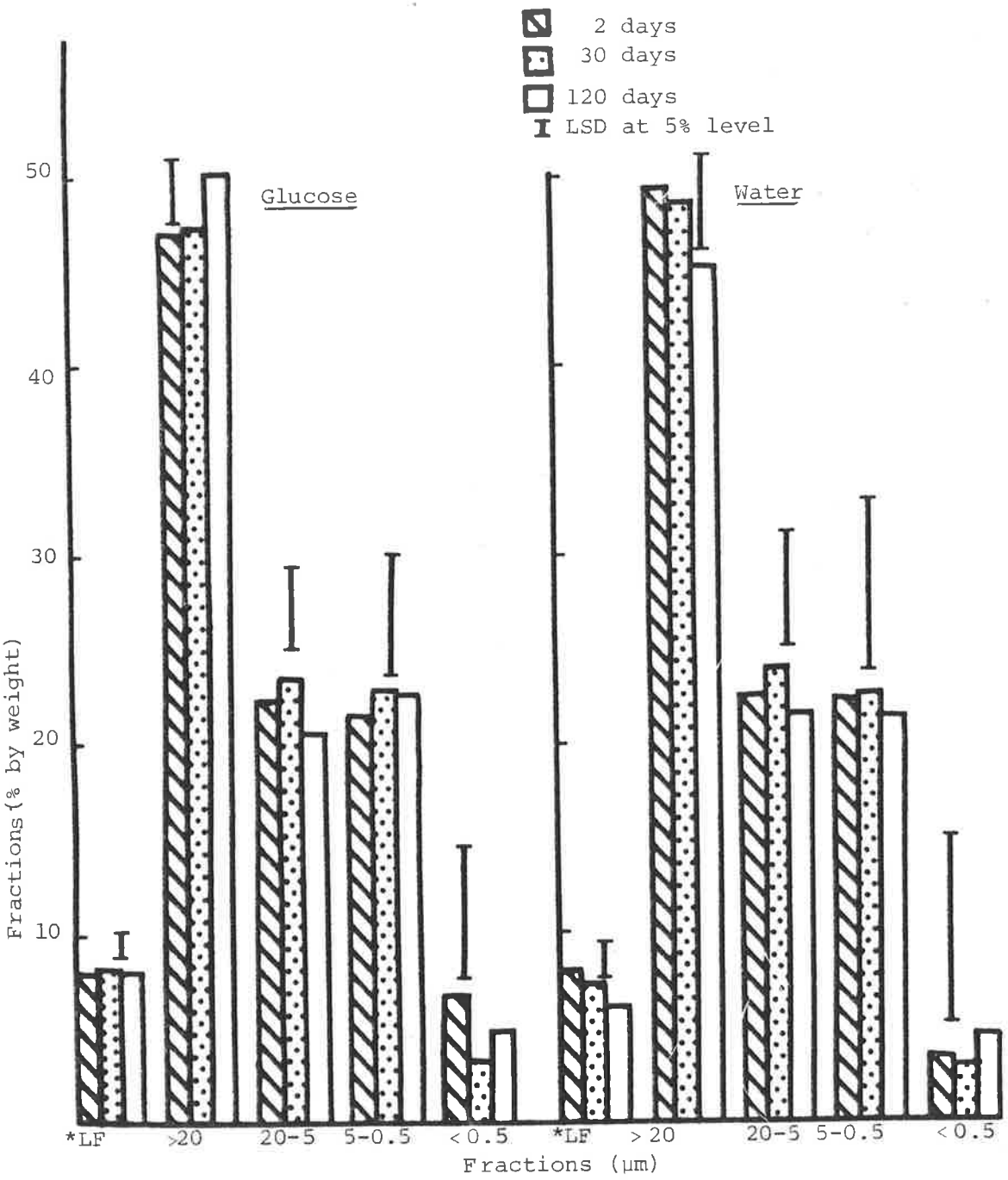

Fig. 14 Distribution of fractions in the Urrbrae fsl after addition of ^{14}C-glucose and water

* The actual value was multiplied by 10

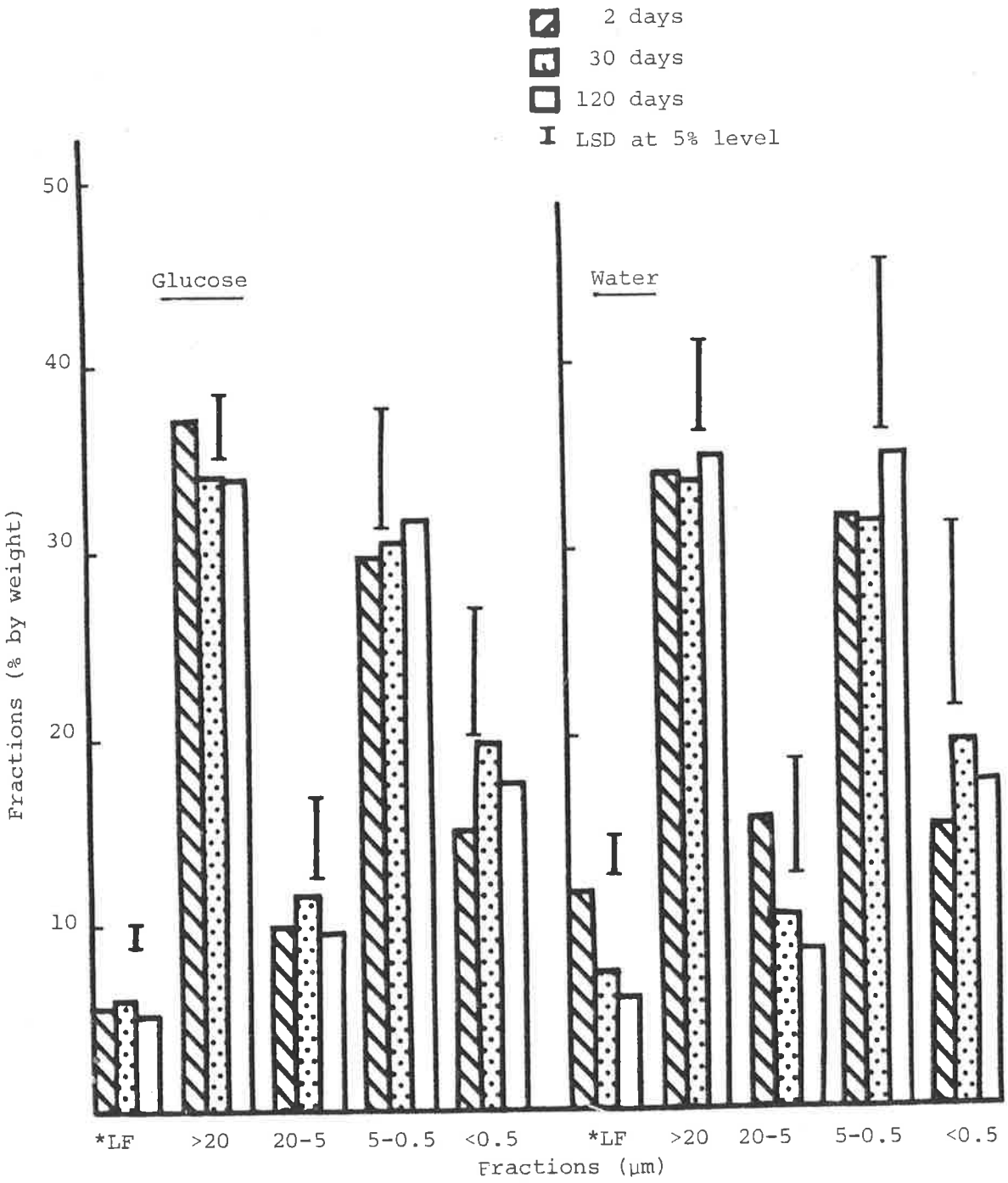

Fig. 15 Distribution of fractions in the Northfield cl after addition of ^{14}C-glucose and water

*The actual value was multiplied by 10.

were stored at -20°C until required since it was not possible to fractionate the soils immediately after incubation due to the time consuming operations involved in fractionation. Substantial losses in the content of ATP from the soils occurred due to storage at -20°C as well as during fractionation (Table 24). During storage and fractionation presumably a large proportion of the organisms died due to the action of freezing and thawing, rupture of the cells due to ultrasonic vibration, centrifugation and drying procedures. The distribution of ATP in the soil fractions (not corrected for all these losses) of the Urrbrae fsl and the Northfield cl incubated with glucose and water are presented in Figs. 16-19. In the Urrbrae fsl, incubated with water, the concentration of ATP in the fractions was in general inversely related to the size of the soil fractions and did not change significantly with time. There was a consistent decline with time in the content of ATP in the light fraction and the 20-5 μm fraction, but changes were not statistically significant ($P<0.05$).

In the glucose amended samples, the ATP concentration (μg ATP g^{-1} of the fractions) increased approximately three times in the <0.5 μm fraction and then declined significantly with time accompanied by a concomittant increase in the concentration of ATP in the 5.0-0.5 μm fraction. The concentration of ATP in the light fraction and the 20-5 μm were also increased considerably but no significant change was observed with time. As the fraction weights did not change significantly during incubation the amounts of ATP in the fractions (Fig.17) also showed similar trends to the concentration of ATP in the soil fractions.

In the case of Northfield cl incubated with water at day 2 the highest concentration of ATP was located in the 5.0-0.5 μm fraction and fractions with diameter 20-5 μm and the <0.5 μm fraction had lower concentrations of ATP (Fig. 18). The ATP concentration of the light fraction in this soil was much higher than the light fractions of the Urrbrae fsl. With

Table 24 Recovery of ATP from the fractions

Incubation period (days)	ATP content (μg g^{-1})					
	Glucose amended			Water amended		
	Determined immediately	After storing at 20°C	*ΣFractions	Determined immediately	After storing at -20°C	*ΣFractions
			Urrbrae fsl			
2	13.31	5.60	2.46	1.63	1.33	1.42
30	4.90	3.95	2.40	1.63	1.03	0.89
120	3.20	2.74	1.92	2.02	1.44	1.13
			Northfield cl			
2	22.30	18.27	4.35	5.02	4.72	1.83
30	8.70	6.49	3.03	4.02	3.37	1.80
120	5.70	4.06	1.50	4.74	4.14	1.68

*ΣATP content (μg) of the fractions g^{-1} soil fractionated

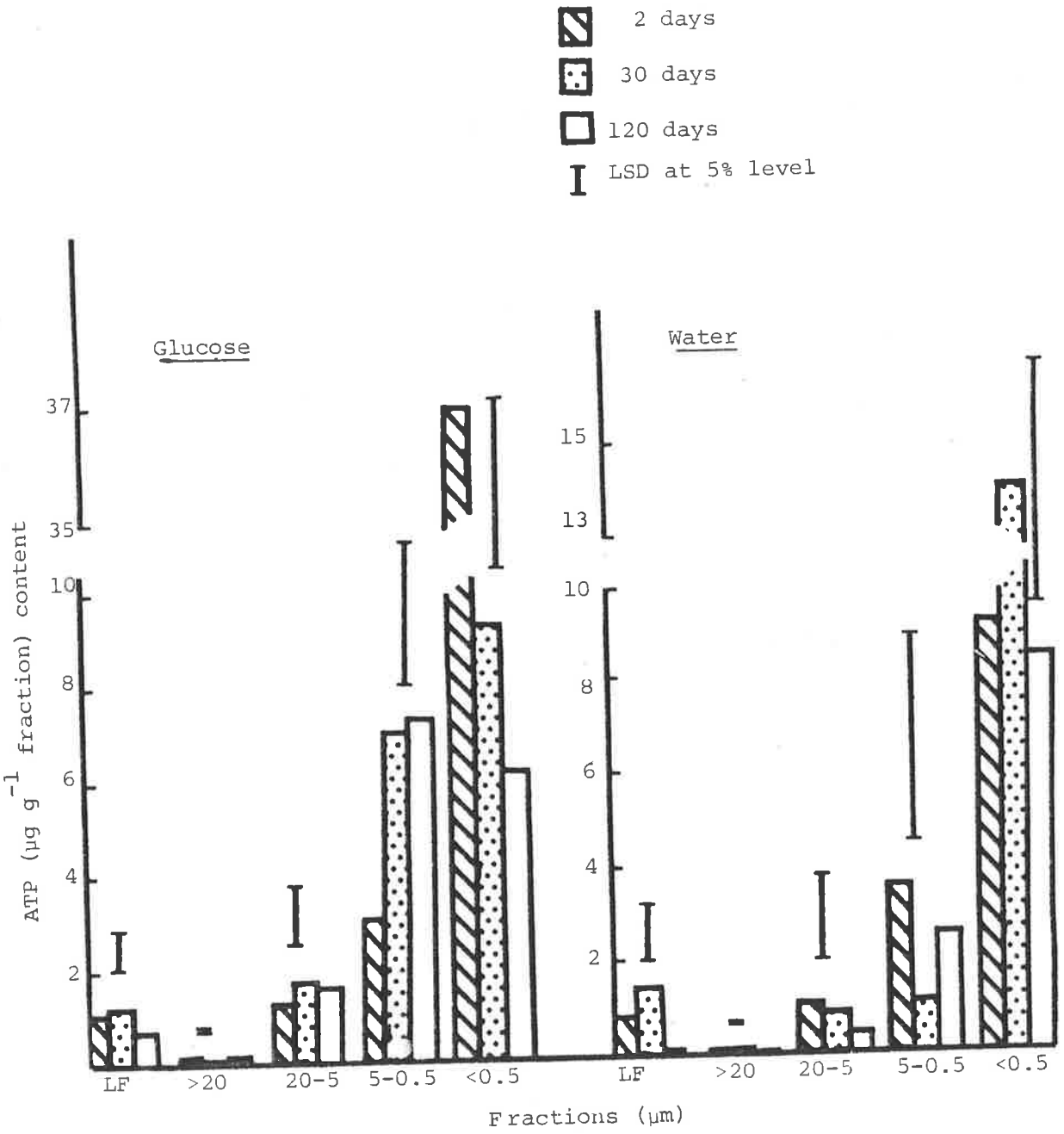

Fig. 16 Distribution of ATP in fractions of the Urrbrae fsl after addition of ^{14}C-glucose and water

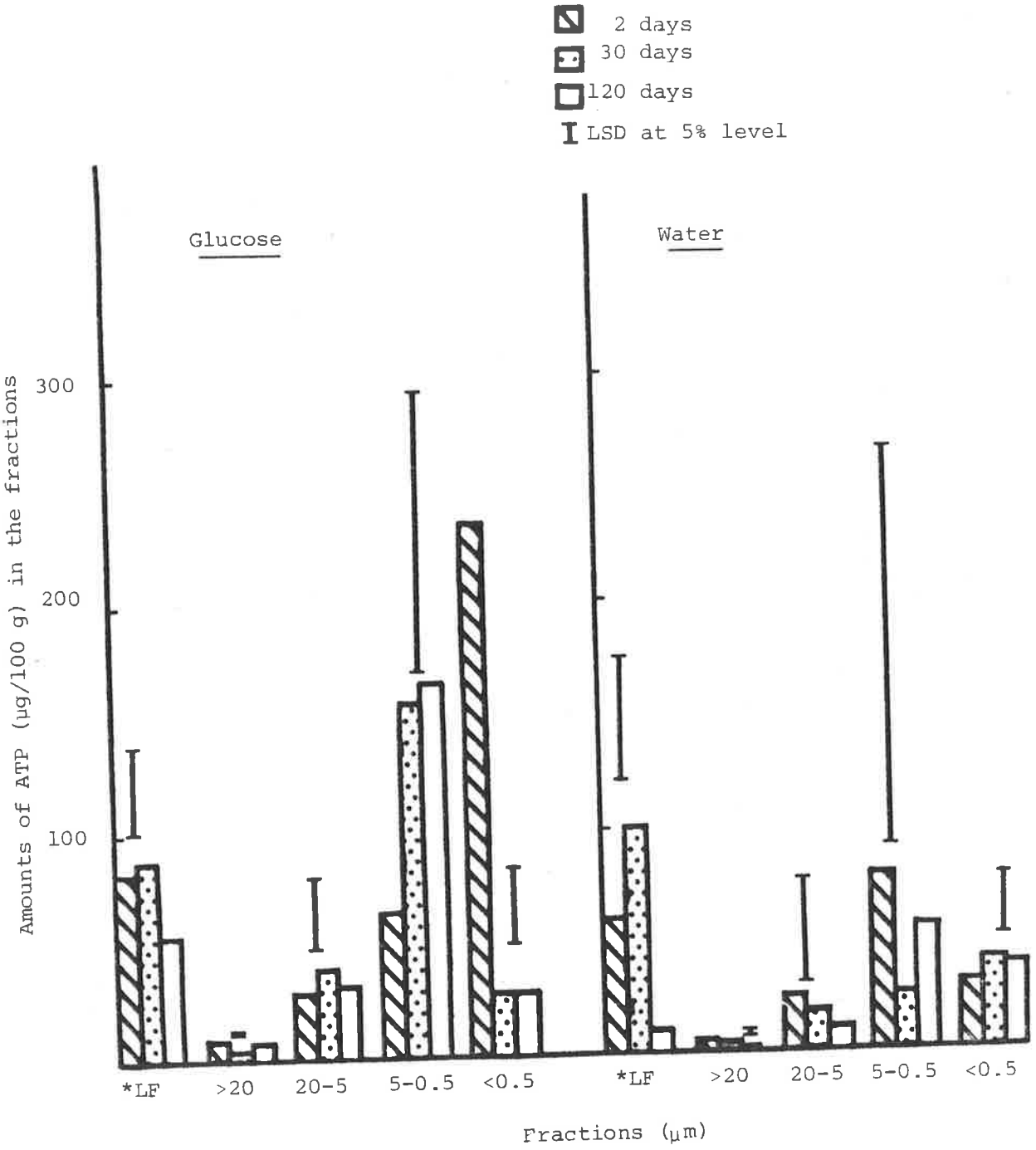

Fig. 17 Changes in the amounts of ATP in fractions of the Urrbrae fsl after addition of ^{14}C-glucose and water

*The actual value was multiplied by 100 .

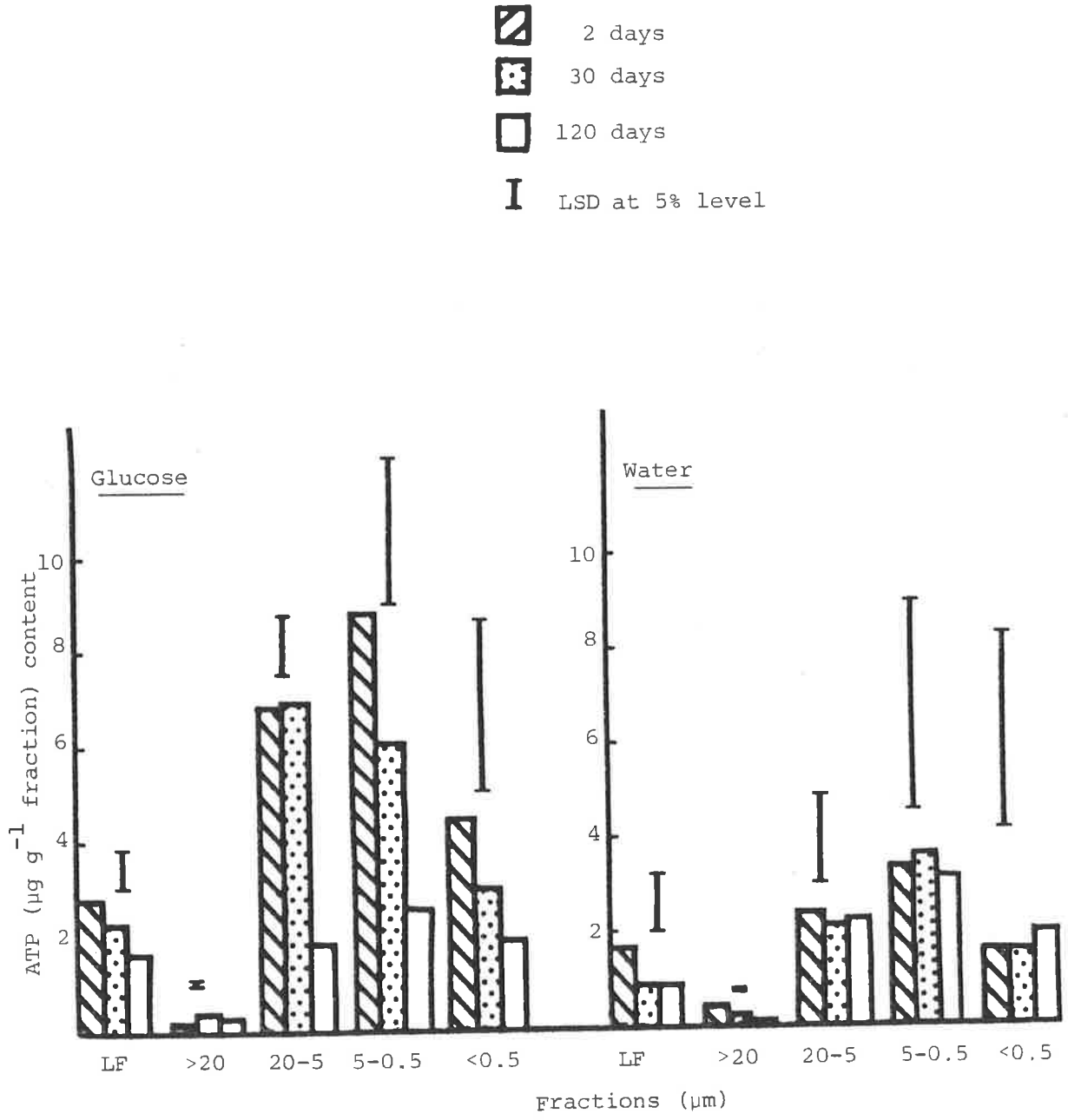

Fig. 18 Distribution of ATP in fractions of the Northfield cl after addition of ^{14}C-glucose and water

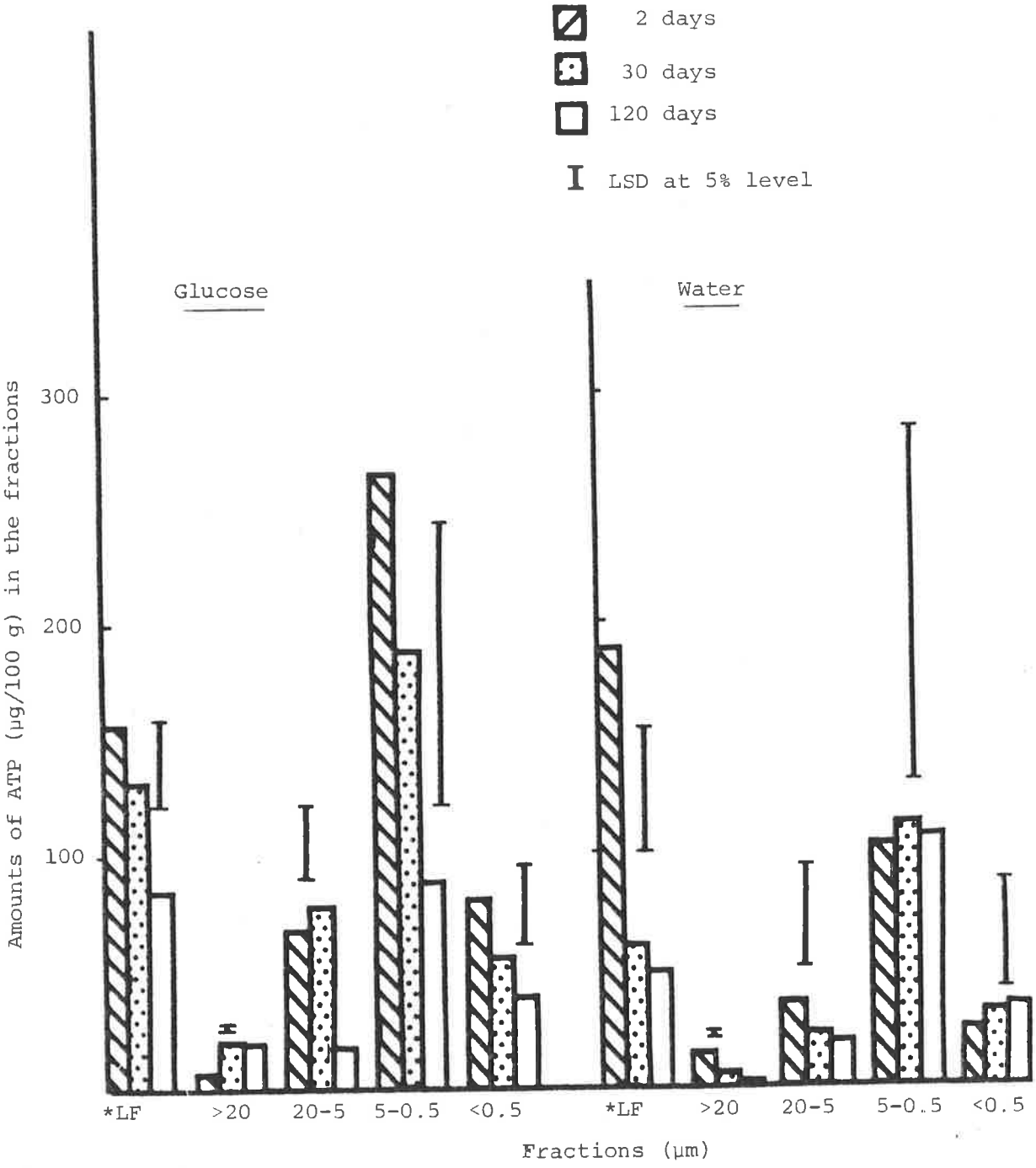

Fig. 19 Changes in the amounts of ATP in fractions of the Northfield cl after addition of ^{14}C-glucose and water

*The actual value was multiplied by 100.

the exception of the >20 μm fraction where there was a significant decline in the concentration of ATP, all other fractions showed no change in the concentration of ATP with time. But when the amounts of ATP (Fig. 19) in the fractions were considered there was a loss in the amounts of ATP in the light fraction, >20 μm and the 20-5 μm fraction although for the latter fraction, the decline was not statistically significant. The amounts of ATP in the 5.0-0.5 μm and <0.5 μm fractions remained stable throughout the incubation period.

Two days after glucose addition, the ATP concentration of all the fractions in the Northfield cl increased approximately twice that of the soils incubated with water, with the exception of the >20 μm size fraction. Thereafter the ATP concentration gradually declined in these fractions during incubation. Similar trends in the amounts of ATP in the fractions were also observed with time. These results are in agreement with the report of Amato and Ladd (1980) who observed a gradual decline in the ^{14}C biomass of silt, coarse clay and the fine clay fraction although the decline was statistically significant only for the latter fraction.

During the slower phase (2-30 days) of decomposition of glucose in the Urrbrae fsl, the rapid decline in the concentration and the amount of ATP in the <0.5 μm fraction greatly influenced the decline of the ^{14}C-biomass from the Urrbrae fsl. On the other hand in the later phase (30-120 days) the 5.0-0.5 μm and the 20-5 μm fractions became enriched with microbial biomass which remained stable during the period of incubation. Moreover, it was observed that at the end of 120 days of incubation, the concentrations and the amounts of ATP in these two fractions were higher than the comparable fractions of the soils incubated with water.

By contrast the decline of ^{14}C in the biomass of the Northfield cl was rapid and was associated with the decline in the content of ATP from all the fractions except the >20 μm fraction.

5.3.5.3 ^{14}C in the fractions

^{14}C in various soil fractions (expressed as μg C g^{-1} fraction) of the Urrbrae fsl and Northfield cl are presented in Fig.20. The recovery of ^{14}C from the fractions varied between 72.4 and 99.9% of the unfumigated soil (Table 25). Such variable recoveries of ^{14}C from the fractions might have resulted due to the loss of ^{14}C as $^{14}CO_2$ due to metabolism during the time of storing the fractions as suspensions at 2°C for more than a week or during the process of fractionation or perhaps some of the materials lost were heavily labelled fractions.

It was observed that the ^{14}C retained in both the soils was distributed rapidly in all the soil fractions. However during the early phase of decomposition (day 2), most of the ^{14}C was contained in the <0.5 μm fraction of the Urrbrae fsl and the amount declined significantly throughout the incubation period. Although the concentrations of ^{14}C (Fig.20) in the light fraction of both the soils were very high, it represented only a small pool of the immobilized ^{14}C (Fig.21) which declined initially rapidly and then became fairly stable after 30 days of incubation. After 2 days incubation of the Northfield cl, the concentration of ^{14}C was similar for the 5.0-0.5 μm and <0.5 μm fractions and declined significantly during the incubation period. After 30 days incubation there was a significant gain in the amount of ^{14}C in the 20-5 μm fraction of the Northfield cl and the 20-5 μm and 5.0-0.5 μm fractions of the Urrbrae fsl (Fig.21). The amounts of ^{14}C in these fractions declined significantly during the latter part of the incubation period (30-120 days). These results are similar to the findings of Amato and Ladd (1980) who reported loss of ^{14}C from most soil fractions, with greatest losses from the fine clay and light fraction. They also reported a considerable gain of ^{14}C in the coarse clay fraction of a clay soil, but the results presented in Fig. 20 and 21 reveal that a comparable fraction (5.0-0.5 μm) of the Northfield cl showed a continued decline

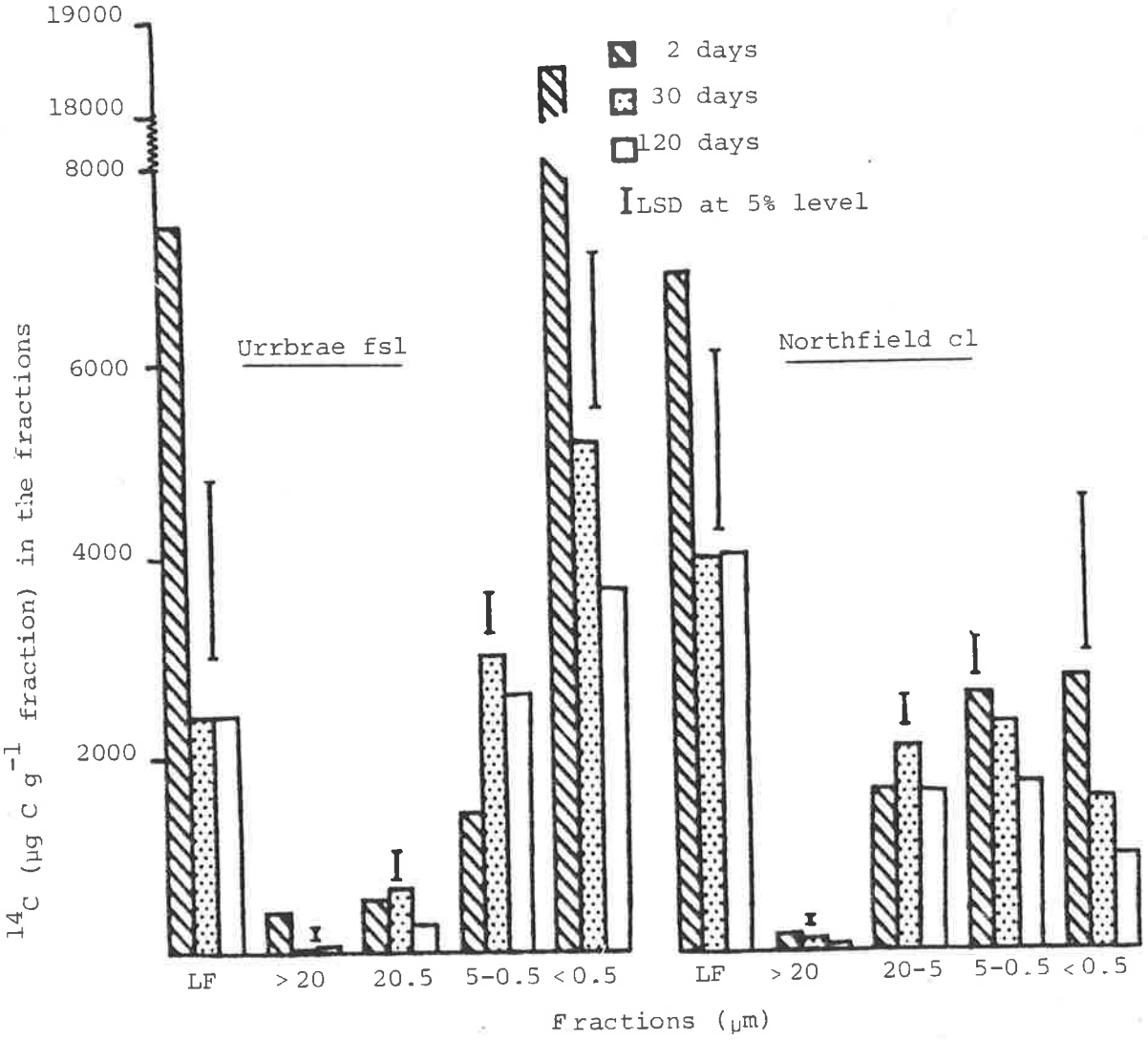

Fig. 20 Distribution of ^{14}C in fractions of the Urrbrae fsl and the Northfield cl with time

Table 25 Recovery of ^{14}C (expressed as µg C g^{-1} soil fractionated) from the fractions

Incubation period (days)	Labelled C (µg g^{-1})	
	Unfractionated soil	*ΣFractions
	Urrbrae fsl	
2	1858	1857
30	1252	1058
120	898	894
	Northfield cl	
2	2163	1566
30	1573	1258
120	1209	938

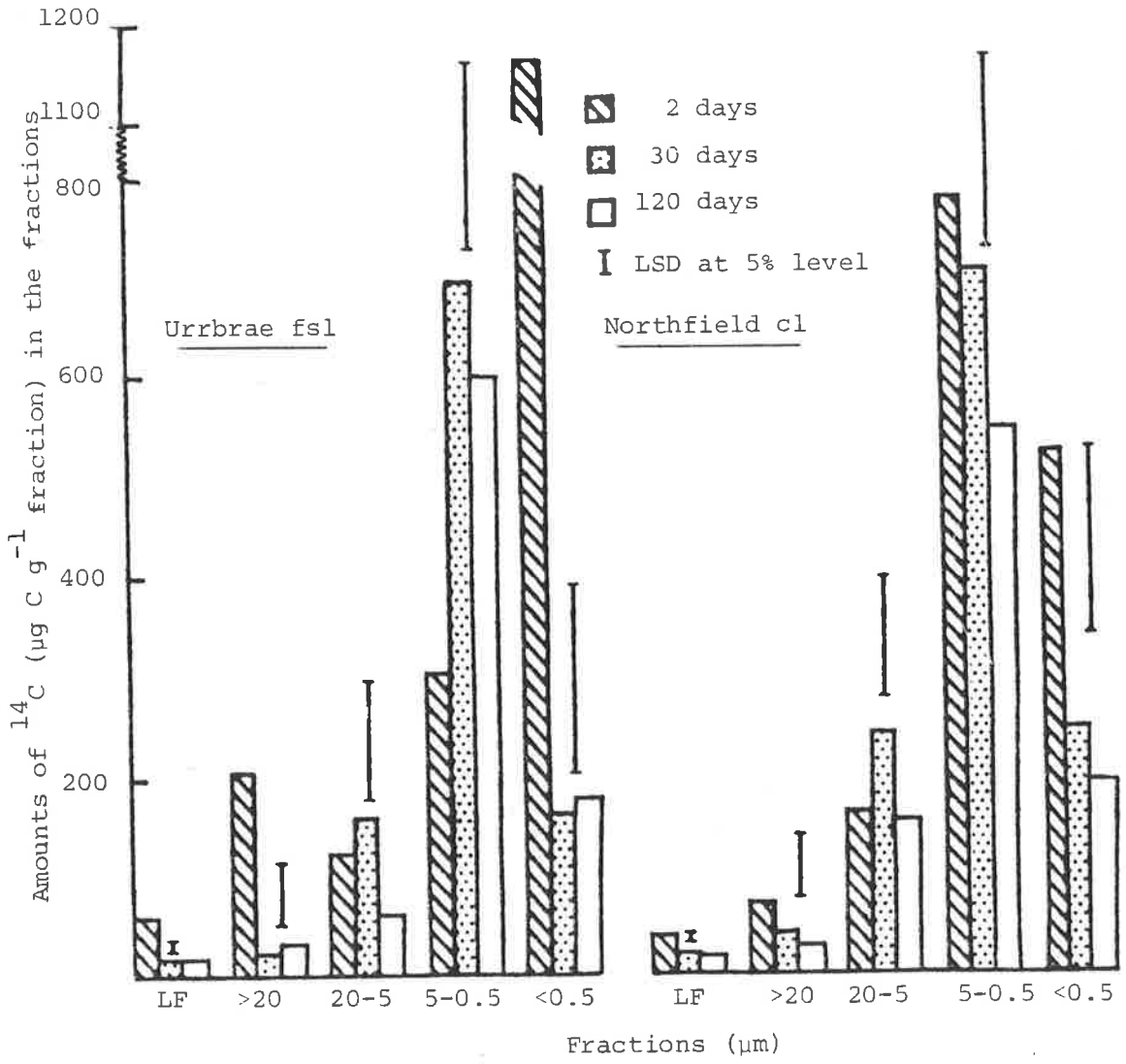

Fig.21 Changes in the amounts of ^{14}C in fractions of the Urrbrae fsl and the Northfield cl with time

as compared to the Urrbrae fsl where both gains and losses of ^{14}C occurred throughout the incubation.

5.3.5.4 Organic carbon

The content and the amount of organic carbon ($^{12}C + ^{14}C$) in the soil fractions are presented in Figs. 22-25. In both the soils incubated with water the organic carbon in all but the >20 μm fraction did not change significantly ($P < 0.05$) during incubation. Addition of ^{14}C-glucose increased considerably the content and the amount of organic carbon in the >20 μm, 5.0-0.5 μm and the <0.5 μm fractions of the Urrbrae fsl and in the >20 μm, 5.0-0.5 μm and the <0.5 μm fractions of the Northfield cl.

The labelled carbon (^{14}C) represented considerable proportions of organic carbon in the 5.0-0.5 μm and the <0.5 μm fractions of the Urrbrae fsl. As the native organic carbon (^{12}C) in these fractions remain unchanged and the concentration and the amount of labelled carbon in the <0.5 μm fraction declined significantly ($P < 0.05$) as compared to an increase in the 5.0-0.5 μm fraction during incubation, the latter fraction accumulated relatively more of resistant products of microbial metabolism (Ladd *et al.*,1977b). In contrast to other fractions where the native organic carbon (^{12}C) was unaffected by glucose addition, the decomposition of the >20 μm and the 20-5 μm fractions were influenced significantly.

In the Northfield cl relatively high proportions of the labelled C in the organic carbon were present in the 5.0-0.5 μm and the <0.5 μm fractions but the amounts declined significantly during incubation. On the other hand the proportion of labelled C in the 20-5 μm fraction was considerably smaller and remain unchanged during incubation. Therefore it may be assumed that the 20-5 μm fraction was enriched with resistant materials (Ladd *et al.*,1977b). The native organic carbon of the >20 μm fraction also decreased similarly as in the Urrbrae fsl.

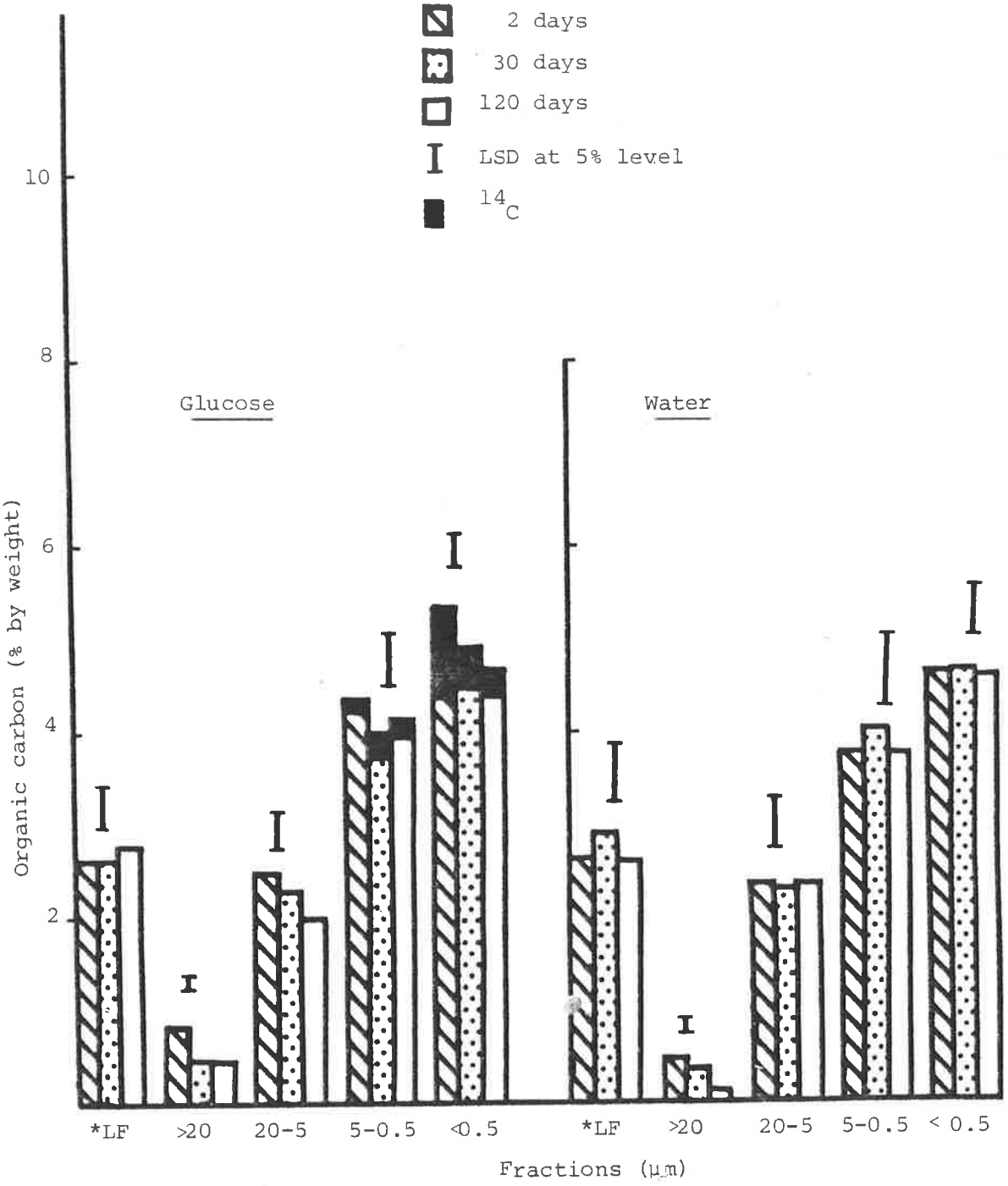

Fig. 22 Distribution of organic carbon in fractions of the Urrbrae fsl after addition of ^{14}C-glucose and water

*The actual value was multiplied by 1/10

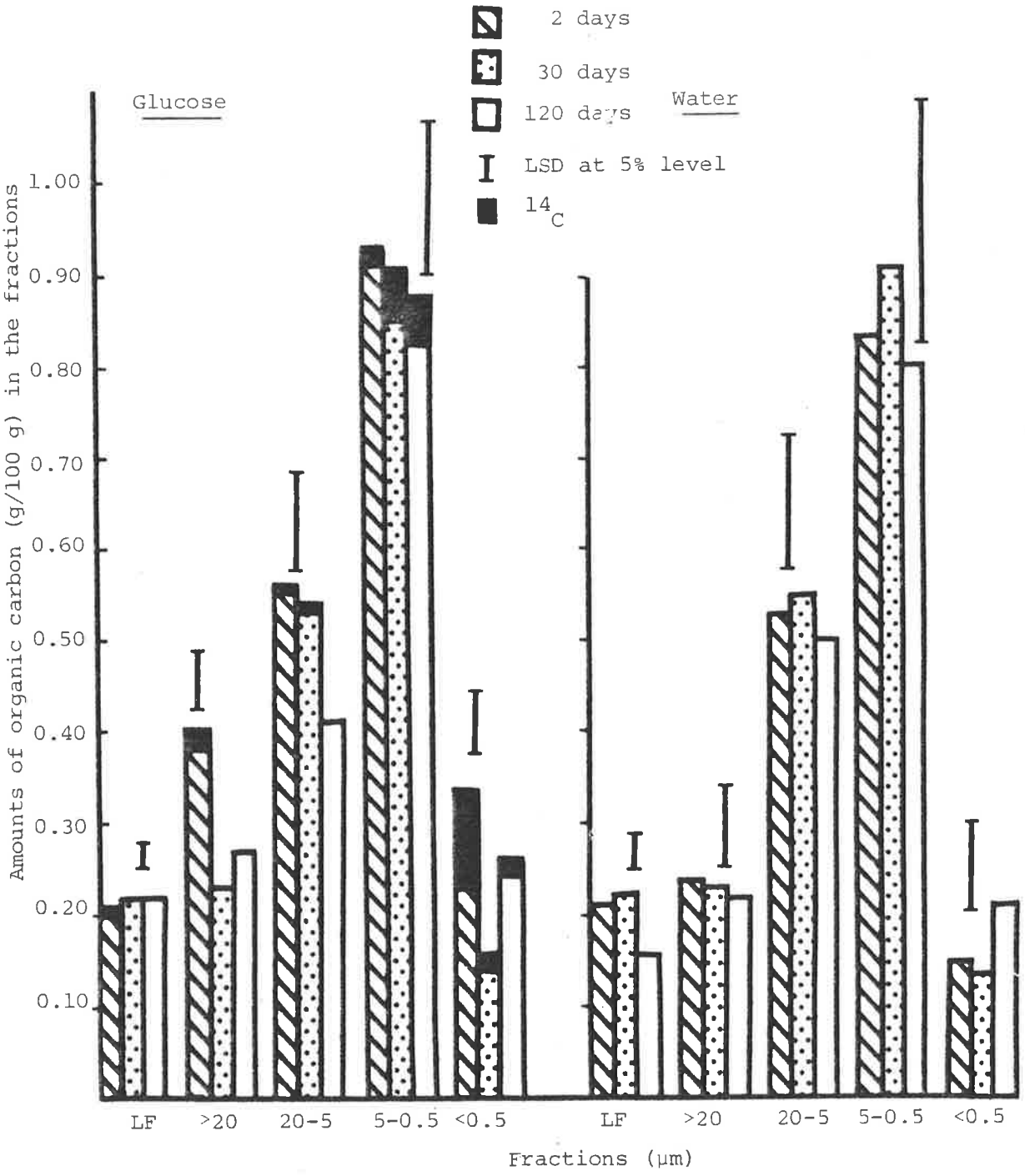

Fig. 23 Changes in the amounts of organic carbon in fractions of the Urrbrae fsl after addition of ^{14}C-glucose and water

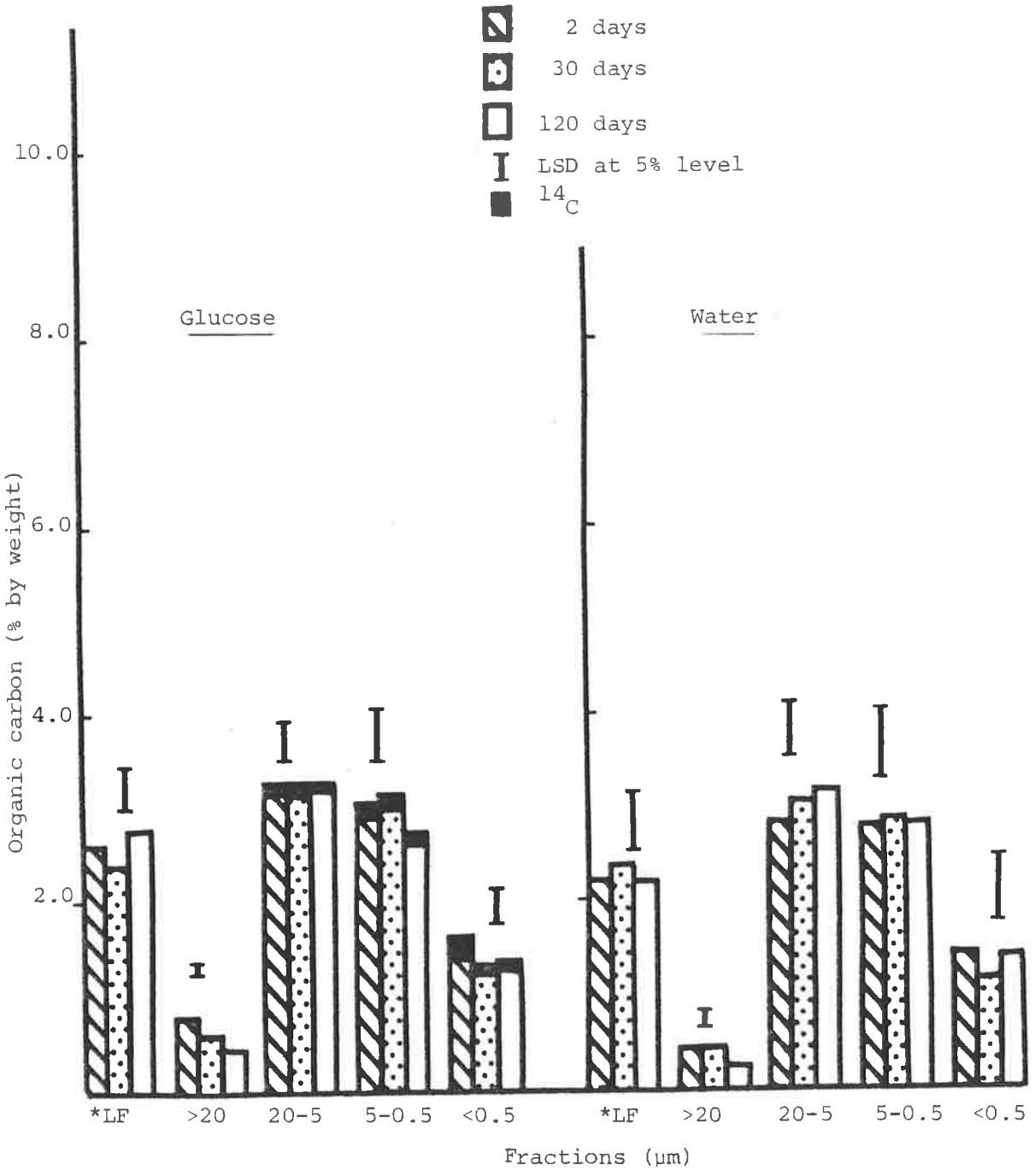

Fig. 24 Distribution of organic carbon in fractions of the Northfield cl after addition of ^{14}C-glucose and water

*The actual value was multiplied by 1/10

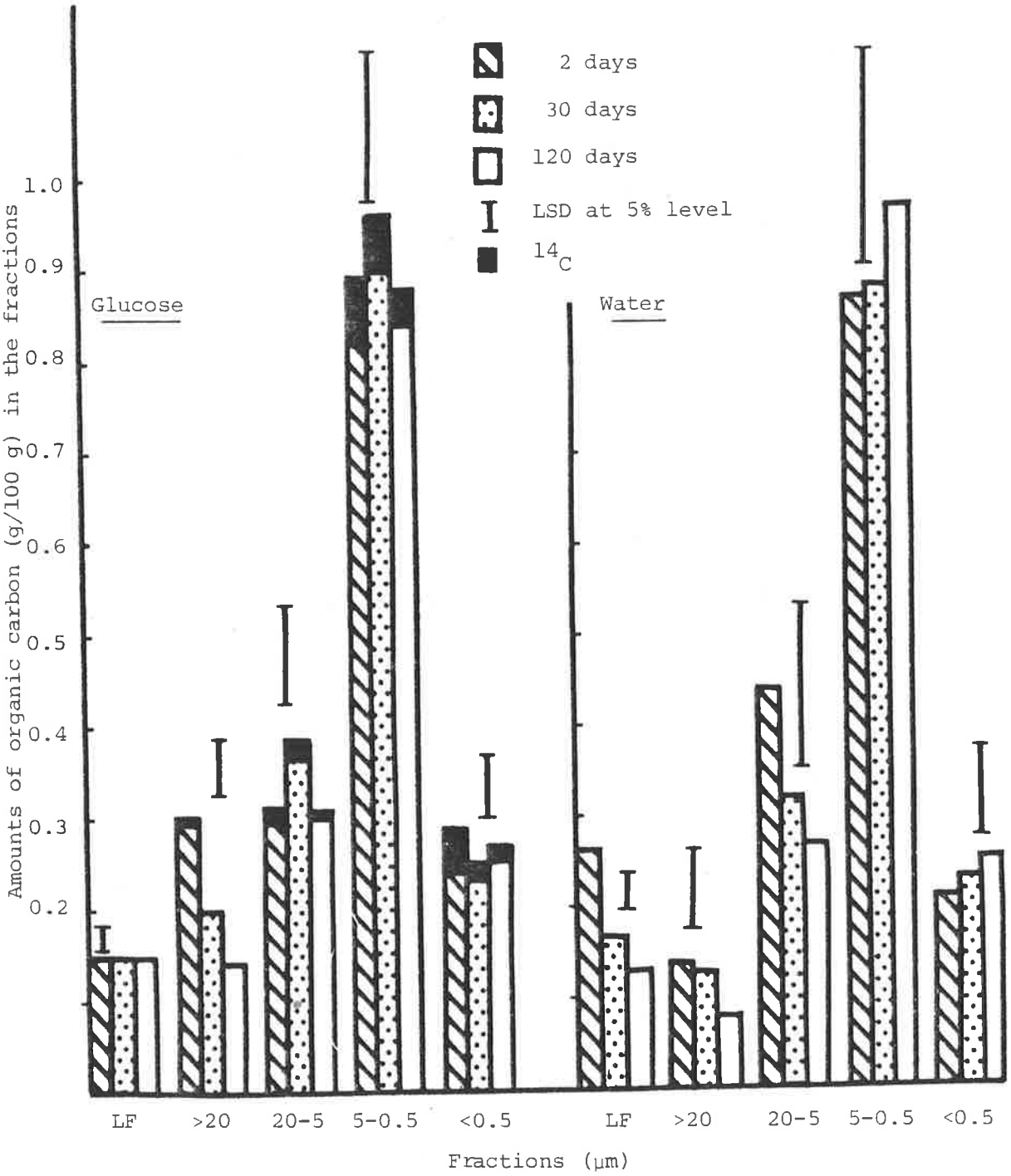

Fig. 25 Changes in the amounts of organic carbon in fractions of the Northfield cl after addition of ^{14}C-glucose and water

5.3.5.5 Reliability of ATP data in carbon metabolism studies

An appropriate estimate of the labelled biomass C in the fractions may be calculated from the net gain of ATP recovered in the fractions after glucose addition by assuming that the biomass C/ATP ratios of the labelled population in the unfractionated soil (Table 26) are applicable to the fractions for the same soil sampled at the same time. To compensate for the losses of ATP during fractionation (which usually took about a week) of the soils sampled at day 2, the average value of the labelled biomass C present in the unfractionated soil (Table 22) for a period of up to 8 days of incubation was considered to be a better control.

On this basis, after 2 days incubation 86% of the labelled biomass (as calculated from ATP data) of the unfractionated Urrbrae fsl was present in the <0.5 µm fraction and with continued incubation, there was a shift in the proportion of the labelled biomass to the 5.0-0.5 µm fraction (Table 27). At 120 days of incubation the labelled biomass in the 5.0-0.5 µm fraction was impossibly high. This was probably due to errors in the estimation of ATP at this time due to some unavoidable technical problems. It may be possible that the organisms developed after 2 days of incubation were separated into the <0.5 µm fraction based on the density and size. Later in the incubation the dominant microbial population changed considerably and was found in the 5.0-0.5µm fraction. After 2 days the proportion of the labelled C present as biomass in the fractions >20 µm were similar and the 5.0-0.5 µm fraction showed a consistent increase in the proportion of ^{14}C biomass it contained as the incubation proceeded. It was pointed out earlier that in the Urrbrae fsl the decline of ^{14}C in the biomass did not lead to more mineralization of ^{14}C from this soil, especially later in the incubation period. This was probably due to the greater cumulative effects of immobilization into larger organisms in the soil fractions.

Table 26 Biomass C/ATP ratios used in calculating the amounts of labelled-C in the fractions

Incubation period (days)	Urrbrae fsl	Northfield cl
2	29	44
30	99	133
120	193	394

Table 27 Flux of biomass in the Urrbrae fsl after addition of ^{14}C-glucose

Fractions	2 day			30 day			120 day		
	Proportion of labelledbiomass C*1 of the unfractionated soil present in the fractions (%)	Proportion of labelled C*2 of the unfractionated soil present in the fractions (%)	Proportion of labelled C present as biomass*3 in the fractions (%)	Proportion of labelled biomass C of the unfractionated soil present in the fractions (%)	Proportion of labelled C of the unfractionated soil present in the fractions (%)	Proportion of labelled C present as biomass in the fractions (%)	Proportion of labelled biomass C of the unfractionated soil present in the fractions (%)	Proportion of labellec C of the unfractionated soil present in the fractions (%)	Proportion of labelled C present as biomass in the fractions (%)
LF	0.28	3.16	1.88	-	1.58	-	0.59	2.16	6.92
>20 µm	2.80	11.21	5.45	0.44	1.89	5.94	7.67	4.03	46.61
20-5 µm	9.23	6.62	30.34	14.98	12.65	30.61	31.54	6.85	?
5-0.5 µm	19.15	16.35	25.47	84.51	55.39	39.47	142.98	66.65	54.45
<0.5 µm	85.94	62.59	29.85	-	13.03	-	-	19.82	-
Σ	117.40	99.93		99.93	84.54		182.80	99.51	

*1 $\frac{\Delta ATP \times \text{Biomass C/ATP ratio of the labelled population} \times \% \text{ Fraction}}{\text{Labelled biomass of the unfractionated soil } (^{14}C \text{ in the biomass})} \times 100$

*2 $\frac{\text{Labelled C in the fractions } (\mu g\ C\ g^{-1} \text{ fraction}) \times \% \text{ Fraction}}{\text{Labelled C in the unfractionated soil}} \times 100$

*3 $\frac{\text{Labelled C } (\mu g\ C\ g^{-1} \text{ fraction}) \text{ in the biomass}}{\text{Labelled C } (\mu g\ C\ g^{-1}) \text{ in the fraction}} \times 100$

- indicates net loss after glucose additions; ? doubtful

There is evidence in the literature on the change in the population dynamics after glucose addition to soil (Nannipieri *et al.*,1978; Shields *et al.*,1973). The consistent increase in the proportion of the labelled biomass C and the proportion of the labelled C present as biomass in the 5.0-0.5 μm fraction also suggests that the organisms might have moved to this fraction from some other fractions (Amato and Ladd, 1980). However it appears that the net decomposition of the labelled C in the Urrbrae fsl was mainly accounted by losses of labelled C present as non-biomass from the <0.5 μm and the 20-5 μm fractions.

The proportion of the labelled biomass of the unfractionated Northfield cl present in the <0.5 μm fraction increased while the proportions in 5.0-0.5 μm, 20-5 μm and >20 μm fractions decreased markedly (Table 28). The decrease in the amounts of labelled biomass of the unfractionated soil may be due to the large reductions in the proportions of labelled C present as biomass in the 20-5 μm and 5,0-0.5 μm fractions. Also the possibility of shift to some extent from the above two fractions to the <0.5 μm fraction could not be ignored.

However it is apparent from Table 27 that the recovery of labelled C in the biomass of the fractions (calculated from ATP data) were reasonably good, indicating that the biomass C/ATP ratios of the newly developed population were not in serious error. Application of such ratios made it possible to trace the dynamics of the biomass carbon during the metabolism of the added carbon in soil. It was also possible to recognize some labile pools of newly synthesized organic materials immobilized in the soil fractions.

Table 28 Flux of biomass in the Northfield cl after addition of ^{14}C glucose

Fractions	2 day			30 day			120 day		
	Proportion of labelled biomass C*1 of the unfractionated soil present in the fractions (%)	Proportion of labelled C*2 of the unfractionated soil present in the fractions (%)	Proportion of labelled C present as biomass*3 in the fractions (%)	Proportion of labelled biomass C of the unfractionated soil present in the fractions (%)	Proportion of labelled C of the unfractionated soil present in the fractions (%)	Proportion of labelled C present as biomass in the fractions (%)	Proportion of labelled biomass C of the unfractionated soil present in the fractions (%)	Proportion of labelled C of the unfractionated soil present in the fractions (%)	Proportion of labelled C present as biomass in the fractions (%)
LF	0.24	1.78	6.25	0.57	1.54	14.79	2.16	1.80	37.57
>20 µm	-	3.28	-	3.19	2.81	45.11	0.29	2.23	3.79
20-5 µm	2.46	7.40	15.44	37.21	15.26	96.72	2.27	12.69	5.62
5-0.5 µm	46.79	35.78	60.71	59.91	44.88	52.96	30.39	45.22	20.99
<0.5 µm	15.03	24.14	28.86	18.34	15.50	46.92	50.17	15.69	65.11
Σ	64.52	72.38		119.22	79.99		85.28	77.63	

*1 $\frac{\Delta ATP \times \text{Biomass C/ATP ratio of the labelled population} \times \% \text{ Fraction}}{\text{Labelled biomass of the unfractionated soil } (^{14}C \text{ in the biomass})} \times 100$

*2 $\frac{\text{Labelled C in the fractions } (\mu g\ C\ g^{-1} \text{ fraction}) \times \% \text{ Fraction}}{\text{Labelled C in the unfractionated soil}} \times 100$

*3 $\frac{\text{Labelled C } (\mu g\ C\ g^{-1} \text{ fraction}) \text{ in the biomass}}{\text{Labelled C } (\mu g\ C\ g^{-1}) \text{ in the fraction}} \times 100$

- indicates net loss after glucose addition

6. DISCUSSION AND CONCLUSIONS

Microbial biomass in soil is recognized as one of the important labile pools of soil organic matter (Persson, 1968; Jenkinson, 1971). Estimations of microbial biomass by indirect methods such as the determination of ATP or the fumigation technique were considered suitable for studying the metabolism of carbon in soils. However it was necessary to determine the effect of various pretreatments associated with sampling soils in the field and handling in the laboratory, before the ATP data could be interpreted with any confidence.

6.1 Determination of ATP

Soils used in this study were the same samples of from the same sites as those used by Jenkinson and Oades (1979). Soils collected from the field were moist and ATP and biomass C were determined immediately without any preincubation. The contents of ATP in the field moist soils were similar to the values reported by Jenkinson and Oades (1979). The same soils subjected to air-drying yielded less ATP and the effect was evident shortly after the commencement of air-drying. The effect of air-drying occurred within 6 hours and thereafter no appreciable change in ATP content took place irrespective of time. A significant decrease in the biomass carbon in the air-dried soils indicated that the large decline in the ATP content of the air-dried soils was partly due to the death of some cells resulting from air-drying.

Freeze-drying of the field moist soils resulted in a small drop in the ATP content but increased the ATP content of the air-dried soils almost to the ATP contents in the field moist soils. Freezing of the soils was found to be lethal to the living cells and therefore substantial losses of ATP occurred. The observed increase in the content of ATP in the air-dried soils subjected to freeze-drying could not be explained by any change in the dispersion of the soils due to freeze-drying. Freeze-drying of the soils included a short wetting phase and thus the effect of the length of the

wetting period on the content of ATP was investigated. The ATP content of the soil increased about two-fold within 10 minutes of wetting. During this short space of time little synthesis of ATP took place and the reactions associated with wetting were largely responsible for the improved extraction of ATP from the air-dried - freeze-dried soils. Synthesis of ATP in the air-dried soils did not start until one hour after wetting and thereafter the increases in the content of ATP in the air-dried soils were due to synthesis as well as other reactions caused by wetting.

Air-drying of soil results in an immediate decrease in the total number of organisms and an increase in the proportion of spore forming organisms (Stotzky *et al.*, 1962; Birch, 1960; McLaren and Skujins, 1967). The rapid decline of ATP in the air-dried soils and concomittant increase after wetting may indicate that the ATP extracted from the air-dried soils represented only the non-resting soil population. Freeze-drying of the air-dried soils increased the ATP content partly because of the reactions which render the cell material of the resting organisms susceptible to disintegration by the ultrasonic vibration. By contrast the ATP content of the newly developed population developed in a soil subjected to two cycles of fumigation, inoculation and incubation did not increase after freeze-drying but decreased instead. Such differences in the content of ATP after freeze-drying may be explained by the reactions caused by wetting of the air-dried soils. In other words the efficiency of extraction of ATP from the organisms depends on their physiological state.

It has been a common practice to freeze-dry vaccines and pure cultures to preserve longevity during transportation and storage (Majer *et al.*,1976). The ATP in the freeze-dried soils however was not stable irrespective of the temperature used for storage.

6.2 Determination of Biomass Carbon by the Fumigation Technique

The determination of biomass carbon by the fumigation technique involves the measurement of CO_2 released from the unfumigated and fumigated soils over an equal period of time. Soils collected fresh from the field or which had a preincubation treatment, gave reliable estimates of biomass carbon, by measurement of the CO_2-evolved from the unfumigated soil from 0 to 10 days of the incubation. However soils pretreated otherwise may show differences in the evolution of CO_2 (Singh and Gupta, 1977). The results presented in this thesis (section 3.3.6) showed that there may be vast differences in the estimates of biomass carbon, especially for the pretreatments such as freeze-drying if the determination of biomass C is based on the measurement of CO_2 from the unfumigated soils during either 0-10 or 10-20 day incubation periods. There may be death of a portion of the soil organisms resulting from freeze-drying. Jenkinson and Powlson (1976b) suggested that the measurement of the amount of CO_2 released from the unfumigated soils during 10-20 day incubation period may compensate such losses to some extent but was not entirely satisfactory. However the use of the 0-10 day incubation period for the measurement of the amount of CO_2 released from the unfumigated soils may underestimate the biomass carbon to some extent.

Biomass carbon of the soils decreased significantly after air-drying and freeze-drying. The decline in the biomass C after air-drying was probably due to the death of some cells (Jackson *et al.*,1967). Rapid formation of intracellular ice crystals in the cells probably were responsible for the death of a portion of the organisms. Powlson and Jenkinson (1976) suggested that difficulties may be encountered in the estimation of biomass C of the soils after air-drying because of the enhanced decomposition of the physically protected non-biomass C. Although the nature of the changes brought about by freeze-drying of the soil is not known, similar factors may also contribute to the errors in the estimation of biomass carbon in the freeze-dried soils.

6.3 Soil Pretreatments and the Concentration of ATP in the Biomass

The concentration of ATP in the biomass of the field-moist, air-dried - freeze-dried and the soils incubated for 4 days with water did not differ greatly from the value reported by Oades and Jenkinson (1979) and subsequently by Jenkinson *et al.*(1979). The concentration of ATP in the soils after air-drying resulted in an apparent decrease because of the incomplete extraction of ATP from the air-dried soils. However increases in the concentrations of ATP were observed after freeze-drying of the field moist soil and the soils incubated with water. Such increases in the concentration of ATP in the biomass of the soils after freeze-drying may either be due to the errors from the measurement of the amount of CO_2 released from the unfumigated soils or due to the death of a portion of biomass, but the ATP in the dead cells did not degrade due to the low temperature used in the freeze-drying procedure.

6.4 Chemical and Biological Properties of the Fractions

Detailed fractionations following dispersion with a Spex shaker or the ultrasonic probe yielded fractions with organic matter concentrated in finer fractions for the Urrbrae fsl. Particles with diameter 5.0-0.5 µm in the Urrbrae fsl were stable against the disruptive action of the ultrasonic vibration. These microaggregates contained significant proportions of fine clay rich in organic matter which probably contributed towards the greater structural stabilities in these microaggregates.

For the Northfield cl particles with diameter 15-5 µm had the highest concentration of organic materials. The greater structural stabilities of these particles with diameter 15-5 µm in the Northfield cl were probably influenced by the cementing action of the carbonates and the organic matter present in particles 10.0-0.5 µm diameter. These trends in the distribution of organic carbon and nitrogen are in good agreement with the report of Turchenek (1975).

Of the two methods of dispersion examined, dispersion was more complete

after shaking on the Spex mixer than after ultrasonic treatment but the poor recovery of soil ATP and similarity in the content of ATP amongst various soil fractions indicated that the amount of energy input from the Spex shaker was excessive. It was also observed that the abrasive action of the Spex shaker not only killed the cells but also separated the disrupted cells from some of the soil fractions and distributed them evenly amongst the soil fractions causing an undesirable smearing effect. On the other hand higher recovery of soil ATP and dissimilarity in the contents of ATP in the soil fractions obtained after ultrasonic dispersion made it more suitable and desirable for a fractionation scheme.

The observed similarity in the trends of C/N ratios, galactose + mannose/arabinose + xylose ratios and the content of ATP in the fractions with diameter <5 μm in the Urrbrae fsl and the fractions >1 μm in the Northfield cl may indicate the origin of the materials. However, living cells and metabolic products may be separated as shown by the inconsistencies between ATP and other criteria of microbial activity.

It was necessary to consider the amounts of ATP, organic carbon and nitrogen contained in various soil fractions in order to obtain fractions enriched with plant or microbial materials. Organic materials in the light fraction were dominated by plant-like materials as indicated by low ATP contents and wide C/N ratios. Fractions with diameter 5.0-0.5 μm were a major source of ATP and organic materials. Fractions with diameters 20-5 μm and <5 μm were characterized by smaller amounts of ATP and organic materials. It is suggested that the coarse clay and fine silt fractions contain living cells and microbial debris (Turchenek and Oades,1979;Cameron and Posner,1979).

6.5 Metabolism of ^{14}C-glucose in soil

Decomposition of ^{14}C-glucose in the two soils under consideration yielded results characteristic of the decomposition of simple substrates in soil (section 1.1.1). The efficiency of carbon utilization by the microbial population in the Urrbrae fsl was similar to that reported for pure cultures

Payne ,1970) but higher efficiencies may have occurred in the Northfield cl.

Although the proportion of ^{14}C retained by the two soils did not differ greatly, more biomass-^{14}C was synthesized in the clay soil than in the loam (Ladd et al.,1981). The relatively high rate of decay of the biomass-^{14}C compared with the non-biomass-^{14}C was largely responsible for the disappearance of ^{14}C from the Northfield cl On the contrary the non-biomass-^{14}C contributed almost entirely to the disappearance of ^{14}C from the Urrbrae fsl especially during the later phases of incubation.

The similarities in the trends of biomass C and the ATP of the two soils incubated with glucose, indicated that determination of ATP in the soil fractions may provide a reasonable estimate of the biomass. Also the biomass C/ATP ratios of the labelled population provided information about their physiological state and enabled calculations of the amount of biomass carbon in the soil fractions. The ATP data for the soils incubated with glucose should be interpreted with caution due to the succession of microbial populations (Nannipieri *et al.*,1978).

It has already been pointed out that the ATP contents of the soils were greatly influenced by pretreatment of the soil resulting from storage and handling in the laboratory. However, due to the volume of work involved in short time, the soil samples had to be stored until required. This along with other procedures inherent in the fractionation probably were responsible for the poor recoveries of ATP from the soil fractions. However the fractionations were carried out as carefully as possible to reduce the loss of ATP.

Fractionation of the soils into various particle size fractions indicated that the additional ATP synthesized in the soils after addition of glucose was rapidly distributed in all the fractions and was related to the ATP content before glucose addition. It may thus be assumed that the ready availability of the substrate led to multiplication of the existing organisms and an increase in the ATP content of the fractions. Thus at day 2 in the Urrbrae fsl incubated with ^{14}C-glucose, both ATP and ^{14}C were

concentrated in the <0.5 μm fraction. The contents of this fraction declined rapidly (Amato and Ladd, 1980) and a concomittant increase in ATP and ^{14}C was observed in the 5.0-0.5 μm fraction. The proportion of the ^{14}C in the biomass as calculated from the ATP data may also suggest a gradual shift of the living microbial bodies to the coarser soil fractions. This shift in the microbial population was probably due to the development of larger organisms later in the incubation or perhaps the development of colonies and associated mucilages. However, mineralization of the ^{14}C in the Urrbrae fsl was largely due to the loss of non-biomass-^{14}C from the <0.5 μm and 20-5 μm size fractions. In both the soils there was a significant reduction in the concentration of ^{14}C in the light fraction, but this pool was small compared to other fractions.

In the Northfield cl most of the ^{14}C at the beginning of the incubation period was immobilized in the soil fractions in the form of microbial biomass which then declined significantly during incubation. Calculation of the proportion of the ^{14}C in the biomass of the soil fractions (from ATP data) as for the labelled C in the biomass of the unfractionated soil, indicated that the reduction of the labelled biomass carbon in the unfractionated soil was attributable to the loss of the labelled biomass C from the 5.0-0.5 μm and the 20-5 μm size fractions.

Determination of the labelled biomass together with ATP made it possible to compute the concentration of ATP in the newly developed labelled segment of the microbial population. Subsequent application to the soil physical fractions enabled examination of the flux of the added ^{14}C and identification of some components of mineralizable organic carbon.

6.6 Suggestions for Further Study

It has been recognized that the estimation of biomass carbon of the soils after various pretreatments by the fumigation technique is not reliable because of the difficulties in the determination of CO_2 from the unfumigated soils. Therefore it would be desirable to compare the effects of such pretreatments on the biomass carbon content of the soils by some other method where such difficulties are not encountered, i.e. the physiological method of Anderson and Domsch (1978b). Such measurements may help to establish the biomass C/ATP ratios of the soils after pretreatments. The ratios derived from such studies may be used to estimate the biomass carbon content of soil fractions and could be used in studies of the dynamics of carbon in soil.

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