STUDIES ON THE DYNAMICS OF ORGANIC SULPHUR AND CARBON

IN

PASTORAL AND CROPPING SOILS

A thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Soil Science at Massey University

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2000

ABSTRACT

Soil organic matter (SOM) can be depleted or regenerated by altering land management practices. Soil tests capable of reporting the size of dynamic SOM fractions may be useful for indicating the environmental cost of landuse and management practices. Information on the effect of land management practices on soil organic S content and turnover is scarce. This study evaluated the ability of a sequential chemical fractionation procedure to characterise changes in soil S and C organic fractions on a range of pasture and cropping soils with different management histories. The fractionation involved an initial extraction with ion exchange resins followed by dilute (0.1 M NaOH) and concentrated (1 M NaOH) alkali. In addition, recently rhizodeposited [14]C (root+exudate derived) produced during a short–term (one week) [14]CO$_2$ pulse–labelling study of intact soil cores growing ryegrass/clover pastures, was used to trace the fate of root–derived C in both chemical and density fractionation procedures.

In pasture and cropped topsoils, the major amounts of soil S and C were either extracted in 0.1 M NaOH (49–69% S and 38–48% C) or remained in the alkali–insoluble residual fraction (17–38% S and 46–53% C). These two fractions were more sensitive to change caused by different landuse and management practices than the resin and 1 M NaOH fractions. With a large amount of dynamic soil C remaining in the residual fraction it was concluded that increasing strengths of alkali were not capable of sequentially fractionating S and C in SOM into decreasingly labile fractions.

The chemical fractionation allocated recent root and root–released ^{14}C amongst all the fractions. Again, most root ^{14}C appeared in the 0.1 M NaOH and residual fractions. Although small in amount, C of higher specific activity (more recently synthesised root C) was preferentially extracted by resin and 1 M NaOH extracts.

Density separation was not capable of recovering recent root and root–released ^{14}C in a single fraction. Root–derived ^{14}C was distributed between light (mostly fibrous root debris) (42%) and heavy (organics attached to clay and silt) (45%) fractions. The dispersing reagent soluble fraction recovered <13% of the [14]C. An anaerobic incubation and various acids and oxidising agents were tried, in order to recover a greater proportion of root and root–released ^{14}C as a single identity. These were not very successful in either

extracting or increasing the alkali solubility of the root C fraction. A 30% H_2O_2 pre-treatment of soil plus roots, or hot 1 M HNO_3 treatment of the residual fraction, were more efficient extractants of the root C fraction and should be investigated further to check their ability to better characterise soil organic S and C fractions with a change in management practices.

The $^{14}CO_2$ pulse labelling study of pasture swards showed a greater allocation of recently photo–assimilated ^{14}C to the topsoil layer with a greater proportion of ^{14}C recovered in roots than in the soil. An *in situ* soil solution sampling technique with mini Rhizon Soil Moisture Samplers^{TM} effectively monitored the rapid appearance of a $^{14}CO_2$ pulse in soil water at various depths. A comparison of the $^{14}CO_2$ pulse labelling study under light and dark conditions indicated that, in the light lysimeters, $^{14}CO_2$ photo–assimilation/translocation/rhizosphere respiration was the main pathway for CO_2 generation at various soil depths. In the dark lysimeters, $^{14}CO_2$ diffusion was the main mechanism and ^{14}C assimilation (either photo–assimilation or assimilation by chemolithotrophs in rhizosphere soil) was small.

The $^{14}CO_2$ activity in soil water from four soil depths of dark and light soil cores, and a CO_2 diffusion model, were used to identify the $^{14}CO_2$ contribution from rhizosphere respiration in the light lysimeters. A model was developed, but the unknown geometry of the air–filled pore space in the undisturbed soil cores made it impossible to precisely calculate the contribution made by root respiration to soil water $^{14}CO_2$ activity.

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CHAPTER 1   INTRODUCTION

Maintaining and improving soil quality is crucial if agricultural productivity and environmental quality are to be sustained for future generations. The soil organic matter (SOM) content has been widely used as a measure of soil quality because it serves numerous functions important to crop production, is responsive to change in management, and also affects ecosystem performance. Soil organic matter is important as a reservoir of essential macro–nutrients such as nitrogen (N), sulphur (S) and phosphorus (P) and, upon mineralisation, plant available forms are released. Soil organic matter also contributes significantly to the formation and stabilisation of soil structure.

Management and landuse practices can affect SOM by influencing the quantity and quality of crop residues (tops and roots), which are returned to the soil, and the rate of decomposition of added residues and native SOM (Gregorich *et al.*, 1994; Paustian *et al.*, 1997b, 2000; Haynes, 1999; Kumar and Goh, 2000). Changes in both quantity and quality of SOM are considered as major influences on the cycling and transformations of carbon (C), N, P and S in soils (Parton *et al.*, 1988).

Short– and medium–term changes in the quantity of SOM induced by management are sometimes difficult to detect because soil variability obscures small changes in relatively large amounts of SOM. Measurement of smaller, more labile fractions of SOM have, however, been used to identify early changes in SOM as influenced by soil management practices (Sparling, 1992; Haynes, 1999, 2000). Such labile organic matter fractions include microbial biomass C and N, light fraction organic matter, readily mineralisable C and N, easily extractable C and N, and carbohydrates (Gregorich *et al.*, 1994; Gregorich, 1996; Haynes, 1999, 2000). These labile fractions are important components of SOM quality, which influence crop productivity.

The labile component of SOM is a primary source of mineralisable N, P and S, and therefore plays a prominent role in nutrient dynamics (Parton *et al.*, 1988) and nutrient sustainability of agricultural systems (Stewart and Cole, 1989). Like C, changes in nutrient (N, P, and S) content associated with the labile portion of SOM can be considered as an important indicator of soil degradation or restoration. In contrast to N and P, information

on the effect of short– and medium–term changes in landuse practices and management on characterising the changes in labile SOM fractions that supply S to plants is scarce. Only sporadic attention has been given to S, a secondary plant nutrient, because few crops give positive yield responses to applied S and those in only a few geographical areas. Inputs of S either as by–products of chemicals and other fertilisers (e.g. superphosphates in New Zealand and Australia; Walker and Gregg, 1975), or as atmospheric deposition from natural and anthropogenic emissions (e.g. UK, Europe; Syers et al., 1987) have often met crop S requirements.

The provision of adequate S is increasingly important for plant production in Europe (Schung, 1991; Syers et al., 1987), South East Asia and the South Pacific (Blair and Till, 1983). The reasons for this are significant reduction in SO_2 emission in Europe and the use of low S–containing fertilisers in South East Asia and Australia. In Europe, the problem of inadequate S supply can become more widespread if the downward trend in SO_2 emission continues (Syers et al., 1987; Schung, 1991). In Australasian agricultural systems, S is an essential element for the development and maintenance of ryegrass (*Lolium perenne* L.)/white clover (*Trifolium repense* L.) based pastures (Lewis et al., 1987; Nguyen and Goh, 1992a). Sulphur is required for the synthesis of amino acids and proteins in plants (Duke and Reisenauer, 1986). Due to continuing losses of S through leaching (Smith et al., 1983; Heng et al., 1991; Sakadevan et al., 1993c, 1994), runoff (Fleming and Cox, 1998), and removal in animal products (Saggar et al., 1990b; Boswell, 1994), productivity of grazed pastures in New Zealand and Australia may not be sustainable (Saggar et al., 1998; Boswell and Gregg, 1998). Consequently, deficiencies of S may become increasingly prevalent. In agricultural systems, where the input of S from fertiliser and atmospheric deposition is low, the release of S from organic forms is important for the supply of S to plants. Therefore, it is desirable to identify and quantify biologically significant fractions of organic S that supply plant available S.

The classical chemical fractionation technique of separating soil S into HI–reducible S and C–bonded S has shown limitations in characterising soil organic S into fractions that have distinct mineralisation potentials. The conventional view is that HI–reducible S represents the labile organic S fraction and therefore accounts for much of the S mineralised. Some studies support this view (Beiderbeck, 1978; Saggar et al., 1984; McLaren et al., 1985) but others have demonstrated that HI–reducible S (ester sulphate)

may be no more important than C–bonded S as a source of mineralisable S (Freney *et al.*, 1975; McLaren and Swift, 1977; Ghani *et al.*, 1988, 1991; Castellano and Dick, 1991). Similarly, some workers have developed physical (Anderson *et al.*, 1981), and a mix of chemical and physical (Bettany *et al.*, 1979, 1980; Keer *et al.*, 1990; Eriksen *et al.*, 1995a, 1995b; Eriksen, 1997) separation techniques to characterise the complex nature of soil S irrespective of its bonding relationships to carbon. Again, the fractionations conducted were not entirely satisfactory, perhaps because of the heterogeneity of S substrates in SOM. Therefore, there is a need to attempt alternative techniques that divide soil organic S into a few biologically meaningful labile fractions important for short– and long–term supply of S to plants.

The sequential extraction procedure for soil P, introduced by Hedley *et al.* (1982), is frequently being used to characterise changes in labile P fractions with short– and long–term changes in management practices (Tiessen *et al.*, 1982, 1983; Perrott *et al.*, 1989; Huffman *et al.*, 1996; Schmidt *et al.*, 1996; Trolove *et al.*, 1996). Therefore, one of the aims of this present study is to test whether Hedley et al's soil P fractionation technique can identify labile soil organic S and C fractions in soils where change has been induced by: differences in fertiliser application to the pasture soils, by short– and long–term cultivation, or by short–term pasture cropping (see Chapter 4). The results of this fractionation were that labile soil organic S and C were extracted by dilute alkali (0.1 M NaOH) but also a large component remained unextracted in a residual fraction. The role of recent root–derived C in creating the residual fraction is investigated using ^{14}C pulse-labelled root material. The ^{14}C pulse-labelled root material is generated by conducting a $^{14}CO_2$ pulse labelling experiment on undisturbed pasture soils. Alternative fractionation procedures are trialed and modified based on their ability to extract ^{14}C labelled root material. As a consequence of the $^{14}CO_2$ pulse labelling experiment, methods are developed to measure the allocation of recently fixed ^{14}C to shoot, root and soil at different depths. The use of mini Rhizon Soil Moisture Samplers^{TM} (RSMS) are evaluated as a non–destructive technique for monitoring the turnover of root and root–released ^{14}C at the plant/soil–water interface in intact soil cores growing pasture. Finally, an experiment is conducted in an attempt to model the input of root–released C to soil at different depths under pasture.

CHAPTER 2   LITERATURE REVIEW

Manipulating the factors that regulate SOM inputs and decomposition can have important influence on the sustainability of agricultural systems (Syers and Craswell, 1995; Paustian *et al.*, 1997b). In investigating the effect of landuse and management practices on SOM quality and quantity, it is important that appropriate measurements of SOM are made which indicate the size of labile and stable pools of SOM. Although organic matter content of the soil changes with the change in management practices, of particular concern is the change in the more labile C fractions, which are associated with soil nutrient dynamics (Parton *et al.*, 1988; Janzen *et al.*, 1992) and stabilisation of soil structure. In the last 20 years, concern with global warming caused by increased atmospheric CO_2 levels has raised the interest in developing a further understanding of the role of SOM as a sink or source of atmospheric CO_2. This area of study also requires dynamic SOM fractions to be identified.

A major goal in the study of SOM is to relate information regarding the size and composition of the labile and stable pools of SOM to soil quality and productivity. For the modellers of global CO_2 change, the rate of turnover of the more labile SOM fractions must be known. In the following sections, various chemical and physical approaches or combination of these are discussed in the context of understanding turnover rates and nutrient availability of SOM pools. The focus of this thesis is to assess changes in soil organic S and C fractions with the change in landuse practices (see Chapter 4); therefore, the nature and forms of S present in the soil are specifically considered.

2.1   CHARACTERISATION OF SOIL ORGANIC MATTER RELATIVE TO ITS TURNOVER AND NUTRIENT AVAILABILITY

2.1.1   *Chemical characterisation of SOM*

Classical chemical fractionation of SOM yields three major fractions: humic acids, fulvic acids, and humin (Kononova, 1966). This technique is based upon differences in solubilities of humic substances in alkaline and acid solutions. Fulvic acids are soluble in both alkali and acids, humic acids are soluble in alkali but precipitated by acid, and humins are insoluble in both alkali and acid. Humic substances differ in molecular weight,

elemental composition, acidity, and cation exchange capacity. In contrast to humic acids, the low molecular weight fulvic acids have higher oxygen but lower C contents, and they contain considerably more acidic functional groups, particularly –COOH (Stevenson, 1994). Polysaccharide content is high and allows some separation by adsorption on charcoal or gel and resin chromatography. However, other than these, Fulvic acids and humic acids have similar chemical structures i.e. they composed of a variety of phenolic and benzene carboxylic acids that are held together by hydrogen bonds to form stable polymeric structures (Schulten and Schnitzer, 1997). The humic acids also tend to contain more hydroxyphenols, hydroxybenzoic acids, and other aromatic structures with linked peptides, amino compounds, and fatty acids (Schnitzer, 1978). Soil humins are considered to be non–extracted humic type polymers that form strong association with mineral fractions and are not as easily separated by alkaline reagents (Schnitzer, 1978). The humins in soils contain polysaccharides, phenolic or methoxyl–substituted aromatic structures (most likely derived from the lignin of vascular plants) and paraffinic structures (derived from algal or microbial sources) (Hatcher *et al.*, 1985). Almendros and Gonzalez–villa (1987) found an unexpectedly high proportion of polymethylene compounds (mainly fatty acids) in the humins, in both loosely and strongly associated forms. They consider that these aliphatic chains are stable humin constituents, physically or chemically associated with a highly oxidised, demethoxylated and disordered matrix entrapping a significant proportion of non–polar compounds. Almendros and Gonzalez–villa (1987) also believe that some of the aliphatic components seem to be inherited from cuticular waxes of higher plants and further suggest that during the biodegradation of lignins the lipid polymers are altered, then contributing to the formation of humin. The use of ^{14}C dating technique has shown that fulvic acids, on average, are much younger (usually have turnover times of hundreds of years or less), whereas humic acids, on average, approach several thousand years, especially when humics associated with the metal ions in soil are isolated. Humins, on average, are intermediate in age (Campbell *et al.*, 1967; Paul and van Veen, 1978).

However, the separation of SOM into fulvic acids, humic acids and humin, is far from precise (Allison, 1973). As mentioned above, the acid–soluble materials classified as fulvic acid invariably contains organic substances classified as non–humic, such as proteins and carbohydrates (Stevenson and Elliott, 1989), which can be removed using 6 M HCl (Schnitzer and Preston, 1983). Therefore, recent C can occur in all fractions, e.g. Stott

et al. (1983) found that the majority of polysaccharides and protein C in wheat (*Triticum aestivum*) residues became associated with the fulvic acid fraction of new organic matter, but 36–54% of the C derived from wheat straw lignin was found in the humic acid fraction. The humin fraction has been reported to contain both old (i.e. stable) and young (i.e. labile) forms of SOM as shown by using "bomb" ^{14}C enrichment (Goh *et al.*, 1976).

The complexity of fulvic acids, humic acids, and humin fractions means that they each contain a wide range of chemical forms with very different turnover rates. These fractions are based largely on solubility and molecular weight differences, and thus may have limited value in studies of SOM dynamics (Stevenson and Elliott, 1989).

Stevenson and Elliott (1989) stressed that a sequence of extractions using reagents specific for defined biologically active component(s) may be preferred over more complete extraction with a single reagent. A wide range of fractionation procedures has been used, but the meaningfulness of the fractionation is often uncertain, and research in this area is required to improve the relevance of fractionation techniques.

Various attempts to develop more relevant fractionation of SOM into classes that vary in their dynamics, and are important in explaining the cycling of N, P and S, are around in the literature (see Schnitzer and Khan, 1978; Stevenson and Elliott, 1989; Stevenson and Cole, 1999). This review will concentrate on recent work involved in organic S and C.

2.1.1.1   Identification of a labile fraction(s)

In recent times, to characterise dynamic SOM, Murata *et al.* (1995) and Murata and Goh (1997) have fractionated soils using various pre–extractions such as cold water shaking, and hot water shaking followed by seven shakings with a mixture of acids (0.1 M HCl/0.3 M HF). Hot water extractable C was considered an indicator of labile SOM, whereas HCl/HF extractable C represented an indicator of change in more stable SOM. Unextracted soil C was subsequently separated into fulvic acids, humic acids and humin using a series of five 0.1 M $Na_4P_2O_7$ and three 0.5 M NaOH extractions. The final residual C fraction (humin) was considered to include a mixture of stable organic forms such as humic acid materials (see earlier discussion), and also the remains of crop residues and dead microorganisms (Murata *et al.*, 1995; Murata and Goh, 1997). The use of acid pre-

treatments (e.g. HCl/HF) and neutral salts (e.g. $Na_4P_2O_7$) enhance SOM extraction and affect its release from soil minerals (Bremner and Lees, 1949; Choudhri and Stevenson, 1957; Goh and Reid, 1975). By using this fractionation, the scientists hoped to produce SOM quality indicators to compare the effects of: a) long–term superphosphate application to irrigated pasture (Murata *et al.*, 1995) and b) cropping systems (Murata and Goh, 1997) on SOM quantity and quality. Long–term superphosphate application to irrigated pasture increased hot water soluble C but did not have significant effects on the distribution of fulvic and humic acid C ($Na_4P_2O_7$/NaOH) fractions. In the sequence of cropping and pasture rotations studied by Murata and Goh (1997), the hot water soluble C decreased as a percent of total C after 6 years of continuous cultivation. In the pasture phase however, over the same period, all C fractions [hot water soluble, HCl/HF, fulvic and humic acids ($Na_4P_2O_7$/NaOH) and humin] increased in some soils. So despite using these pre-treatments in a SOM extraction scheme, Murata *et al.* (1995) and Murata and Goh (1997) found that labile SOM still appeared in both the extracted and unextracted fractions.

The goal in the present study is not to extract all the SOM but is try to recover labile SOM in a fewer distinct fractions that change with changes in landuse practices. Also, there is a need to look at some alternative pre– and post–alkali extraction treatments that tend to reduce the size of dynamic SOM in the unextracted fraction.

2.1.1.2   *Acid hydrolysis*

An alternative to classical chemical fractionation is acid hydrolysis. The most common methods either successively extract soils with acids e.g. hot 0.5 M HCl followed by 6 M HCl hydrolysis (Martel and Paul 1974a) or use single treatment such as 1 M HCl (Xu *et al.*, 1997) or 6 M HCl (Goh *et al.*, 1984; Xu *et al.*, 1997; Paul *et al.*, 1997; Collins *et al.*, 2000; Rovira and Vallejo, 2000). Acid hydrolysis of soil readily provides acid–soluble fractions ranging from 25–60% of the soil C and a nonhydrolysable fraction constituting 40–75% of the C (Martel and Paul, 1974a; Martel and Lasalle, 1977; Jawson and Elliott, 1986; Xu *et al.*, 1997; Collins *et al.*, 2000). Pre-treatment with 0.5 M HCl is recommended because it removes sugars and organic substances of relatively low molecular weight, which would otherwise form black precipitates due to their condensation reaction with nitrogenous compounds during direct boiling with 6 M HCl to form insoluble complexes (Onger, 1969 – as cited by Martel and Paul, 1974a).

An important methodological detail is that if hot 3 M H_2SO_4 is used (e.g. Jawson and Elliott, 1986), Cl^- interferences in the C determination using a dichromate digestion can be avoided (Quinn and Saloman, 1964). Acid hydrolysate usually consists of amino acids, amino sugars, carbohydrates, and other biomass components (McGill and Cole, 1976; Stevenson and Cole, 1999). However, acid hydrolysis does not completely solubilise the lignin, phenols, and cellulose of plant residues (Martin *et al.*, 1980; Paul *et al.*, 1997). Acid hydrolysis left 42% of the C of wheat (*Triticum aestivum* L.) straw and 34% of the C of maize (*Zea mays* L.) residues undissolved (Follett *et al.*, 1997). It also has differential effects on soil fulvic acids and humic acids (Schnitzer and Khan, 1972). Posner and Creeth (1972) demonstrated that boiling humic acids in 6 M HCl induced the formation of higher molecular weight humic substances. Stevenson and Goh (1971) reported that treatment of soil fulvic acids with hot 6 M HCl produced changes in their acid solubility and infrared spectral characteristics.

Although acid hydrolysis of soils seems to hold some hope for separation of active from the resistant fraction of soils (Campbell *et al.*, 1967; Martel and Lasalle, 1977; Goh *et al.*, 1984; Xu *et al.*, 1997; Rovira and Vallejo, 2000), it can also hydrolyse old organic materials present in soil (Martel and Paul, 1974b). Martel and Paul (1974a) found that although the ^{14}C age of soil increased considerably [i.e. from 250 years BP to 710 years BP (BP = before present i.e. before 1950)] after 60 years of cultivation of a northern grassland soil (Oxbow Association, Udic Boroll), there was no measurable change in the age distribution of the acid hydrolysable and nonhydrolysable materials, suggesting that all fractions decomposed at a similar rate after cultivation independently of their ease of acid hydrolysis. Collins *et al.* (1997) also indicated that there are some artefacts of acid hydrolysis e.g., modern lignin ends up in the non–hydrolysable plant fraction, as reported in some experiments with ^{14}C involving both C dating and enriched samples. Collins *et al.* (2000) have shown an increase in size of acid nonhydrolysable resistant fraction by 20% where no–till was conducted compared to conventional–till (inversion) treatment in the past 30 years on corn cultivated forest derived soils. They attribute the increase to some of the lignin from modern plant residues that is recovered in the non–hydrolysable fraction. However, there still is usually a difference of 1000 years or more in the average C age of the soluble and acid–insoluble fractions giving an estimate of the size and tracer age of the resistant old fractions (Campbell *et al.*, 1967; Martel and Lasalle, 1977; Paul *et al.*, 1997).

Various forms of acid hydrolysis may be worth further investigation because of their ability to separate polysaccharides and amino acids from more lignified material.

2.1.1.3   Oxidation techniques

Rather than assessing the chemical forms of SOM by extraction techniques, measurements of the rate of breakdown have also been used to assess the quality of SOM. The decomposition of SOM normally involves uptake of oxygen and liberation of carbon dioxide. Solutions of potassium permanganate ($KMnO_4$) have been recently used for the oxidation of organic compounds and measurement of the more easily oxidised C fractions (Blair *et al.*, 1995; Lefroy *et al.*, 1995; Bell *et al.*, 1998; Conteh *et al.*, 1998; Whitbread *et al.*, 1998; Blair and Crocker, 2000). The method assumes that the oxidative action of $KMnO_4$ on soil organic C is comparable to that of the enzymes of soil microorgamisms and other enzymes present in the soil. The rates and extent of oxidation of different substrates is governed by their chemical composition (Hayes and Swift, 1978) and the concentration of $KMnO_4$. The hypothesis is often that the lower the concentration of $KMnO_4$ required for oxidation, the more labile the organic component (Loginow *et al.*, 1987). Blair *et al.* (1995) standardised the original $KMnO_4$ oxidation procedure of Loginow *et al.* (1987) and simplified the technique to use only one concentration of $KMnO_4$ (0.333 M), thereby dividing soil C into labile ($KMnO_4$ oxidisable) and non–labile carbon (i.e. obtained by difference of total and labile C). The method was used by Blair *et al.* (1995) to monitor changes that occur in the total and labile C as a result of the agricultural practices (wheat/lucerne pasture rotations or sugarcane cropping), with increased importance attached to changes in the labile, as opposed to non–labile, component of the SOM. The reduction in the labile C due to cropping was proportionally greater than the decline in total or non–labile C. The labile C ($KMnO_4$ oxidisable) recovered between 14–25% of the total C, which can only monitor small, relatively short–term changes in the amount and quality of SOM in different soils under different management condition (Blair *et al.*, 1995). However, for monitoring both short– and long–term changes in SOM, a more detailed fractionation is required which can provide SOM pools of distinct lability.

Whilst the use of chemical fractionation technique has greatly enhanced our knowledge and understanding of soil organic matter substances, chemical fractionation of

SOM may suffer several limitations. For example, chemicals used in fractionating SOM may modify SOM structure and hence influence the magnitude of different SOM fractions (Stevenson, 1982; Stevenson and Elliott, 1989). Also, chemical extractants isolate SOM that may be physically protected from microorganisms and not readily available for decomposition (Duxbury *et al.*, 1989). Nevertheless, the application of chemical fractionation procedures as a characterisation technique to monitor the effect of treatments on both quantity and quality of SOM is quite useful. These procedures offer a useful approach in assessing the relative distribution of labile and stable SOM components among fractions. However, the success of chemical fractionation and characterisation methods is always limited by the fact that SOM is of very complex nature. An enormous array of compounds exists in soil, ranging from recent plant materials through a continuum of metabolic products of microorganisms to compounds of stable humus (Stevenson and Elliott, 1989). Currently, no single chemical technique has been developed to classify labile SOM into a few distinct fractions.

2.1.2 *Physical characterisation of SOM*

Although chemical extractions have been used to characterise solubility and some functional groups of SOM, they have not been very useful in identifying specific SOM pools that diminish upon intensive management. This is because the turnover rates of different pools of SOM, controlled partly by microbial decomposition, are affected by both the *chemical* and *physical* nature of SOM. The stability of humic substances has been attributed to their chemical structure, interactions with metal cations and clay minerals and inclusion within soil aggregates (Stout *et al.*, 1981). It is often suggested that this stability is due more to the physical or physicochemical characteristics of interactions with metal cations and clay minerals, and inclusion in soil aggregates than their chemical structure (Anderson, 1979).

Physical fractionation procedures have been usually used either a) to determine the type of organic matter involved in the formation of water–stable aggregates (Turchenek and Oades, 1979; Tisdall and Oades, 1982; Oades, 1984), b) to examine the more detailed nature, distribution and biological significance of organic matter in the various size fractions (sand, silt and clay size separates) of the soil (Anderson *et al.*, 1981; Oades *et al.*, 1987; Christensen, 1987), or c) to recover a "light fraction" consisting largely of

undecomposed plant residues and their partial decomposition products (Skjemstad *et al.*, 1986; Boone, 1994; Golchin *et al.*, 1994; Magid *et al.*, 1996).

A review of mechanisms involved in the formation of stable soil aggregates has been given by Oades (1984). Tisdall and Oades (1982) and Oades (1984) proposed three types of physical fractions in mineral soil based on the association of different types of organic matter and soil i.e. a) free primary particles comprising mainly microbial and plant derived polysaccharides, b) macroaggregates including roots and fungal hyphae, and c) microaggregates including aromatic humic material associated with Fe and Al, and other polyvalent metal cations. The metals act as clay–organic matter and organic matter–organic matter bridges. Tisdall and Oades (1982) further suggested that microaggregates are bound together into macroaggregates by roots, fungal hyphae and polysaccharides of microbial and plant origins.

The distribution of organic matter within physical fractions of soil must involve disrupting soil structure followed by the separation of fractions by either particle size or density gradients (Christensen, 1992). Physical fractionation procedures based on size and density separation have already been reviewed elsewhere (Stevenson and Elliott, 1989; Elliott and Cambardella, 1991; Christensen, 1992; Collins *et al.*, 1997). A typical fractionation procedure based on size–density separation is shown in Figure 2.1. The light fraction is usually separated using a liquid of high density such as sodium polytungstate (ρ = 1.4–1.85 Mg m^{-3}) (Cambardella and Elliott, 1992, 1993; Magid *et al.*, 1996, 1997), sodium iodide (ρ = 1.7 Mg m^{-3}) (Strickland and Sollins, 1987; Barrios *et al.*, 1996), and Ludox, a colloidal silica solution (ρ = 1.13–1.37 Mg m^{-3}) (Hassink, 1995; Meijboom *et al.*, 1995). Following light fraction removal, further separations are made by sieving and sedimentation. Common fractions are sand, silt, coarse silt, fine silt, coarse clay, and fine clay. Besides the densitometric separation (Barrios *et al.*, 1996), various physical fractions have been subjected to chemical (Anderson *et al.*, 1981; Oades *et al.*, 1987; Shang and Tiessen, 1997), microbiological (Ahmed and Oades, 1984), and microscopic examination (Tiessen and Stewart, 1988).

Soil

┌── **Dispersion, density separation** ──────────→ **Light fraction**

├── **Wet sieve, 300 mesh** ────────────────────→ **>53 μm**   (Coarse sand
                                                                                                and aggregates)

├── **Gravity separation** ──────────────┬──→ **20-53 μm**   (Fine sand)
│   ├──→ **5-20 μm**   (Coarse silt)
│   └──→ **2-5 μm**   (Fine silt)

└── **Centrifugation** ──────────────────┬──→ **0.4-2 μm**   (Coarse clay)
                                                                  ├──→ **0.1-0.4 μm** (Medium clay)
                                                                  └──→ **<0.1 μm**   (Fine clay)
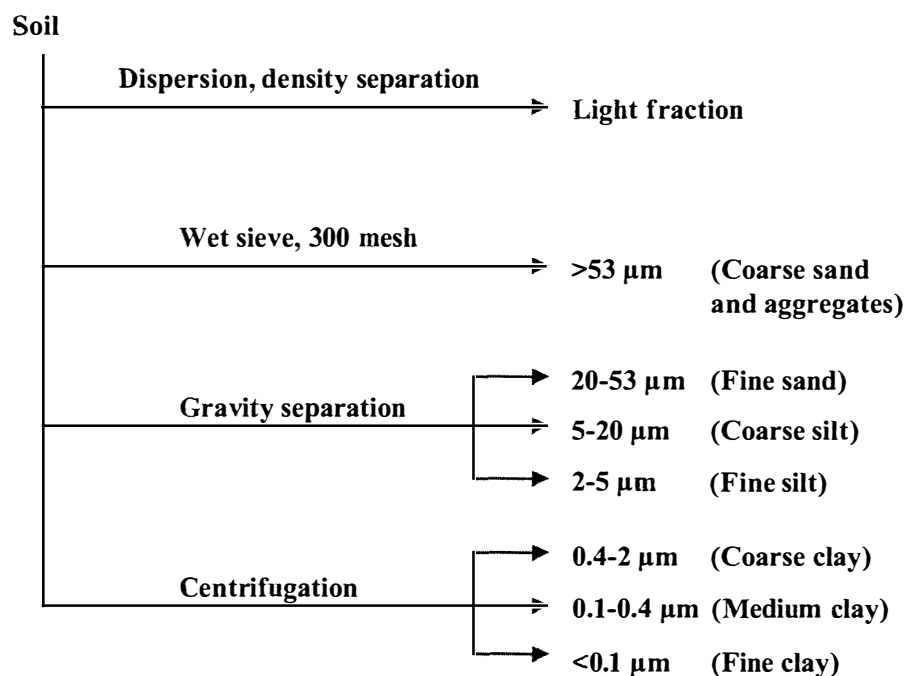
Figure 2.1: A scheme for the physical fractionation of soil organic matter (after Stevenson and Elliott, 1989).

More recently, Magid *et al.* (1997) used the size–density fractionation procedure where materials <100 μm are discarded after chemical dispersion because a study using young ^{14}C labelled *Lolium perenne* shoot material indicated that such material did not contribute considerably to the "active" organic matter (Magid *et al.*, 1996). The lighter particulate organic matter of >100μm is separated by several vigorous rotations followed by decantation, which is then separated by density into light ($\rho < 1.4$) and heavy ($\rho > 1.4$) particulate organic matter fractions. After chemical dispersion of soil (amended with ^{14}C labelled *Lolium perenne* shoot material), Magid *et al.* (1996) used sodium polytungstate in combination with centrifugation (at 10 000 ×g for 1 h) to separate fractions of different density of whole soil without size separation. They recovered the added C in both the light ($\rho < 1.4$) and heavy ($\rho > 1.4$) fractions. They attributed lower than expected recovery in the light fraction to the artefact of centrifugation at high–density field for long duration (1 h), which probably caused liberation of entrapped air in plant material, and therefore, increased the density of young, light organic matter (Magid *et al.*, 1996), or alternatively caused aggregation and precipitation of soil and root together.

Various steps to obtain different size/density fractions or components are discussed in the following subheadings.

2.1.2.1   Mechanical disruption

Mechanical disruption of soil particles is commonly achieved using sonication or shaking (Elliott and Cambardella, 1991; Christensen, 1992). Sonication produces vibration energy to the soil suspension causing cavitation. Cavitation involves three steps: nucleation (bubble formation), bubble growth, and implosion (bubble collapse). The collapse of these bubbles produces shock waves, which disrupt bonding agents (Gee and Bauder, 1986; Gregorich *et al.*, 1988, 1989). Currently, there are no standard protocols for sonic dispersion. A review of various experimental methods showed that sample weights ranging from 10–100g, vibration periods from 10–30 minutes and energy disruption per ml of solution ranging from 90–5350 J ml^{-1} (60–600 W) were mainly used (Christensen 1992). The disadvantage of the disruption technique is that the degree of dispersion and therefore the type of C recovered in each separate varies enormously between experiments making comparisons difficult. For example, Christensen (1985), working on Danish soils, showed that clay yield increased and silt decreased with increased sonication time, indicating the progressive disruption of silt–size particles. The duration of ultrasonic treatment may have to vary between soil types to achieve complete dispersion. Hinds and Lowe (1980a) concluded that ultrasonic dispersion [for 20 minutes at a rated maximum power output of 300 W (2140 J ml^{-1})] of Gleysolic soils in electrolytes (NaCl, CaCl$_2$ or BaCl$_2$) would produce higher yields of less altered organo–clay complexes by considerably reducing dissolution of Fe, Al, Si and organic C.

One of the greatest problems with the use of sonication in SOM studies is the potential for redistribution of organic matter among size/density fractions (Christensen, 1992; Whitbread 1995). Increasing the energy levels (Gregorich *et al.*, 1988) and duration (Christensen, 1992) of ultrasonication results in the recovery of increasing amounts of organic matter in the fine silt and clay fractions. However, limited ultrasonic treatments provide incomplete dispersion of soils, causing microaggregates of smaller size particles to be included in silt and sand size separates (Christensen, 1992).

Shaking is a more gentle alternative dispersion method to sonication (Christensen, 1992). Simple shaking in water, however, is unlikely to provide complete dispersion even after prolonged treatment periods (Genrich and Bremner, 1974; Young and Spycher, 1979). The duration of shaking and addition of chemical dispersants can contribute to

improved uniformity with which soil is disrupted. Various chemical pre-treatments have been used to aid dispersion or to remove a specific component responsible for the stabilisation of aggregates. Soil samples are often saturated with Na^+ by treatment with sodium hexametaphosphate (Magid *et al.*, 1996) or sodium chloride (Magid *et al.*, 1997) to facilitate dispersion as well as remove polyvalent cations such as calcium, which would otherwise form insoluble calcium polytungstate salt (density reagent) during size/density separation (Magid *et al.*, 1996). However, whether used in sonication or shaking systems, some researchers suggest the use of chemical dispersants during physical disruption may create changes in some SOM components through chemical transformations, solubilisation or oxidation, and should be avoided unless the action of the chemical is specific and well documented (Christensen, 1992).

2.1.2.2   *Sieving*

Soil fractionation by sieving is based on the separation of organic matter components on the basis of aggregates and/or particle size. The techniques range from dry sieving to wet sieving after complete dispersion of aggregates by physical and chemical methods (Christensen, 1986, 1992). Sieving is usually used to separate out fractions >50 μm to 60 μm in diameter before or after isolation of clay and silt. While sieving may be very useful for a short–term study of decomposition of litter layers or crop residues; over longer periods, where SOM has few 'macro' characteristics, it may be of little value.

2.1.2.3   *Sedimentation*

Fractionation using sedimentation separates particles that can vary in size, shape or density based upon their equivalent spherical diameter. Sedimentation dynamics are described by Stokes Law. Many organo-mineral particles are porous and when these pores are filled with water or air, the density of the sedimenting particle is then the density of the solid plus water or air. Thus, the fractions obtained by sedimentation are not necessarily homogeneous with respect to density and the type of organic matter they contain (Tiessen and Stewart, 1983; Balesdent *et al.*, 1988).

Size separates between 60 and 2 μm are isolated by gravity sedimentation. Various centrifugation techniques are used for subdividing separates <2 μm to speed up

sedimentation time of small clay particles compared with gravity sedimentation. This may be advantageous in order to reduce the possible microbial activities in soil suspensions (Christensen, 1992).

Organic matter associated with different primary particles has been shown to have varying rates of turnover (Tiessen and Stewart, 1983). Sand size separates have been reported to contain more labile particulate organic matter (i.e. relatively less decomposed vegetative fragments – Tiessen and Stewart, 1983; Cambardella and Elliott, 1992; Christensen, 1992; Buyanovsky *et al.*, 1994). Results are inconsistent, however, as Christensen (1987) has shown that C and N of the silt fraction were more recalcitrant than that of the clay fractions. There is a large body of information supporting both the quantitative importance and the resistant nature of the SOM associated with the silt/coarse clay fraction and sometimes faster turnover rate of the fine clay separates obtained from sonication and sedimentation (Anderson *et al.*, 1981; Tiessen and Stewart, 1983; Balesdent *et al.*, 1988; Shang and Tiessen, 1997). This, however, may be somewhat an artefact of the fractionation technique in which labile microbial constituents and other compounds become adsorbed upon the fine clays during fractionation (Shang and Tiessen, 1997).

2.1.2.4   *Density separation*

Density separation, which is independent of size or shape, can be applied to whole soil, to particles–size fractions with density gradients, or to solutions with a specific density, usually from 1.4 to 2.2 Mg m^{-3}. Greatest concentration of young organic materials is usually obtained in the lightest fractions (see discussion below). Meijboom *et al.*, (1995) stated that fresh plant material has a density of around 1 Mg m^{-3} so accordingly they used a density of heavy liquid (silica suspension) close to 1 (1.13 and 1.37).

A light fraction consists of relatively mineral free, incompletely decomposed plant and animal debris, and associated microorganisms (Janzen *et al.*, 1992; Golchin *et al.*, 1994). The light fraction of soil is usually defined as having a density ranging from <1.4–2.0 Mg m^{-3} (i.e. lower than that of soil minerals), a relatively wide C:N ratio and is a biologically active fraction with a relatively rapid turnover rate (Janzen, 1987; Christensen, 1992; Barrios *et al.*, 1996; Magid *et al.*, 1997). The rapid turnover rate of the light fraction is due to the labile nature of its constituents, such as easily decomposable carbohydrates

(Skjemstad *et al.*, 1986; Golchin *et al.*, 1994), and the lack of protection by soil colloids (Meijboom *et al.*, 1995).

A heavy fraction consists of organic matter adsorbed to mineral surfaces or contained within organo-mineral microaggregates (Sollins *et al.*, 1984; Strickland and Sollins, 1987). The heavy fraction has a relatively narrow C:N ratio, representing already partially decomposed material with a slower turnover rate and a higher specific density than the light fraction due to its intimate association with soil minerals (Sollins *et al.*, 1984; Meijboom *et al.*, 1995).

The degree of sample dispersion is critical to the outcome of density fractionation. Compared to size fractionation, density fractionation methods appear to rely on less vigorous dispersions. However, very little can be concluded on the degree of dispersion obtained in various studies (Christensen, 1992) because of incompletely defined procedures, which are not evaluated for their effectiveness. But like size separation, limited dispersion causes each fraction to include a wider mixture of noncomplexed SOM, SOM in organo-mineral complexes, and SOM in aggregates of finer particles. Microaggregates become included in light fractions because entrapped air and adsorbed water lower their effective density (Christensen, 1992), or in heavy fraction if pore space is open.

As mentioned above, specific density of heavy liquids differs widely among studies. Light fraction yields are very sensitive to changes in the density of fractionation liquids (Ladd and Amato, 1980; Dalal and Mayer, 1986). The use of high specific densities cause the light fraction (noncomplexed SOM) to invariably include a high ash content with relatively high proportion of macro–organic matter (Christensen, 1992).

The light fraction has been used as an indicator of changes in labile organic matter as affected by tillage, cropping practices, addition of crop residues, and environmental factors affecting microbial activity (Spycher *et al.*, 1983; Dalal and Mayer, 1986; Skjemstad *et al.*, 1986; Janzen, 1987; Janzen *et al.*, 1992; Magid *et al.*, 1997; Shang and Tiessen, 1997). Janzen *et al.* (1992) found that the light fraction accounted for between 2.0–17.5% of the organic matter in soils from three long–term crop rotation studies in Saskatchewan, Canada. Within each study, the light fraction content was generally highest in treatments with continuous cropping or perennial forages and lowest in those with a high frequency of

summer fallow. Janzen *et al.* (1992) further reported that the light fraction content was strongly related to soil respiration rates, suggesting it as a ready source of C and energy for soil microorganisms. They considered the light fraction as a sensitive indicator of the effects of cropping on SOM content and composition but because of its transient nature, it probably only reflects short–term effects. Magid *et al.* (1996) hypothesised that the air–filled porosity is an important feature in determining the densities of larger organic particles, and further suggested that the least decomposed tissues would retain the largest amount of air when subjected to density agents, at least under normal gravity. As mentioned above (also in Section 2.1.2), Magid *et al.* (1997) separated the particulate organic matter (>100 μm) into light ($\rho < 1.4$ Mg m^{-3}) and heavy ($\rho > 1.4$ Mg m^{-3}) fractions and studied the effect of additions of different amounts (0, 4 or 8 tonnes) of straw material on these fractions for 20 months. During the first 4 months, the particulate organic matter decreased quite rapidly in the straw amended treatments, which slowed down in the remaining 16 months. The heavy fraction C was completely unaffected by the application of residues, and apparently consisted mainly of 'native' organic matter with a low rate of decay. Magid *et al.* (1997) concluded that the difference in particulate organic matter between the treatments could be attributed completely to differences in the light fraction.

The above review suggests that physical separation procedures may yield the most useful information when considering the fate of a distinctly 'light' SOM fraction like a crop residue or plant shoot or root residues.

2.1.3   Chemical characterisation of SOM in physical fractions

Since organic matter can be protected from breakdown by both physical and chemical characteristics, it is possible that procedures which involve both physical and chemical aspects to the extraction of SOM may reveal more meaningful pools of SOM, which are more closely related to pool turnover rate. Christensen (1992) reviewed various conventional chemical analysis and ^{13}C– nuclear magnetic resonance spectroscopy (NMR) studies that were intended to elucidate chemical characteristics of SOM in various physical fractions. Christensen (1992) concluded from his review that the stabilisation and subsequent turnover of SOM in different size separates are the result of physical as well as chemical processes, which occur concurrently and affect organic substrates in all stages of

decay. Organic materials physically protected from microbial access with soil aggregates may be labile or recalcitrant. If labile substrates are released from physical protection, mineralisation may be rapid, whereas mineralisation may be slow where recalcitrant SOM is released from the disruption of aggregates. For these reasons, physical fractionation followed by chemical or biological assays of lability is a useful approach to the study of SOM dynamics.

Halstead *et al.* (1966) tried physical (ultrasonic) dispersion of SOM in the mild acetylacetone extractant and found the increased effectiveness of the reagent to extract organic matter and various nutrient fractions particularly organic P and S and to lesser extent N from a variety of soils. More recently, Swift *et al.* (1988), Keer *et al.* (1990), Eriksen *et al.* (1995a, 1995b), and Eriksen (1997) have extracted organic S by ultrasonic dispersion of soil in aqueous acetylacetone extractant (see Section 3.1 for more details). Recently, Shang and Tiessen (1997) used physical fractionations in combination with the permanganate–oxidation technique (Blair *et al.*, 1995 – see Section 2.1.1.3) and examined the changes in labile fractions of SOM caused by cultivation. Results indicated that sand–size floatable organic matter (22% of total soil C) was highly labile and spatially variable (i.e. declined by 40% upon cultivation). However, silt–size organic matter containing a large proportion of total soil C (about half), declined by 13% during cultivation. Shang and Tiessen (1997) further indicated that absolute and percent C losses in clay–associated C (27% of total soil C) were lowest. Chemical oxidation with 0.03 M $KMnO_4$ [10 times lower than the one used by Blair *et al.* (1995) – see Section 2.1.1.3] was able to predict the proportion of labile C in native (20%) and cultivated (25%) soils, and various particle size (sand 8–42%; silt 29–46%; clay 29–46%) fractions. After cultivation, there was a decrease in the sand–size fraction labile C, which indicated lower stability of sand–size organic matter and potentially more rapid turnover in the cultivated soil, whereas the loss of labile C, as a result of cultivation, was similar for the silt and clay size fractions. However, the loss of nonlabile C (non–oxidisable by $KMnO_4$) from clay was less than that from silt, suggesting that a portion of organic matter associated with clay was more stable.

Combined size (or density) fractionation and chemical extraction although obviously time consuming may hold potential for the classification of labile SOM, particularly in cultivated soils, where cultivation may cause size or stability changes in soil aggregates.

2.1.4   *Nutrients (N, P and S) in soil organic matter*

Soil organic matter contains a significant amount of N, S and to a somewhat lesser extent, P. Considerable research has already been carried out on the fractionation of organic N and P in soils (see reviews by Stanford, 1982; Bundy and Meisinger, 1994; Hedley *et al.*, 1995; Kuo, 1996). The sequential extraction procedure for soil P, introduced by Hedley *et al.* (1982), is frequently being used to characterise changes in labile soil organic P fractions with the change in soil management and cropping practices (Huffman *et al.*, 1996; Schmidt *et al.*, 1996) and plant uptake (Hedley *et al.*, 1994; Trolove *et al.*, 1996). Hedley et al's soil P fractionation relies on differing strengths of NaOH to extract soil organic phosphate of differing lability. One-tenth molar NaOH appears to extract a dynamic soil organic P fraction that change with P uptake by plants (Trolove *et al.*, 1996) and microbial decomposition (Hedley *et al.*, 1982). It is uncertain whether a procedure similar to Hedley et al's soil P fractionation can be used to sequentially fractionate labile soil S fractions in the same manner. Nguyen and Goh (1992b) have reported that the alkaline solutions [0.5 M $NaHCO_3$ (pH 8.5) and 0.1 M NaOH] could extract considerable proportions of total soil S (see Section 3.1). In this study, the aim is to investigate whether soil S fractions extracted in NaOH of increasing concentration represent S pools of decreasing lability. Firstly, we must consider the forms of S in soil.

2.1.4.1   *The nature of S in soils and organic S fractionation*

Sulphur occurs in soils in both organic and inorganic forms. Essentially most of the S (95–99%) in soils of humid and semi–humid regions occurs in organic forms (Tabatabai and Bremner, 1972; David *et al.*, 1982; Freney, 1986; Stevenson and Cole, 1999). Although inorganic S constitutes a very small proportion of total soil S, it is mostly and commonly contains $SO_4^=$ ions, which can be readily taken up by plants (Metson, 1979a). In contrast, virtually all of organic S is unavailable to plants until it is mineralised to $SO_4^=$ (Freney, 1986).

Classification of plant available $SO_4^=$ in soils is relatively easy. Various chemical extractants (e.g. chloride and phosphate based) that remove soluble and adsorbed S have met with some success as an indicator of plant available soil S status in unimproved grasslands (Saunders *et al.*, 1988). However, in well–developed grasslands, where organic

S has accumulated over a period of time and its contribution to plant growth is significant, soil tests based on $SO_4^=$ alone do not adequately predict the temporal change in plant available soil S status (Saunders *et al.*, 1988; Nguyen *et al.*, 1989). This is because of the continued mineralisation of $SO_4^=$ from SOM, which is often a function of the SOM content, fertiliser history and environmental factors. For this reason, researchers in both New Zealand and Australia have developed soil testing methods, which attempt to measure both the labile $SO_4^=$ pool and the potentially–mineralised soil organic S in well developed pastures (Spencer and Freney, 1960; Watkinson and Perrott, 1990; Blair *et al.*, 1991; Watkinson *et al.*, 1991; Anderson *et al.*, 1992; Watkinson and Kear, 1996) (see Section 3.1 for detailed review).

The soil organic S pool is considered to comprise of two pools, the rapid organic cycling or active pool (10–50% of soil organic S) and the inert, recalcitrant cycling pool (50–90% of soil organic S) (Till and May, 1970, 1971; Clark *et al.*, 1980; Nguyen, 1990; Goh and Gregg, 1982). The turnover rate of the active pool, which consists of labile, readily mineralisable plant litter, animal residues and microbial biomass, is important in providing S for plant nutrition (Till and May, 1970; Sarathchandra *et al.*, 1988). In contrast, the turnover rate of the inert organic S pool, which represents the more recalcitrant, chemically stabilised soil organic matter is too slow to contribute a significant amount of S to the S cycling pool (Till and May, 1970, 1971; Goh and Gregg, 1982).

The chemical nature of organic S has not been fully characterised. Most of the S compounds in soils are susceptible to decomposition and therefore, do not accumulate in uncombined form and are not readily detected (Freney, 1986). A significant proportion (up to 30%) occurs as amino acids bound to soil colloids (Freney *et al.*, 1970). Small amounts of S may occur in free amino acids, sulphated polysaccharides, and sulpholipids.

In order to define unclear, heterogeneous nature of organic S in soils, many researchers have characterised organic S into broad, functionally distinct groups. The most widely used is separation based on susceptibility to reduction by reducing agents: a) HI–reducible S (ester sulphate S), defined as S that can be reduced by hydroiodic acid to H_2S, including ester sulphates (–C–O–S–), sulphamates (–C–N–S), and the second S^* in S–sulphocysteine (–C–S–S*–), where the S and C atoms are separated by O, N, or S atoms, respectively, and b) C–bonded S, which includes all compounds in which S is covalently

bonded to C (Freney, 1986) such as cysteine (HOOC–CH(NH$_2$)–CH$_2$–SH) and methionine (HOOC–CH(NH$_2$)–CH$_2$– CH$_2$–S–CH$_3$) amino acids (Stevenson and Cole, 1999). The conventional view is that ester sulphate, which typically accounts for approximately 50% of soil organic S, represents the labile organic S fraction and is associated largely with low molecular weight materials (Beiderbeck, 1978; Saggar *et al.*, 1984; McLaren *et al.*, 1985). It is also suggested that C–bonded S is primarily associated with the aromatic core of humic acids because of its resistance to several extractants, and correlation with certain humus properties (Bettany *et al.*, 1973; McGill and Cole, 1981).

Considerable effort has been devoted to characterising the biochemical source of mineralised S (McLaren and Swift, 1977; Bettany *et al.*, 1979; Saggar, 1980; Anderson *et al.*, 1981; McGill and Cole, 1981; Maynard *et al.*, 1985; Ghani *et al.*, 1988; Keer *et al.*, 1990; Eriksen *et al.*, 1995a, 1995b; Eriksen, 1997). These studies indicated that the classification of S on the basis of bond classes might not have enough sensitivity to distinguish between labile and refractory fractions (Castellano and Dick, 1991; Lou and Warman, 1992; Eriksen *et al.*, 1995c). Similarly, physical separation (Anderson *et al.*, 1981) and a combination of physical and chemical fractionation techniques (Bettany *et al.*, 1979; Keer *et al.*, 1990; Eriksen *et al.*, 1995a, 1995b; Eriksen, 1997) characterising the more labile S from the more inert components irrespective of its bonding relationships to C have met with limited success, perhaps because of the heterogeneity of S substrates in SOM (refer to detailed review in Chapter 3).

Limited work has been conducted on identifying labile organic S pools by alkaline chemical extraction (Nguyen and Goh, 1992b). Soil incubation and mineralisation procedures have been used (Ghani *et al.*, 1988, 1991; Sakadevan *et al.*, 1993a). As yet these procedures have been relatively unsuccessful in characterising soil labile S fractions.

2.1.5 *Tracer use in SOM studies*

Characterisation of SOM relative to its turnover rate and stability has also been investigated by measuring the rate or degree of incorporation of isotopically labelled plant material into SOM. Stable and radioisotopes, when available, have been used in these studies for carbon (^{14}C and ^{13}C), nitrogen (^{15}N), phosphorus (^{32}P and ^{33}P) and sulphur (^{34}S

and ^{35}S). Detailed isotopic methods for the study of SOM dynamics are available (Middleboe *et al.*, 1976; Wolf *et al.*, 1994).

The radioisotope ^{14}C (half–life of about 5730 years) has been used in many aspects of SOM research. For the study of C transformations, the fate of uniformly ^{14}C labelled plant materials (Jenkinson, 1977a, 1977b) can be used to study both the residues decomposition rates and the contribution that residue addition makes to various already identified soil C pools (Buyanovsky *et al.*, 1994). Substantial experimental efforts have been made where decomposition of uniformly labelled plant (shoot and/or root) materials and ^{14}C labelled substances has been followed in the laboratory or field by changing variables, such as soil type, plant cover, organic matter addition, type of organic material, elevated CO_2, moisture, different climatic and environmental conditions, or pasture phase within crop rotation to assure results of high accuracy (Jenkinson, 1964; Nyhan, 1975; Jenkinson, 1977a, 1977b; Jenkinson and Ayanaba, 1977; Ladd *et al.*, 1985; van Veen *et al.*, 1985; Martin, 1987; Raina and Goswami, 1988; Sallih and Bottner, 1988; Cheng and Coleman, 1990; Saggar *et al.*, 1996; Chotte *et al.*, 1998; van Ginkel and Gorissen, 1998; Parshotam *et al.*, 2000). In general, these studies have shown that the residues are attacked rapidly at first, but after a few months, the rate slows substantially although considerable amounts of plant derived C remain in the soil. The decreased rate over time was due to the differences in the rate at which the various plant components decompose (e.g., lignin decomposes quite slowly) and to the resynthesis (during decomposition) of the more readily degraded constituents (e.g., soluble and cellulose) into microbial C components that are more resistant to decay than the original plant material (Stevenson and Cole, 1999).

At the termination of an experiment, ^{14}C labelled SOM can also be fractionated, with each component being analysed for ^{14}C. This may aid in evaluating useful chemicals for fractionation procedures. Sorensen (1963) fractionated SOM after allowing ^{14}C–labelled barley straw to decay in soil for 100 days at 20°C. Residual ^{14}C was found mainly in humin (49–55%), 12–19% in fulvic acid, 20–26% in humic acids, and 5–13% unaccounted for in these SOM fractions. These results suggest that compounds other than lignin contributed to formation of humic substances. Recently, Magid *et al.* (1996) elucidated the decomposition of ^{14}C–labelled plant residues (*Lolium perenne*) by using different density

and size–density fractionation methods. They showed that the residual ^{14}C remaining in soil could be largely recovered from the soil by separations based on size and density.

The radioisotope ^{14}C have also been used to characterise the partitioning of recently photo–assimilated C in the plant shoot–root–soil system (Rattray *et al.*, 1995; Saggar *et al.*, 1997, 1999), and to measure the extent of rhizodeposition (see review by Lynch and Whipps, 1990). This has been achieved in number of studies either by pulse labelling or continuous labelling of plants with $^{14}CO_2$ (Martin and Merckx, 1992; Zagal, 1994; Rattray *et al.*, 1995; Saggar *et al.*, 1999). The stable isotope (^{13}C) can be used in a similar manner (Stewart and Metherell, 1999). Accordingly, formation of new humus can be followed by tracing the flow of ^{13}C from the added residues to the older organic matter (Balesdent *et al.*, 1987; Skjemstad *et al.*, 1990). Stewart and Metherell (1999) pulse labelled pasture field with $^{13}CO_2$ to follow ^{13}C uptake and allocation in pasture plants. Combined with chemical fractionation of the soil profile, information can be gained on the distribution of rhizodeposited and root C in various fractions.

The short half–lives of ^{32}P (14 days), ^{33}P (25 days) and ^{35}S (87 days) make them less appropriate than ^{14}C (5730 years) for studies of SOM dynamics, particularly long–term studies in the field. However, despite these limitations, they have been successfully used in studies of the transformations of organic and inorganic materials labelled with ^{33}P (Friesen and Blair, 1988) and ^{35}S (Saggar *et al.*, 1981; Goh and Gregg, 1982; Maynard *et al.*, 1985; McLaren *et al.*, 1985; Sakadevan *et al.*, 1993b; Lefroy *et al.*, 1994) and in the dynamics of ^{35}S in fractions of SOM (Saggar *et al.*, 1981; Ghani *et al.*, 1992; Eriksen *et al.*, 1995b; Eriksen, 1997). Also, several other S isotope studies have been done using the classical soil S fractionation technique (Freney *et al.*, 1971, 1975; Bettany *et al.*, 1974), which evaluate the usefulness of the S fractionation for biological systems. Again, these fractionations were not entirely satisfactory in that all fractions contributed toward the available or mineralisable S. Therefore; the traditional soil S chemical fractionation procedure must be further refined to establish "biologically meaningful" fractions.

2.1.6   *The importance of measurable pools in SOM modelling studies*

Several scientists have tried to model soil C levels (Paustian *et al.*, 1998; Patwardhan *et al.*, 1998; Grant *et al.*, 1998), mainly to provide data on global C balance and the

consequences of land management practices on atmospheric CO_2 levels. Currently most of these models have conceptual C pools, which are simply arbitrary divisions of the amount of C in an appropriate soil profile depth (Cambardella, 1998). Some, however, are more sophisticated using measures of some soil C pools (e.g. soil microbial C – Jenkinson and Rayner, 1977). Two modelling approaches often cited in the literature are Rothamsted model (Jenkinson and Rayner, 1977) and Century model (Parton *et al.*, 1987, 1988).

The five–pool Rothamsted model (Jenkinson and Rayner, 1977) includes the plant residue component partitioned into two subpools (readily decomposable and resistant), the microbial biomass, and two forms of stabilised organic matter (physically protected and chemically protected). Mean residence times for the various conceptual pools of SOM in the model are given in Table 2.1.

The Century model (Parton *et al.*, 1987, 1988) is a general model of the soil–plant ecosystem that has been used to represent C and nutrient (N, P and S) turnover dynamics for grasslands, crops, forests and savannas. Mean residence times for the various conceptual pools of SOM in the model are given in Table 2.1. In the Century model, incoming plant residues (from shoots and roots) are divided into metabolic and structural pools as a function of lignin to N ratio of the residues, and soil organic fractions are then divided up into three major components which includes active, slow and passive soil C. Active SOM includes live soil microbes plus microbial products. The slow pool includes resistant plant material (lignin–derived) and soil–stabilised plant and microbial material. The passive pool contains physically and chemically stabilised SOM. Typical turnover times for various theoretical pools of SOM are given in Table 2.1. The N, P and S submodels have the same general structure as the C submodel. Critical components are the C:N, C:P and C:S ratios of the different inputs and pools.

A major limitation of current simulation models describing SOM turnover is that the conceptualised pools may not directly correspond to experimentally measurable fractions and thus it is difficult to validate and test the accuracy of the simulation model with field–derived data.

Table 2.1: Comparison of mean residence times of C in theoretical pools of SOM and in soil physical fractions (adapted from Buyanovsky et al., 1994).

Pool	Jenkinson and Rayner (1977) (Theoretical pools)	Parton et al. (1988) (Theoretical pools)	Buyanovsky et al. (1994) – measured physical fractions
I	Decomposable plant material, 0.24 yr	Metabolic plant residues, 0.5 yr	Vegetative fragments (2–0.2 mm), 0.5–1 yr
II	Resistant plant material, 3.33 yr	Structural plant residues, 3.0 yr	Vegetative fragments (> 0.053 mm), 1–2 yr Vegetative fragments (0.053–0.025 mm), 2–3 yr Macroaggregates (2–1 mm), 1–4 yr
III	Soil biomass, 2.44 yr	Active soil C, 1.5–10 yr	Aggregates (1–0.5 mm), 2–10 yr Aggregates (0.5–0.1 mm), 3–10 years Nonaggregated soil, 7 yr
IV	Physically stabilised, 72 yr	Slow soil C, 25–50 yr	Fine silt (internal), 400 yr
V	Chemically stabilised, 2857 yr	Passive soil C, 1000–1500 yr	Fine clay (internal), 1000 yr

An alternative approach is that of Buyanovsky et al. (1994), who followed amongst physical fractions, the distribution of ^{14}C originating from uniformly labelled soybean [Glycine max (L.) merr.] residues incorporated into soil (mesic Udollic Ochraqualf), over a four–year period. These researchers characterised the SOM residues by particle size, using categories of SOM in vegetative fragments (2–0.2 mm, >0.053 mm, and 0.053–0.025 mm), within natural aggregates (2–1 mm, 1–0.5 mm, 0.5–0.1 mm) and non–aggregates, and individual mineral particles such as the fine silt (25–2 µm – internal) and the fine clay (<2 µm –internal). Buyanovsky et al. (1994) compared residence times of each measured fraction with theoretical pools of SOM in Rothamsted (Jenkinson and Rayner, 1977) and Century (Parton et al. 1988) models (Table 2.1). In both the Rothamsted and Century models, conceptual pools I and II are characterised by the quality of plant material with rapid turnover. Vegetative fragments measured by Buyanovsky et al. (1994), with turnover times ranging from 0.5–3 years, correspond to these two pools (Table 2.1).

Macroaggregates (2–1 mm), organised around plant fragments, have approximately the same life span as vegetative fragments 2–0.053 mm, and represent a transient C pool. The physical fractions (macroaggregates, microaggregates and nonaggregated soil) that correspond to pool III [soil biomass (2.44 years – Rothamsted model) or active C (1.5–10 years – Century model)] demonstrate a turnover time of 2–10 years (Table 2.1).

Turnover time of the two most stable pools of SOM, characterised as physically/chemically stabilised C pools by the Rothamsted model or slow/passive pools by the Century model (i.e. pools IV/V – Table 2.1), were obtained from estimates derived by Balesdent *et al.* (1988) and Hsieh (1992) using ^{13}C and ^{14}C natural abundances, respectively in SOM. These estimates were based on the differences in behaviour of C associated with clay and silt fractions (Balesdent *et al.*, 1988; Buyanovsky *et al.*, 1994). These results demonstrated that the residence times of various measured physical fractions were similar to the conceptual pools used in both the Rothamsted and Century models.

The results of Buyanovsky *et al.* (1994) study showed that physical fractionation of SOM might allow direct correlation between modelled and measured SOM pools. This would allow better evaluation and testing of SOM conceptual models.

2.2   CONCLUSIONS

Soil organic matter is a labile source of nutrients that can be depleted or renewed by altering landuse and management practices. A number of studies have been conducted that aimed at separating SOM into fractions with a rapid turnover rates and fractions with a slow turnover rates. Characterisation of young, labile SOM may become more necessary for understanding nutrient availability. A wide variety of techniques have been applied to the measurement and characterisation of SOM, and plant available nutrients contained therein, in relation to their dynamics under various management practices but only limited success has been obtained and no one procedure has been consistently superior to all others. Classical approaches have combined chemical extractions with identification of specific chemical compounds but have not been very successful at separating SOM into pools important in cycling and release of plant nutrients. Results from physical fractionations indicate that nutrient turnover depends not only on the kind and amount of organic matter but on its location within the soil, which combined with functional

found to be more sensitive to differences in management practices. The physico–chemical approaches can play an important role in elucidating resistant or labile nature of organic constituents associated with a particular physical/density fraction. The use of isotopes (e.g. ^{13}C or ^{14}C) in SOM studies has also been successful to follow the fate of specific C sources (e.g. crop residues) against the background of large amounts of SOM, and to identify specific fractions that are biologically active. Predictive models of organic matter decomposition in soil invariably identify several SOM pools with different turnover rates. These SOM pools are often defined in an arbitrary conceptual way to enable functioning of models. An aspect that requires attention is research leading to methods capable of measuring dynamic SOM pools in soils. In this way the accuracy of SOM dynamic models can be tested. Accurate models are needed to explain the fate of C in terrestrial ecosystems.

Many workers have identified nutrient availability of SOM as an important indicator of soil quality. Sulphur is one of the essential elements for plant growth, which like N and to lesser extent P, relies on SOM for its conservation in the root zone. In agriculture systems, where input of S from fertiliser and atmospheric sources is decreasing, the release of S from organic sources would become more important for the supply of S to plants. Historically, not much work has been done to characterise turnover of soil organic S under different conditions. More detailed information is required on the S pools/fractions that are responsive to short– and long–term changes in landuses practices and, therefore, important for the release of plant–available S. Knowledge of how to characterise these fractions will be important in determining SOM quality.

Key information gaps highlighted by this literature review are consistent with the thesis objectives stated in Chapter 1. Further research on the characterisation and measurement of dynamic fractions of SOM would be useful for evaluating sustainable land management practices.

CHAPTER 3   METHODS FOR ANALYSING SULPHUR AND CARBON IN SOIL AND SOIL EXTRACTS

3.1   INTRODUCTION

Plant available S in soils comprises the inorganic sulphate in soil solution and adsorbed on particle surfaces. More than 90%, however, of the S in topsoil is organic, originating from different humus, microbial and plant forms. This S is the main source of plant available S (sulphate) (Nguyen and Goh, 1994), with only a small but variable fraction (approximately 2–8%) of soil organic S being mineralised each growing season (Freney, 1986; Chapman, 1987). This is partly because the nature of the soil fabric and the organic forms limit the accessibility and effectiveness of mineralising microorganisms or extracellular hydrolytic enzymes.

Tests for predicting soil S status have often extracted only the sulphate pool from soil. For example, the calcium phosphate extractable inorganic S soil test (Saunders *et al.*, 1988) extracts little organically bound S which is potentially mineralisable and available for plant uptake. In situations where the mineralisable S pool is large, calcium phosphate extractable sulphate may be a poor indicator of soil S status (Hedley, 1988; Saunders *et al.*, 1988). A soil test, which indicates the size of the mineralisable organic S, may correlate better with S response and uptake (Giddens *et al.*, 1995). Over the last three decades, a number of soil tests have been developed which indicate the size of an organic S fraction that is relatively labile and may be readily utilised by plants. To improve the ability of the phosphate extraction to predict S deficiency in pasture soils, Watkinson and Perrott (1990) and Watkinson and Kear (1996) measured sulphate S and organic S extracted in 0.02 M $Ca(H_2PO_4)_2$ or 0.02 M KH_2PO_4 solutions. They found that approximately half the S extracted was organic and the size of the organic fraction, rather than the sulphate fraction, was correlated better with pasture response to S.

In Australia, the most common method used to indicate soil S status involves heating soil in 0.25M KCl at 40°C for 3 h and measuring the total S rather than inorganic or organic S mobilised in the soil extract (Blair *et al.* 1991). Sulphate is not measured separately, which makes the interpretation of high S fertilised soils difficult. A better

estimate for mineralisable organic S may be the sulphate mineralised during laboratory incubation (Searle, 1992). While incubation would most closely represent mineralisation under field conditions, the time involved would preclude it for use as a routine test. Recently, Pamidi (1999) compared the amount of soil S extracted in either 0.01 M $CaCl_2$, 0.016 M KH_2PO_4, 0.25 M $KCl-40°$, 0.5 M $NaHCO_3-pH$ 8.5, or 0.1 M NaOH to plant S uptake during a short period of 20 weeks of ryegrass growth. Pamidi's (1999) study showed a good relationship between $CaCl_2$, KH_2PO_4, or $KCl-40°$ extractable soil S (which were largely utilised by the 8th week harvest) and plant S uptake, indicating the value of these extractants as rapid soil tests. However, any mineralised S present in subsequent harvests might have originated from organic S pools extracted by $NaHCO_3$ and NaOH, all of which were not reflected in plant S availability in the short–term (Pamidi, 1999).

The common feature of all the tests for plant available S mentioned above is that only a small percentage of the mineralisable S pool is measured. Those wishing to characterise the 'quality' of a soil require a more accurate estimate of the quantity of potentially mineralisable S in the SOM fractions.

Different approaches have been used to separate soil organic S into some broad fractions representing distinct forms and properties. Among these are separation based on bonding relationships with carbon (HI reducible S as C–O–S or carbon bonded S as C–S) (Freney, 1961, 1986; Freney *et al.*, 1969), physical separation into organo-mineral size fractions (Hinds and Lowe, 1980b; Anderson *et al.*, 1981) and chemical extraction followed by physical–chemical separation into humic acids, fulvic acids and humin (Bettany *et al.*, 1979, 1980). The traditional approach of characterising soil organic S according to reactivity with reducing agents into HI–reducible (ester sulphate) and C–bonded S has not been useful in characterising a pool of mineralisable S (Hedley, 1988; Swift *et al.*, 1988). Mineralised S originates from both C–O–S and C–S compounds (Freney *et al.*, 1975; McLaren and Swift, 1977). Although in some conditions the HI reducible S fraction can mineralise at faster rates and is considered more labile (Beiderbeck, 1978; Sakadevan *et al.*, 1993a). Ghani *et al.* (1988) demonstrated that recently formed microbial C–S was readily remineralised leading to the production of HI–reducible and $SO_4^=-S$. This indicated that some forms of soil C–S, possibly consisting of simple amino acid S, can also act as sources of readily mineralisable S (McLachlan and De Marco, 1975; Maynard *et al.*, 1985). However, it could not be inferred that all C–S in soil

has the same characteristics. Furthermore, Lou and Warman (1994) indicated that much of the ester sulphate, while intrinsically labile, might not be accessible to enzymes through adsorption to soil surfaces and aggregate formation.

Swift *et al.* (1988) and Keer *et al.* (1990) extracted organic S from soil with a milder alkaline extractant (aqueous acetylacetone) in conjunction with ultrasonic dispersion. The acetylacetone releases organic matter by complexing with the polyvalent metals in the organo-mineral complexes (Keer *et al.*, 1990). Eriksen *et al.* (1995a, 1995b), using acetylacetone extraction with ultrasonic dispersion, demonstrated that a large part of soil organic S was physically protected in soil aggregates. The decomposition rate of the protected organic S was slow. The use of milder extractants to characterise mineralisable fractions can avoid the problem of degradation of humic substances and modification of organic sulphur forms during extraction. Milder extractants, however, may extract a small amount of S only, leaving a major portion in the insoluble organic matter pool uncharacterised (Eriksen, 1997). However, the small, labile pool of organic S in the non–protected fraction (Eriksen *et al.*, 1995b) is important only in short–term supply of S to plants.

Ideally, techniques are required which divide organic S into a few biologically meaningful labile fractions. Neutral and alkaline extractions of soils have been successful in characterising the labile fractions of SOM that supply phosphorus (P) to plants under short– (Trolove *et al.*, 1996) and long–term cultivation (Hedley *et al.*, 1982, Tiessen *et al.*, 1982, 1983) and those organic P fractions that build up under permanent pasture (Perrott *et al.*, 1989). Nguyen and Goh (1992b) have reported that shaking soil for 16 h with 0.5 M NaHCO$_3$ (pH 8.5) or with 0.1 M NaOH extracted approximately 14–22% and 49–75% of total soil S (inorganic and organic together) respectively, which were correlated more closely with total S, organic S, HI–reducible S, and C–bonded S. These extractants may be suitable for use in a sequential fractionation of soil S in a manner similar to that of P. In this chapter, a soil S and C fractionation procedure is developed by modifying the procedures of the sequential P fractionation of Hedley *et al.* (1982). In particular, analytical procedural changes are required to measure HI reducible S and total S, and total C, in various extracts obtained during fractionation.

3.2   METHODS AND MATERIALS

3.2.1   A sequential soil S and C fractionation technique

The procedure used to sequentially fractionate soil S and C is summarised in Figure 3.1. The fractionation procedure is similar to that was used to measure changes in soil P fractions induced by cropping practices (Hedley *et al.*, 1982) and plant uptake (Hedley *et al.*, 1994; Trolove *et al.*, 1996). The initial design of the sequential fractionation procedure was to determine firstly the exchangeable ionic forms of soil S and C (both soluble and adsorbed and simple organic esters) extracted to ion–exchange resins [resin–S(C)]. Strips of both anion and cation exchange resin membranes (Saggar *et al.* 1992) were used

Soil

2 g soil (ring ground) in 40 ml polypropylene centrifuge tubes, add 18 ml deionised water and resins (both cation and anion). Shake for 2 h at room temperature.

Remove strips, rinse with deionised water and elute them together.

Soil Residue + Water   **Ion-exchange Resin Strips**   Elution in 10 ml of 0.5 M NaCl/ 0.5 M NaNO$_3$ **Resin–S(C)**

Add 2 ml of 1 M NaOH (to generate 0.1 M NaOH), shake for 16 h at room temperature, centrifuge and filter supernatant through Whatmann filter paper No.42/glass microfibre filter (GF/C).

Soil Residue   **Filtrate**   **0.1 M NaOH–S(C)**

Add 20 ml of 1 M NaOH, shake for 16 h at room temperature, centrifuge and filter supernatant through Whatmann filter paper No.42/glass microfibre filter (GF/C).

Soil Residue   **Filtrate**   **1 M NaOH–S(C)**

Dry the soil residue at 35°C and ring grind.

Residual–S(C)

Figure 3.1: A soil S and C sequential fractionation technique

because in soils of variable charge, extraction of soil anions may be more effective when cations contributing to surface positive charge are removed (Curtin *et al.*, 1987; Saggar *et al.*, 1992). Solid phase non–exchangeable forms of S and C were extracted in alkali extractions of various strength. A conceptually 'labile' organic fraction was extracted by

0.1 M NaOH [0.1 M NaOH–S(C)] and a less 'labile' organic fraction extracted by 1 M NaOH [1 M NaOH–S(C)]. The S and C fraction, which could not be extracted with either resins or the two NaOH extractants was termed the residual fraction [(residual–S(C)]. This fraction may represent the more recalcitrant, chemically stabilised soil organic matter.

The soil C fractionation procedure was slightly modified from the reported methods for S in the following way. The anion and cation resins were charged as per the procedure described by Saggar *et al.* (1990a) except anion exchange resin, which was charged as OH^- using 0.1 M NaOH instead of HCO_3^- resin (i.e. by using 0.5 M $NaHCO_3$). This would avoid any interference in the C estimation from HCO_3^- ions. Both cation and anion (in the hydroxide form) resins were eluted in 0.5 M $NaNO_3$ (this eluted the same amount of C from the cation/anion exchange resins as was eluted in 0.5 M NaCl) to avoid Cl^- ion interference during dichromate digestion procedure used for the estimation of total C in the extract (see following Section 3.2.5.1). Also, alkaline extracts were filtered through Whatmann glass microfibre (GF/C) to avoid any cellulose coming out in the alkaline extracts from paper filters (Whatmann No. 42) used during soil S fractionation.

3.2.2 *Analysis of resin extractable S*

Resin extractable S was measured by analysing an aliquot of 0.5 M NaCl elutant using automated HI reduction and the bismuth sulphide turbidimetric finish (Dean, 1966). Any C–bonded S in this would not be measured.

3.2.3 *Analysis of total S in alkaline extracts*

3.2.3.1 *Preparation of standard organic S solutions*

Sulphanilamide and methionine were selected as organic S standards for the development of a method of estimation of total S in alkaline extracts. The C–S bond of these compounds is considered to be quite strong and therefore, difficult to oxidise particularly of methionine (Blanchar *et al.*, 1965). Stock solutions of sulphanilamide and methionine were prepared (1000ppm S) in ethanol.

As a check, the $NaHCO_3$ fusion technique reported for plant and soil samples by Steinbergs *et al.* (1962) was employed for oxidising organic S standard compounds

dissolved in ethanol. The methionine and the sulphanilamide (0.05 ml and 0.08 ml of 1000 ppm S stock solution) were added separately in triplicate to the fusion tubes. A mixture (0.5 g) of $NaHCO_3$ and AgO (12.5:1) was added to each tube, mixed thoroughly using a vortex mixer and then covered with 0.5 g of $NaHCO_3$ and fused at 550°C for 2.5 to 3 h (Steinbergs *et al.*, 1962), and left for few hours. After cooling, 10 ml of 5 N HCl was added slowly in two to three instalments, and mixed thoroughly. The $SO_4^=$ concentration in the digest was determined by reducing $SO_4^=$ to H_2S using HI mixture and measured as bismuth sulphide turbidimetric finish (Dean, 1966) using a Technicon II autoanalyser.

Standard amounts of sulphanilamide and methionine compounds (5 and 8 ppm S) dissolved in ethanol were added in triplicate in 0.1 M NaOH and 1 M NaOH reagents. The S was measured in the NaOBr digested and undigested [see Sections 3.2.3.2 (Method 1) and 3.3.1] alkaline solutions as $SO_4^=$–S (Dean, 1966).

In the following Sections 3.2.3.2 and 3.2.3.3, a range of modifications are tested in attempts to either produce a direct NaOBr oxidation method or modify the original Tabatabai and Bremner (1970) wet oxidation technique for quantitative recovery of organic S in alkaline extracts.

3.2.3.2   *In situ production of NaOBr*

In this procedure, bromine drops were added directly into alkaline solutions to carry out *in situ* production of NaOBr.

Method 1

Two mls of 0.1 M NaOH and 1 M NaOH reagents were taken in digestion tubes in triplicate and two drops of bromine were added directly into 0.1 M NaOH and 1 M NaOH solutions so as to carry out *in situ* production of NaOBr. The stock solutions (0.05 ml and 0.08 ml of 1000 ppm S) of sulphanilamide and methionine were spiked into 0.1 M NaOH and 1 M NaOH in the digestion tubes separately using auto–pipette. These digestion tubes were then placed onto a cool digestion block and the temperature was raised to 150°C and after one hour raised to 200°C for half an hour till dryness and continued heating for another 20 minutes after evaporation to dryness as suggested by Tabatabi and Bremner

(1970). Tubes were removed from the digestion block. After cooling, the digested material in the digestion tubes was neutralised with 0.2 ml and 2 ml of 1 M HCl, respectively and resuspended with water to the 10 ml volume. Alkaline reagents blanks were also digested without sulphanilamide and methionine. The aliquot of the diluted solution was taken and measured for $SO_4^=$–S using autoanalyser as mentioned above. Undigested alkaline solutions containing the sulphanilamide and methionine organic S standards were also analysed for $SO_4^=$–S. For sulphate recovery standards, two different concentrations of $SO_4^=$–S i.e. 50 ppm and 80 ppm S prepared from K_2SO_4 were digested in the same way.

Method 2

Sulphanilamide in alkaline solutions was evaporated to dryness at 200°C in the same way as described above. Once dry, the tube was allowed to cool for 5 minutes and the residue resuspended with 2 ml of water. Two drops of bromine were added to further produce NaOBr *in situ*. The tube contents were then heated at 160°C till dry. Again the residue was neutralised with HCl and resuspended with water in a final volume of 10 ml. The $SO_4^=$ in the sample was reduced with HI to quantitatively recover the inorganic $SO_4^=$– S formed by wet oxidation.

Method 3

Two different concentrations of sulphanilamide (as mentioned above) in 0.1 M NaOH solution were digested at 160°C in the same way as described in Method 1 above (i.e. by carrying out *in situ* production of NaOBr), but condenser funnels were placed at the top of digestion tubes. Four sets (three replicates of each) were placed in the digestion block and condenser funnels from each set of three tubes were removed after 1, 2, 3, and 4 h of digestion. After removal of funnels, the solutions were evaporated to dryness by heating at 160°C for about 1 h. Digests were neutralised with HCl and volumes were made to 10 ml. Aliquot's were taken for the analysis of S as described in previous sections.

Sulphanilamide was initially dissolved in ethanol. The influence of ethanol was evaluated by dissolving sulphanilamide in hot water (50–60°C). A stock solution of 1000 ppm S was prepared in hot water. Then, 0.05 ml and 0.08 ml of this solution, and the ethanol solution, were spiked separately into 0.1 M NaOH placed in the digestion tube and

two drops of bromine were added and digested at 160°C till dryness as per the procedure mentioned above.

3.2.3.3   *Tabatabai and Bremner wet oxidation technique with some modifications*

<u>*Sodium hypobromite addition in one instalment*</u>

Two ml of 0.1 M NaOH and 1 M NaOH reagents were neutralised with 0.2 ml of 1 M HCl and 0.2 ml of 10 M HCl respectively, in digestion tubes (Tabatabai and Bremner, 1970). Sulphanilamide and methionine (0.05 ml and 0.08 ml of 1000 ppm S) were spiked in the neutralised solutions. The NaOBr reagent is prepared immediately before use by adding 3.5 to 4 ml of bromine slowly (@0.5 ml/minute) to 100 ml of 2 M NaOH with continuous shaking. Four ml of the freshly prepared NaOBr was added to the digestion tubes in one instalment instead of four instalments as suggested by Phimsarn (1991). The tubes were placed onto a cool aluminium digestion block and the temperature was raised to 150°C in 25 minutes. The digestion was continued for 20 minutes at 150°C until the volume of the digest was reduced by one third of its original volume. The temperature was then raised to 200°C and digestion was continued for another 20 minutes until the volume of the digest was reduced by two third of its original volume. The temperature was then raised 260°C and the tubes were taken to dryness. The tubes were kept at 260°C for another 30 minutes after evaporation to dryness (Tabatabai and Bremner, 1970). The tubes were removed from the digestion block. After 5 minutes of cooling, one ml of formic acid (98–100%) was added to remove excess bromine and the volume was made to 10 ml with deionised water and mixed thoroughly using a vortex mixer. An aliquot was taken for the measurement of S by autoanalyser as described above for resin–S. Potassium sulphate was used as a standard.

<u>*Sodium hypobromite addition in two equal instalments*</u>

Two ml of NaOBr was added initially in the digestion tubes containing neutralised alkali solution and organic S compounds as mentioned above. The tubes were heated to 150°C for one hour and then raised to 200°C till dryness and heated for another half an hour. Digestion tubes were then removed from the digestion block. After 5 minutes of cooling, the remaining 2 ml of NaOBr was added and placed back onto the block.

Temperature was raised to 260°C and heated for another half an hour till dryness. Heating continued for 30 minutes after evaporation to dryness. Excess bromine was removed using formic acid and residue was resuspended in water as mentioned above, and analysed for S using a Technicon II autoanalyser (Section 3.2.3.1).

3.2.4 *Analysis of total S in soil and residual fraction*

The total S in soil and residual fraction was oxidised to $SO_4^=$–S using the $NaHCO_3$ fusion method (see description earlier in Section 3.2.3.1) of Steinberg *et al.* (1962). The digests were analysed for S using a Technicon II autoanalyser (Section 3.2.3.1).

3.2.5 *Analysis of C in different fractions*

A wet oxidation technique for the simultaneous determination of total C and its radioactivity in soil and plant material, using a heating block and digestion tubes, has been described by Amato (1983). This procedure was used for determination of total C in the various soil extracts (including the 0.5 M $NaNO_3$ resin elutant) and the soil residual fraction. A modified digestion tube apparatus incorporating a CO_2 trap was used as described by Sparling *et al.* (1991). The digestion mixture was concentrated H_2SO_4 (60 ml), 80% H_3PO_4 (40 ml) and $K_2Cr_2O_7$ (10g). Three ml of various soil extracts (i.e. 0.5 M $NaNO_3$, 0.1 M NaOH and 1 M NaOH) or approximately 0.3 g of the residual (non–alkali soluble fraction) soil were taken in the screw top Kimax glass sample tubes (25×150 mm, 55 ml volume) and digested using 7.5 ml of the digestion mixture at 135 ± 5°C for 1.5 hrs. A 5 ml graduated tube containing 3 ml of 2 M NaOH and 0.2 M Na_2CO_3 was placed on the twisted glass support in the digestion tube for trapping CO_2 evolved.

An aliquot (0.6–1.4 ml) of the trapping solution was taken for titration against 0.1 M HCl to determine the residual NaOH, after first precipitating out the carbonates by adding 25 ml of 10% $BaCl_2$, using phenolphthalein as indicator.

Initially, recovery of C was checked by the dichromate digestion procedure using standard soil (5.2% C) and plant (49.8% C) samples. Total C in the standard soil and plant samples was determined by combustion using a Leco FP–2000 CNS analyser. Dry, ground, standard soil (between 0.25 and 0.35 g), or plant material (15–20 mg) was added in

triplicate to the bottom of the digestion tube and digested as per the procedure described above. Experiments to check the cap seals of the Kimax digestion tube were then undertaken. Carolyn Hedley (Pers. Comm.) reported that the use of new Neoprene seals prevented CO_2 losses. The original Kimax seals were compared in terms of the C recovery with new Neoprene seals in the screw cap, and with the new Neoprene seal placed over the original.

Glucose ($C_6H_{12}O_6$) was used as an organic C standard to standardise the method of estimation of total C in the resin elutant and other alkaline soil extracting reagents used during the C fractionation procedure. Stock solution of glucose (1000 ppm C) was prepared in different reagents (0.5 M $NaNO_3$, 0.1 M NaOH and 1 M NaOH). Standard amounts of glucose (i.e. 3000 ppm C, 1000 ppm C and 250 ppm C) were added in triplicate to the digestion tubes by taking 3 ml, 1 ml and 0.25 ml respectively of the stock solution of glucose in different reagents. The volume of the samples was adjusted to 3 ml using corresponding blank (without glucose) reagents and digested with 7.5 ml of the digestion mixture as per the procedure described by Sparling *et al.* (1991).

3.2.5.1 Chloride (Cl^-) interference in the resin extract

The presence of chloride in the soil would result in the extraction of Cl^- ions onto resins during resin extraction and upon elution Cl^- can appear in the resin elutant (see Section 4.2.5). Quinn and Saloman (1964) reported that the presence of chloride in the extracts during dichromate oxidation of C caused the reduction of $K_2Cr_2O_7$ (i.e. 1 milli–equivalent of chloride is equivalent in reducing power to approximately 3.5 mg C). They have mentioned that addition of HgO, as a chloride complexing agent (i.e. formation of non–ionised $HgCl_2$) would correct chloride problem. In this study, during standardisation of the dichromate digestion procedure for total C determination (see Section 3.2.5), a chloride standard correction curve (concentrations of Cl^- 0, 0.5, 1, 2, 3, 4 milli–equivalent) was developed using a standard glucose solution (4000 ppm C was used). This curve was used to correct C concentrations in samples having known concentrations of Cl^- ion present. The chloride concentration in a sample was determined using a Corning 925–chloride analyser (Corning, 1980).

3.3   RESULTS AND DISCUSSION

3.3.1   *Calibration of methionine and sulphanilamide organic S standards against standard K_2SO_4 solution*

The $NaHCO_3$ fusion technique is generally used for total S estimation from plant and soil samples. When employed for the estimation of amount of S present in the organic S standards (sulphanilamide and methionine), a recovery of about 100% of organic S was recorded (Table 3.1). This method confirmed the expected amount of S present in these organic S compounds for subsequent use as standards and for evaluation of NaOBr digestion techniques.

Table 3.1: Sulphur recovery from organic S compounds dissolved in alkaline extracting reagents by the $NaHCO_3$ fusion technique.

Conc. (ppm) of organic S standard	Organic S compounds	ppm of S recovered	
		After fusion	No digestion
5	Sulphanilamide	5.1 (0.1)[#]	BD[*]
	Methionine	5.2 (0.2)	BD
8	Sulphanilamide	8.0 (0.2)	BD
	Methionine	8.2 (0.3)	BD

[*]*BD means "Below detection limit";* [#]*Values in brackets are SE of mean*

When undigested alkaline (sulphanilamide and methionine) solutions were analysed using automated HI reduction (Dean, 1966), no S was detected (Table 3.1) confirming no sulphate contamination of the standards.

3.3.1.1   *In situ production of NaOBr*

<u>*Method 1*</u>

The three NaOBr digestion methods employed to test the measurements of organic S standards in alkali solutions were not able to recover the full concentration of organic S. Method 1 gave between 73% and 80% recovery for both concentrations of organic S

(sulphanilamide) present in two different concentrations of alkali reagents. However, for methionine, which has been found more difficult to oxidise (Shaw, 1959; Blancher *et al.*, 1965), recoveries were only 18–26% of the organic S present (Table 3.2). *In situ* produced NaOBr was not capable of breaking the strong C–S bond of methionine at 200°C to give quantitative recovery of the inorganic S formed by this wet oxidation procedure (Melting point of sulphanilamide is 165°C and that of methionine is 272°C).

Methods 2 and 3

It was initially assumed that the probable reason for the low recovery in Method 1 was incomplete formation of NaOBr. Therefore, Method 2 was attempted by resuspending the alkaline residues, adding more bromine and then heating again to dryness. This method

Table 3.2: Sulphur recovery by different methods of *in situ* production of NaOBr.

Methods	Organic S standard	NaOH conc. (moles litre^{-1})	Removal time (h) for funnels	ppm of organic S in the final solution	ppm of S recovered	% recovery
Method 1	Sulphanilamide	0.1	–*	5	3.7 (0.2)[#]	74
			–	8	5.9 (0.4)	74
		1	–	5	4.0 (0.2)	80
			–	8	5.8 (0.1)	73
	Methionine	0.1	–	5	0.9 (0.1)	20
			–	8	1.6 (0.2)	20
		1	–	5	1.3 (0.1)	26
			–	8	2.1 (0.6)	26
Method 2	Sulphanilamide	0.1	–	5	4.0 (0.2)	80
			–	8	6.5 (0.2)	81
		1	–	5	4.3 (0.3)	86
			–	8	6.4 (0.2)	80
Method 3	Sulphanilamide	0.1	1	5	4.0 (0.2)	80
			2		4.1 (0.2)	82
			3		3.8 (0.2)	76
			4		4.2 (0.2)	84
			1	8	5.9 (0.1)	74
			2		6.0 (0.3)	75
			3		6.0 (0.2)	75
			4		6.2 (0.4)	78

– means "No funnels"; [#]Values in brackets are SE of mean.

(Method 2, Table 3.2) gave slightly better recovery of S (80–86%) than Method 1 (73–80%). Similarly Method 3 only recovered 74–84% of the S in sulphanilamide even though samples were digested for longer period of time. This indicates that idea of carrying out *in situ* NaOBr production was unsuitable, as NaOBr was probably not formed efficiently.

One hundred percent of S was recovered when a known amount of S from K_2SO_4 was digested with different concentrations of NaOH in the presence of bromine (Table 3.3). Neither the alkaline nature of solutions nor the ethanol was the cause of low recovery of organic S by *in situ* NaOBr production procedure. Sulphur recoveries were similar from sulphanilamide dissolved in either water or ethanol, but as sulphanilamide concentration increased from 5 to 8 ppm, the *in situ* digests recovered less total S (Table 3.4).

Table 3.3: Sulphur recovery from K_2SO_4 digestion in NaOBr at 160°C.

NaOH conc. (moles litre^{-1})	ppm of inorganic S (source: K_2SO_4) in the final solution	ppm of S recovered	% recovery
0.1	5	5.1 (0.2)[#]	102
	8	8.1 (0.2)	101
1	5	5.1 (0.2)	102
	8	8.0 (0.1)	100

[#]*Values in brackets are SE of mean*

Table 3.4: Sulphur recovery from digests (Method 1) of sulphanilamide dissolved in ethanol or hot water.

NaOH conc. (moles litre^{-1})	Conc. (ppm) of organic S standard	Solvent used	ppm of S recovered	% recovery
0.1	5	Ethanol	4.4 (0.3)[#]	88
		Hot water	4.5 (0.1)	90
0.1	8	Ethanol	6.1 (0.1)	76
		Hot water	6.5 (0.3)	81

[#]*Values in brackets are SE of mean*

3.3.1.2   Tabatabai and Bremner wet oxidation technique with some modifications

Both modified wet oxidation procedures (i.e. addition of 4 ml of NaOBr in one and in two equal instalments and gradually raising the temperature to 260°C) gave between 98–100% recoveries of S from methionine and sulphanilamide (Table 3.5).

Table 3.5: Sulphur recovery by two different addition methods of freshly prepared NaOBr.

Organic S compounds	Methods of NaOBr addition	ppm of organic S	ppm of S recovered	% recovery
Sulphanilamide	One instalment	5	4.9 (0.2)[#]	98
		8	8.0 (0.2)	100
	Two instalments	5	5.1 (0.1)	102
		8	7.9 (0.2)	99
Methionine	One instalment	5	4.9 (0.1)	98
		8	8.1 (0.2)	101
	Two instalments	5	5.0 (0.2)	100
		8	8.0 (0.3)	100

[#]*Values in brackets are SE of mean*

In the original method (Tabatabai and Bremner, 1970), the use of 3 ml of freshly prepared NaOBr for digestion of soil samples containing 10–50 µg of S, leaves brown colours in the digested alkali soil extracts (containing >100 µg S). This leads to doubt about the completeness of oxidation of the organic S extracted in the alkali soil extracts. With 4 ml of NaOBr, the colour of the digested residue was a grey–white. Phimsarn (1991) also observed many incomplete digestions and variable results for plant samples digested using 3 ml of NaOBr and suggested use of 4–8 ml of freshly prepared NaOBr depending on the size of the sample. However, the procedure is laborious and involves addition of NaOBr in 4 instalments during the digestion process. In the present study (see Section 3.2.3.3), the two different methods of adding NaOBr during the digestion process are not different in terms of quantitative recovery of organic S (Table 3.5). Therefore, the use of NaOBr in one instalment and gradually raising the temperature to 260°C was recommended for digestion of alkali soil extracts.

3.3.2   *A wet digestion procedure for C estimation*

3.3.2.1   *Calibration of neoprene seal against original seal in Kimax tube screw cap*

When standard soil and plant samples were digested by the dichromate digestion procedure using Neoprene seals (see Section 3.2.5), it was found to give better (99–100%) recovery of C from both standard soil and plant samples than if the original Kimax seals were used (78–85%) (Table 3.6). The % C recovery was between 94–98% when both

original and Neoprene seals were used together (Table 3.6). These results confirmed the results of Carolyn Hedley (Pers. Comm.) that Neoprene seal should replace the original seal in the screw cap to ensure a better recovery of C by this digestion procedure.

Table 3.6: Calibration of neoprene seal against original seal in the screw cap of the Kimax digestion tube for better C recovery

Seal in the screw caps of the Kimax digestion tube	Materials used as standard	% C in the standard samples	Total C (in %) recovered	% recovery
Original seal	Plant	49.8	38.8 (1.9)[#]	78
	Soil	5.2	4.4 (0.3)	85
Neoprene seal	Plant	49.8	49.4 (0.3)	99
	Soil	5.2	5.2 (0.1)	100
Neoprene and original seal	Plant	49.8	48.8 (0.7)	98
	Soil	5.2	4.9 (0.2)	94

[#]*Values in brackets are SE of mean*

3.3.2.2   *Applicability of the digestion procedure to soil extracts*

The Amato (1983) C digestion method (as described in Sparling *et al.*, 1991) recovered between 97–100% C from different extracting reagents containing different

Table 3.7: Carbon recovery from glucose dissolved in different extracting reagents by the dichromate digestion procedure

Reagents used in the fractionation	Glucose (ppm C) conc. in the aliquot	ppm of C recovered	% recovery
0.5 M NaNO$_3$	250	243 (4.2)[#]	97
	1000	998 (2.2)	100
	3000	2970 (15.1)	99
0.1 M NaOH	250	245 (3.9)	98
	1000	973 (10.1)	97
	3000	2995 (5.5)	100
1 M NaOH	250	248 (2.7)	99
	1000	982 (8.0)	98
	3000	2932 (12.5)	98

[#]*Values in brackets are SE of mean*

concentrations of glucose (Table 3.7).

3.3.2.3   Correction in the measured C for chloride interference

The presence of increasing concentration of Cl⁻ ions in a soil extract sample caused a linear decrease in the amount of HCl used to neutralise residual NaOH (Figure 3.2) in the titration finish to the C determination (Section 3.2.5). A recovery of 99.5% C was obtained in samples having no chloride present. This standard curve was then used to estimate chloride interference from the Cl⁻ ion concentration in the 0.5 M $NaNO_3$ resin elutant (Section 4.2.5) and the 5% NaCl soil extract (Section 6.2.8).

The graph shows: y-axis labeled "ml of 0.1 HCl used" ranging from 0 to 16, x-axis labeled "Chloride conc. (milli-equivalent)" ranging from 0 to 4. Line equation: $y = -3.17x + 14.91$, $R^2 = 0.998$.
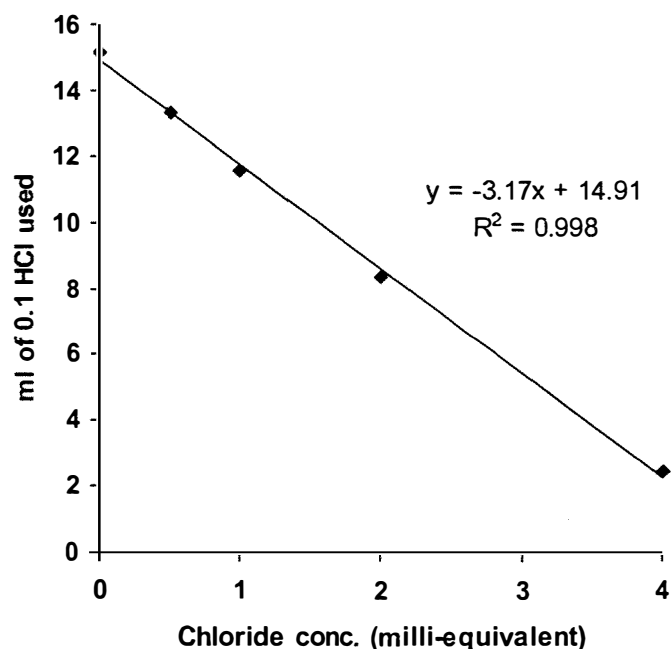
Figure 3.2: Relationship between volume (ml) of 0.1 M HCl used during titration of the unused NaOH and concentration of chloride present in the solution to be analysed for total C.

3.4   CONCLUSIONS

Digestion and analysis of organic S standard solutions of methionine and sulphanilamide have indicated that for analysis of organic S in alkaline extracts the

modified method of NaOBr oxidation by Tabatabai and Bremner (i.e. the use of 4 ml of freshly prepared NaOBr in one instalment) is recommended. $NaHCO_3$ fusion technique also provides completed oxidation of these standards. Standard solutions of methionine (being most resistant to oxidation) are most suitable for quality control standards in determination of total S in soil and plant samples.

The modified dichromate digestion procedure of Amato (1983) for C estimation from soil and plant (as described in Sparling *et al.*, 1991) works well for C estimation from soil extracts. However, Neoprene seals should replace original Kimax digestion tube cap seals to ensure a good recovery of CO_2. Any chloride interference in the extract from soil during dichromate digestion should be corrected using a standard chloride correction curve.

CHAPTER 4   CHEMICAL FRACTIONATION TO CHARACTERISE CHANGES IN SOIL SULPHUR AND CARBON

4.1   INTRODUCTION

Management practices involving different cultivation and fertiliser regimes alter the dynamics of organic matter input and turnover in soils and influence the supply of plant–available nutrients (Murata *et al.*, 1995; Murata and Goh, 1997). Decreases in soil organic matter (SOM) caused by intensive tillage and/or reduced inputs of crop residues are well-documented (Smith and Elliott, 1990). Indicators of both SOM content and quality (form) are needed because a large investment in time, restorative crops, fertiliser and/or manure are required to rebuild SOM reserves (Chater and Mattingly, 1980; Sparling *et al.*, 1992; Paustian *et al.*, 1997b; Reeves, 1997). In specific soil types, soil productivity and the economics of farming operations are influenced by both the amount and quality of the SOM (Scrimgeour and Shepherd, 1998). This influences land value, the long–term sustainability of certain farming operations and equity between generations of landholders. Issues such as these have stimulated interest in the development of SOM quality indicators (Doran, 1996).

Whereas the amount of organic matter in a particular soil type may be a suitable indicator of the important biophysical role played by SOM (Syers and Craswell, 1995), indicators of the nutrient availability (quality) of SOM are also required. Assessment of nutrient availability is also important because soil degradation processes such as excessive cultivation affect the nutrient status of soil. The greatest losses are usually in those nutrients (N and S) that are bound in SOM and are not readily retained by the mineral soil fraction (Logan, 1990; Reeves, 1997). Data from long–term cropping experiments have repeatedly shown that continuous cultivation depleted soil organic C, N and S, and reduced soil quality compared to native vegetation, regardless of cropping system (Greenland and Nye, 1959; Mclaren and Swift, 1977; Odell *et al.*, 1984; Horne *et al.*, 1992; Sparling *et al.*, 1992; Francis and Knight, 1993; Riffaldi *et al.*, 1994; Campbell *et al.*, 1996a, 1996b). Changes in the quantity of SOM are sometimes difficult to detect because of the relatively large amounts of organic matter, which obscures small changes (Gregorich, 1996). Several

fractions of organic matter have been identified as having a relatively rapid turnover including microbial biomass, mineralisable C and N, and light fraction organic matter (Gregorich, 1996). These active fractions of organic matter may hold potential as more sensitive indicators of SOM change because they change more rapidly than total SOM.

Most of the S ($> 90\%$) in surface horizons of well–drained agricultural soils is present in organic forms (Nguyen and Goh, 1994) and a close relationship is reported between organic S and C content of soil (Nguyen and Goh, 1994). The release of S from organic forms is important for both the short– and long–term supply of S, although short–term S cycling involves only a small fraction of total organic S (Freney, 1986; Chapman, 1987). It is therefore, essential to identify the more active fractions to obtain a better understanding of factors determining plant availability and potential loss of S from soils.

Many approaches have been tried to characterise the SOM into labile fractions that supply S to plants (reviewed in Chapter 3) but they were not successful in characterising labile 'organic S and C' as a single identity. This chapter describes the evaluation of a modified alkaline soil P fractionation procedure of Hedley *et al.* (1982) for use in characterising S and C fractions. The objective is to identify organic S and C fractions that are sensitive to changes induced by landuse practices. Variance in the size of these fractions could be useful indicators of SOM quality (Doran, 1996; Gregorich, 1996). This fractionation procedure makes use of alkali as a SOM extractant. However, numbers of workers have reported the use of milder alkaline extractants (Swift *et al.*, 1988; Keer *et al.*, 1990). The use of milder extractants can avoid the problem of degradation of humic substances and modification of organic S forms. But milder extractants often result in the extraction of relatively small proportions of the total organic S present in the soil leaving a major portion of organic S in the insoluble organic matter pool (Eriksen, 1997). According to Stevenson (1982), for certain investigations i.e. characterisation of organic structures, a mild extractant is definitely preferred; for others (such as fractional elemental recovery), a more complete extraction with alkali may be desired even though this will be achieved at the expense of some changes in the organic matter. As a rule, extraction of soil with 0.1 M NaOH or 0.5 M NaOH leads to the recovery of approximately 75–80% of soil organic matter (Stevenson, 1982; Schnitzer and Schuppli, 1989). The amount of organic matter extracted from soil with alkali increases with time of extraction, a result that may be caused by slow depolymerisation of high molecular weight complexes (Stevenson, 1982).

Murata and Goh (1997) sequentially extracted soil samples for labile and stable SOM using cold water, hot water, acid mixtures and alkalis but the procedure is laborious and does not give a labile fraction of SOM as a single identity. In the present study, extraction of ionic forms of S and C (i.e. inorganic $SO_4^=$, $CO_3^=$, HCO_3^- and simple esters) by the ion exchange resins was followed by sequential extractions with alkali of increasing strength, which may give S and C of decreasing lability in a manner similar to the phosphorus fractionation of Hedley *et al.* (1982).

The objective of this study was to evaluate the ability of a modified soil P fractionation procedure of Hedley *et al.* (1982) to characterise changes in soil S and C organic fractions on a range of pasture and cropping soils with different management histories.

4.2   MATERIALS AND METHODS

Soil samples were obtained from three experimental sites representing a range in S fertiliser application (pasture soils, Mt.Thomas), period of cultivation (Kairanga) and pasture restoration crops (Wakanui) (Table 4.1). A detailed description of these sites, experimental treatments, management history and soils is presented elsewhere (Giddens *et al.*, 1995; Francis *et al.*, 1999; Shepherd *et al.*, 2001). A brief description of these soil types, soil sampling and preparation, treatments and soil texture pertinent to this work is given below.

4.2.1   Description of field sites

4.2.1.1   Mt. Thomas site (fertilised pasture)

The fertiliser treatments at this initially S deficient permanent pasture site (Craighead *et al.*, 1990; Giddens *et al.*, 1995) involved sulphate S applied as single superphosphate (SSP) or SSP/gypsum, a mix of sulphate and elemental S in 'sulphur super extra' (SSX) and elemental S^0 granulated with bentonite applied tri–annually over a 6 year period (Table 4.1). There were four replicates of each fertiliser treatment per block. Soil samples (0–7.5 cm depth) were collected with a 2.5 cm diameter probe by taking 20 soil

Table 4.1: Soil classification and treatment description for the Mt. Thomas, Kairanga and Wakanui sites.

Soil name (Application)	Soil group[+]		Treatment	Treatment code	Application rate $(kg\ ha^{-1}\ application^{-1})$	
	New Zealand (Genetic)	USDA			$SO_4^{=}$	S^0
Mt. Thomas silt loam (Tri–annual)	Allophanic brown soil (High country yellow brown earth)	Udic Dystrochrept	Control	Ct	0	0
			Single superphosphate/gypsum	SSP/G	28	0
			Bentonitic S^0	B28	0	28
			Sulphur super Extra	SSX	19	37
			Bentonitic S^0 56	B56	0	56
			Sulphur super extra (reapplied)	SSXr	27	91
Kairanga silty clay loam (Annual)	Typic orthic gley soil (Gley recent soil)	Typic Endoaquept	Permanent pasture	PP	22[*]	0[*]
			Maize 4 years	M4Y	35[*]	0[*]
			Maize 11 years	M11Y	NA[*]	NA[*]
			Barley 30 years	B30Y	NA[*]	NA[*]
			Maize 11 years followed by 10 years of pasture	M11Y+P10Y	44[*]	0[*]
Wakanui silt loam (Annual)	Immature pallic soil (Yellow grey earth)	Udic Ustochrept	Annual ryegrass grazed and conventionally cultivated	ARc	17	0
			Annual ryegrass grazed and direct drilled	ARd	17	0
			Perennial ryegrass grazed	PRg	17	0
			Perennial ryegrass mown	PRm	17	0

NA = Not available.

[*]The S regimes given are those applied only during the sampling year. Although S has been previously applied in these treatments either as ammonium sulphate or as SSP, the data for these applications were not available from the farmers.

[+]Hewitt, 1992.

cores from each replicate. All S treatments produced marked growth responses in legume yield in the first four years of the trial (Craighead *et al.*, 1990).

4.2.1.2   *Kairanga site (short– to long–term cultivation)*

This site involved a permanent pasture (PP) regime and a number of continuous cultivation systems with different times since last in pasture i.e. 4 years of maize cultivation (M4Y), 11 years of maize cultivation (M11Y) and 30 years of barley cultivation (B30Y). Also included was a site that had been ploughed for 11 years of maize cultivation and then brought back to pasture for 10 years (M11Y+P10Y) (Table 4.1). Soil samples (from each paddock) were taken from 0–10 cm depth of soil using a 2.5 cm diameter probe.

4.2.1.3   *Wakanui site (short–term pasture cropping)*

This site involved a short term mixed cropping trial, which included a range of permanent pastures, annual pastures and arable crops started in 1989 on a Wakanui silt loam soil after 11 years of arable cropping (Francis *et al.*, 1999). Four treatments providing differences within and between the annual and perennial pastures (Table 4.1), replicated four times, were included in the present study because different crops in the trial confound any other comparisons. At the sixth year, soil samples (0–5 cm depth) were collected by taking 10 cores from each replicate using a 2.5 cm diameter probe.

4.2.2   Preparation of soil samples

Organic material such as visible live and dead roots was removed from the soil samples collected from all the experimental sites. The samples were sieved (< 2 mm) and air–dried at 30°C ± 2°C. The air–dried samples were then finely ground for 10 seconds using a ring grinder (Rocklabs, Auckland, New Zealand) for fractionation and other soil analysis. Unless otherwise stated all field soil samples were analysed in duplicate in the various extractions described below.

4.2.3   A sequential soil S and C fractionation technique

The procedure used to sequentially fractionate soil S and C is summarised in Figure 3.1.

4.2.4   Analysis of S in different fractions

Detailed descriptions of the S analytical methods in different fractions are mentioned in Chapter 3. Resin–S was measured using autoanalyser (Dean, 1966). The modified wet oxidation technique of Tabatabai and Bremner (1970) described in Section 3.2.3.3 (i.e. NaOBr addition in one instalment) was used to digest solid phase non–exchangeable organic S extracted in various alkali soil extracts. Total S in the soil and residual fraction was measured using the $NaHCO_3$ fusion method of Steinberg et al. (1962).

4.2.5   Analysis of C in different fractions

Total C in soil samples was determined by combustion using a Leco FP–2000 CNS analyser. The oxidation and digestion technique of Amato (1983) as described by Sparling et al. (1991) was used to determine C in the various soil extracts and the residual soil (see Section 3.2.5).

Chloride interfered during the dichromate digestion of resin extracts from Kairanga soil for total C determination. The interference effect from chloride was determined to be linearly related to chloride concentration (see Section 3.3.2.3) by spiking a standard glucose solution with different concentrations of chloride (see Section 3.2.5.1). The chloride interference was corrected using the linear relationship given in Figure 3.2.

4.2.6   Statistics

The significance of relationships between soil S and C fractions was determined by the simple linear regression model in $SAS^®$ for $Windows^{TM}$ using PROC GLM (SAS Institute, 1990). The significance of differences between treatment means in replicated experiments (i.e. Mt. Thomas and Kairanga) was tested (at 5% level of significance) using the $SAS^®$ GLM procedure (SAS Institute, 1990) to conduct analysis of variance, and the means were separated by Duncan's multiple range comparison procedure.

4.3   RESULTS

The chemical fractionation technique recovered between 90 and 115% of the total soil S in all samples analysed and between 102 and 110% of the total soil C. Recoveries greater

than 100% presumably result from either summation of errors in the fractionation of total S or underestimation of soil total S content by $NaHCO_3/AgO$ oxidation.

4.3.1 Changes in soil S and C

4.3.1.1 Mt. Thomas site (fertilised pasture)

Total soil S and C increased with increasing rates of fertiliser S application (Mt. Thomas data, Figure 4.1). This relationship ($r = 0.43$) between total soil S and C with increasing rates of S application, however, was not significant.
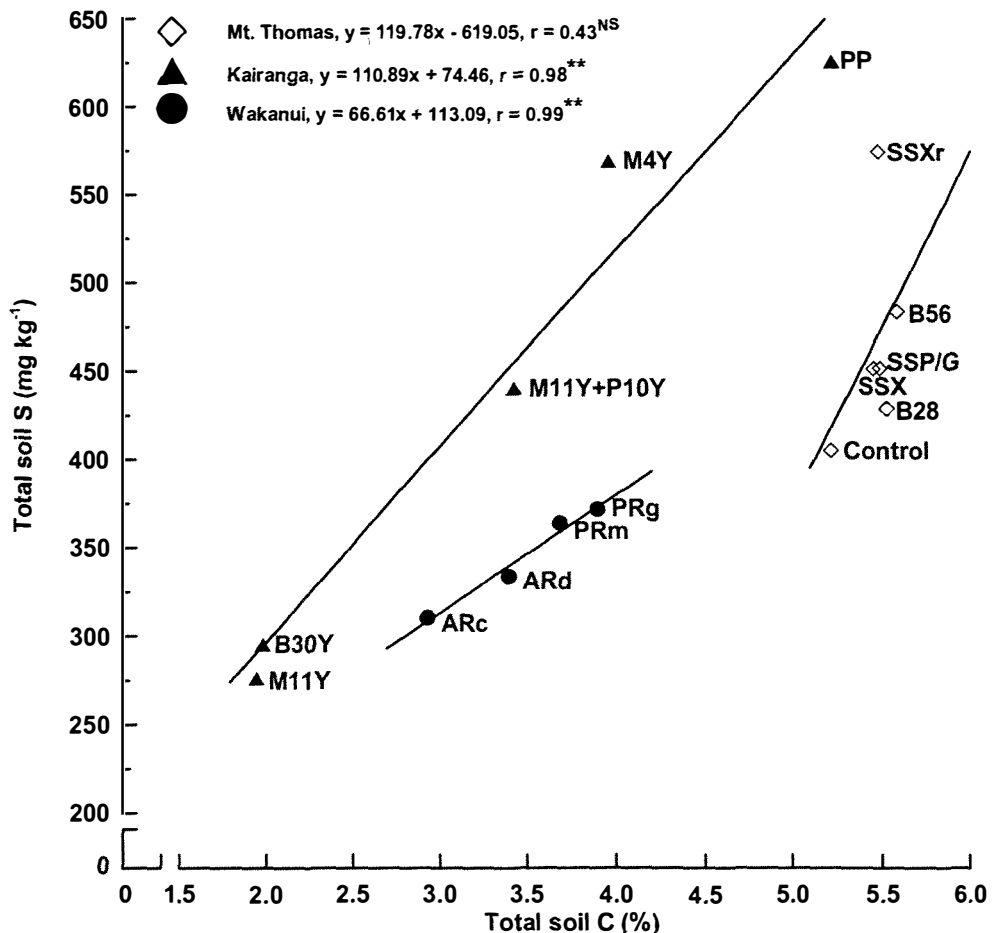
Figure 4.1: Relationship between total soil S and soil C as influenced by different management practices. The total soil S at Wakanui site is the sum of all the S fractions recovered by the sequential fractionation procedure. *Treatment codes are given in Table 4.1.*

Sulphur in all the fractions increased with the increase in total soil S from increasing amounts of fertiliser application (Figure 4.2). The rate of increase in 0.1 M NaOH–S was significantly (Section 4.2.6) greater ($P < 0.001$) than that in residual–S and both were significantly ($P < 0.001$) greater than increases in resin–S and 1 M NaOH–S. One–tenth molar NaOH extracted a major portion (57–66%) of the total soil S. Resins extracted
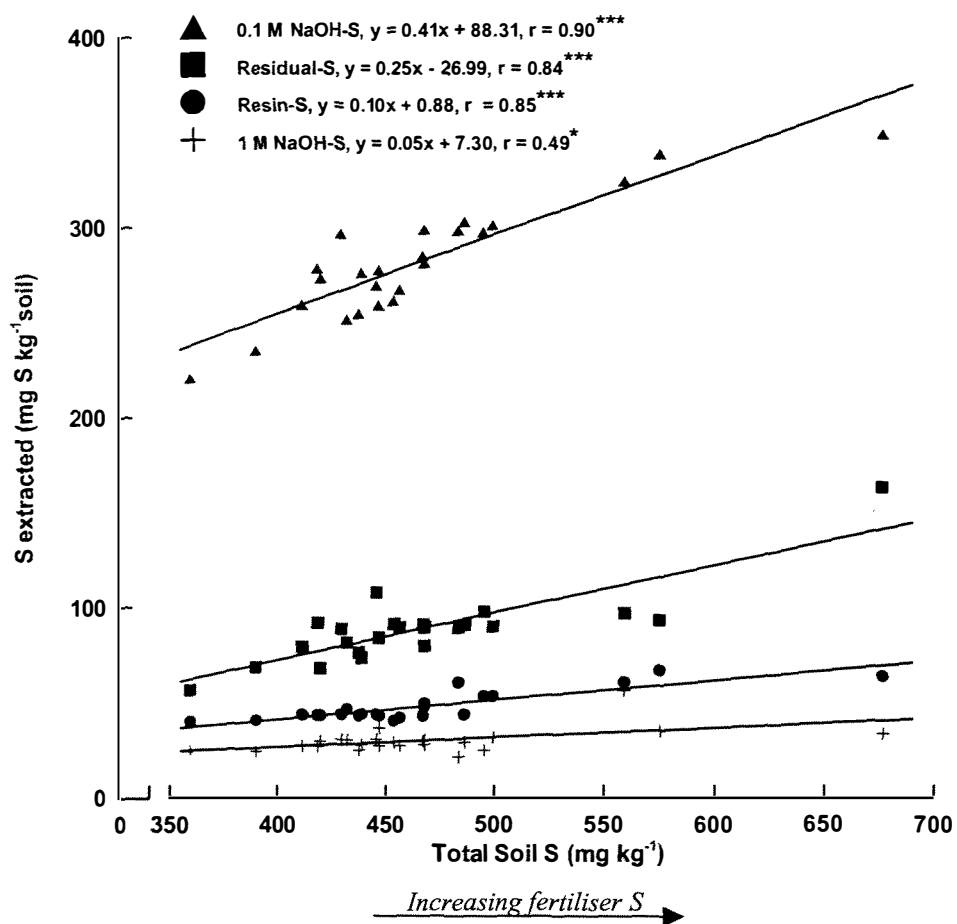
Figure 4.2: Changes in amounts of S fractions as total soil S increases from increasing amounts of fertiliser application to the permanent pasture soil (Mt. Thomas) over a six–year period.

between 9–13% and 1 M NaOH extracted between 5–11% of the total soil S. Between 17–27% remained unextracted as residual–S.

There was a greater accumulation of total S in the SSXr treated soil followed by soil treated with B56, SSX, B28, SSP/G and Ct (see Table 4.1 for treatment codes), indicating a better conservation of S^0 into organic S or an accumulation of residual S^0. When applied at

the same rate, the different forms of fertiliser applied had no significant effect on any particular S fraction, except resin–S. Treatment B56 increased resin–S significantly above that obtained from SSX treatment (Figure 4.3) despite both the sources supplying equal amounts of S (Table 4.1).

Treatment SSXr (S applied at the rate of 236 kg ha^{-1} over 6 years) had significantly higher resin–S and 0.1 M NaOH–S than the other treatments. The amount of S that remained in the residual fraction was significantly higher in the SSXr treatment compared to the control (Figure 4.3). This may be attributed to the unoxidised S^0 that remained from re–topdressing. The 1 M NaOH–S was not influenced significantly by the various fertiliser treatments (Figure 4.3).
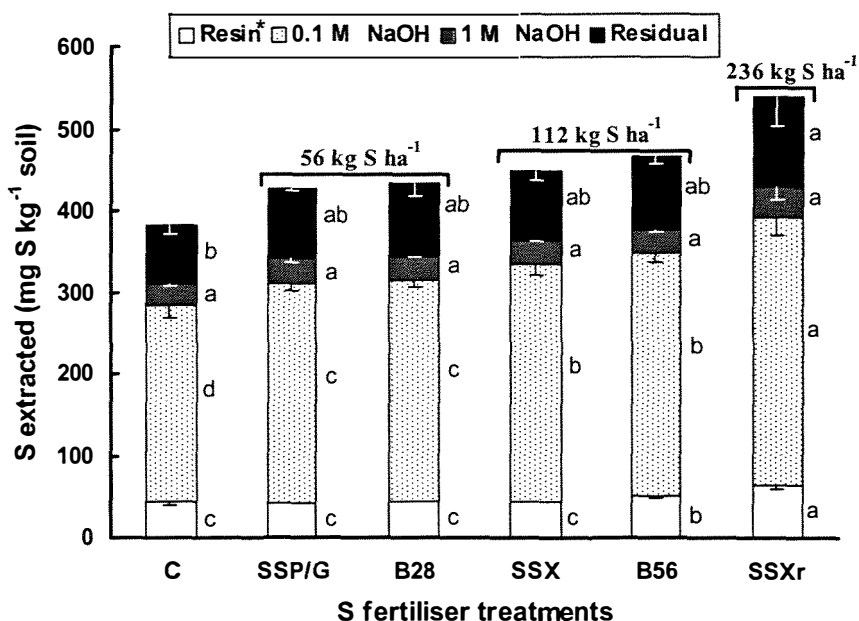
Figure 4.3: Effect of S fertiliser application to pasture on different S fractions in the 0–7.5 cm depth of Mt. Thomas soil. *Treatment codes are given in Table 4.1. Error Bars: SE of treatment mean.*

Comparing across fertiliser treatments, fractions in the figure followed by same letter are not significantly different at P<0.05.

4.3.1.2   Kairanga site (short– to long–term cultivation)

The permanent pasture had greater total soil S and C than the cultivated soils. Paddocks that were cultivated longer had considerably lower total soil S and C (Figure 4.1). The decrease in soil S was significantly ($P < 0.01$) related to decrease in soil C ($r = 0.98$). There was a greater depletion of total soil S and C in the M11Y and B30Y treatments than in the M4Y treatment. When the cultivated soil (M11Y) was brought back to pasture for ten years (P10Y), the soil S and C accumulated again (compare M11Y with M11Y+P10Y) (Figures 4.1 and 4.4).
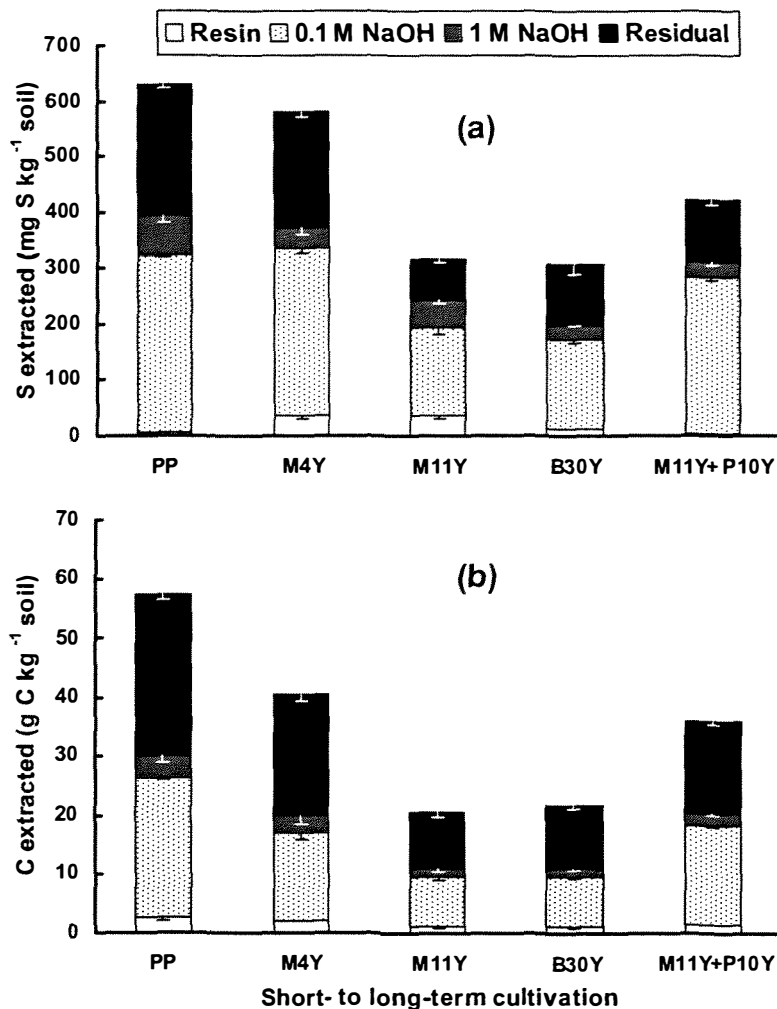
Figure 4.4: Effect of short– to long–term cultivation on different (a) S and (b) C fractions in the 0–10 cm depth of Kairanga soils. *Treatment codes are given in Table 4.1. Error Bars: SE of sample mean.*

The major portion of total soil S was found in 0.1 M NaOH–S (49–66%) followed by residual–S (23–38%), 1 M NaOH–S (6–15%) and resin–S (1–12%) in the 0–10 cm depth of the Kairanga soils (Figure 4.4a). The PP regime had the greatest amounts of soil S in all the fractions (except resin–S) (Figure 4.4a). Short–term cultivation (M4Y) caused 0.1 M NaOH–S, 1 M NaOH–S and residual–S to decrease. However, resin–S increases in the M4Y and M11Y treatments, and decreases in the B30Y treatment and when the M11Y site was brought back to 10 years of pasture (M11Y+P10Y). The amount of 0.1 M NaOH–S in the medium– to long–term cultivation treatments (M11Y or B30Y) was approximately 50% of that in the PP site and increased to 88% of the initial level (as was found in the PP site) when the cultivation site was restored back to pasture (M11Y+P10Y). Residual–S decreased to one–third in the M11Y and to half in the B30Y treatments as compared to the PP treatment. Residual–S also increased when the cultivated soil (M11Y) was brought back to pasture (M11Y+P10Y) but the increase was small (i.e. increased to 47% of the initial level in the PP site) compared to the increase in 0.1 M NaOH–S. Trends in the change of 1 M NaOH–S with the change in cultivation duration were irregular (Figure 4.4a). Moreover, 1 M NaOH–S changed little when the cultivation site (M11Y) was brought back to pasture (M11Y+P10Y). The rates of change in both 0.1 M NaOH–S and residual–S with the change in total soil S were similar and were significantly greater ($P <$ 0.01) than the rates of change in resin–S and 1 M NaOH–S (see methods Section 4.2.6).

The major portion of total soil C was recovered in residual–C (46–53%) followed by 0.1 M NaOH–C (38–48%), 1 M NaOH–C (6–8%) and resin–C (5–6%) from the 0–10 cm depth of the Kairanga soil (Figure 4.4b). Cultivation (short– or long–term) of PP caused the C in all fractions to decline. In the short–term (M4Y), the decline was more in 0.1 M NaOH–C and residual–C than in resin–C and 1 M NaOH–C. Medium– to long–term cultivation treatments (M11Y or B30Y) caused the C in all fractions to decrease to about one–third of the C in the native PP phase. One–tenth molar NaOH–C increased twofold in the pasture restoration phase i.e. M11Y+P10Y treatment (Figure 4.4b). Resin–C, 1 M NaOH–C and residual–C also increased in the pasture renovation phase but the increases were small compared to the increase in 0.1 M NaOH–C. The rates of change in both 0.1 M NaOH–C and residual–C were similar and were significantly ($P <$ 0.001) greater than the rates of change in resin–C and 1 M NaOH–C (Figure 4.2).
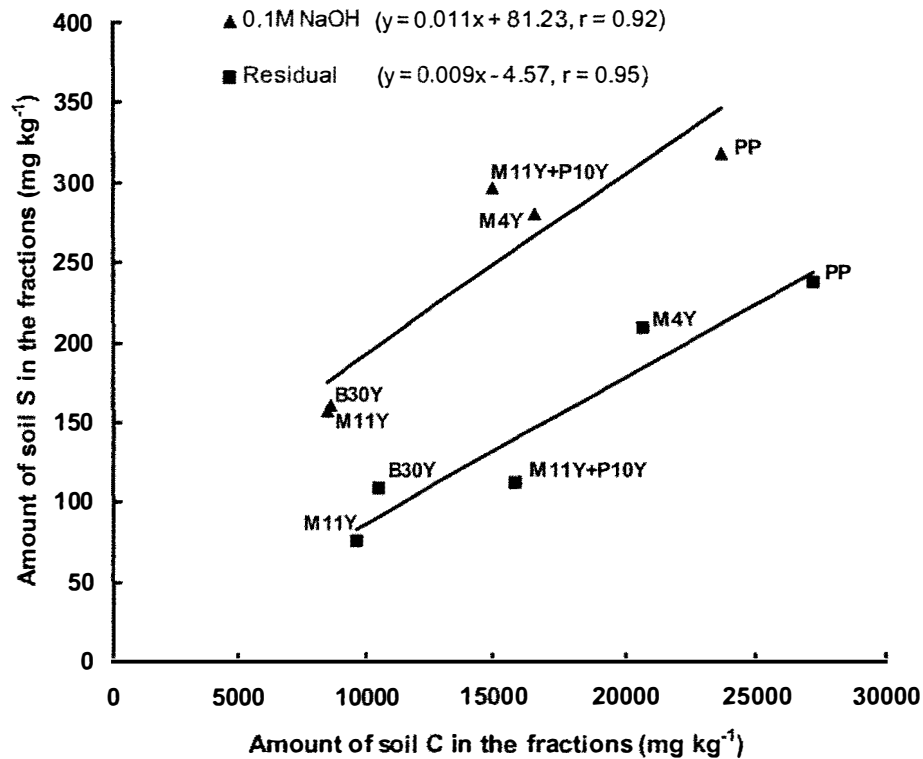
Figure 4.5: Comparison of S and C recovered in 0.1 M NaOH and residual fractions from 0–10 cm depth of Kairanga soils. *Treatment codes are given in Table 4.1.*

The 0.1 M NaOH fraction had a narrow C:S ratio (50–75:1) compared with the wide C:S ratio (96–141:1) of the residual fraction (Figure 4.5) but losses and gains of S and C in both these fractions occurred at the same rate (as indicated by similar slopes in Figure 4.5) with the change in cultivation practices.

4.3.1.3   *Wakanui site (short–term pasture cropping)*

The significant linear relationship between the change in the total soil S and C observed in the long–term cropped Kairanga soil was also observed in the short–term cropped Wakanui soil (Figure 4.1). The Kairanga soil, however, has a narrower C:S ratio (67–83:1) compared with the wider C:S ratio (93–105:1) of the Wakanui soil (Figure 4.1). The PRg soil had greatest amount of the total soil S and C and the ARc soil had lowest (see Table 4.1 for treatment codes).

As in the Mt. Thomas (Figure 4.3) and Kairanga soils (Figure 4.4a), the major portion (61–69%) of total soil S was extracted in 0.1 M NaOH (Figure 4.6). Resin–S and 1 M NaOH–S constituted between 1–8% and between 6–16%, respectively. The remaining 20–27% was in residual–S. The sizes of 1 M NaOH–S and resin–S were not influenced significantly by different short–term pasture cropping (Figure 4.6). Within the annual and the perennial pasture treatments, the size of 0.1 M NaOH–S was not significantly different (Figure 4.6). When comparing between the annual and perennial treatments, 0.1 M NaOH–S was significantly lower in the ARc treatment compared to the PRm and PRg treatments.
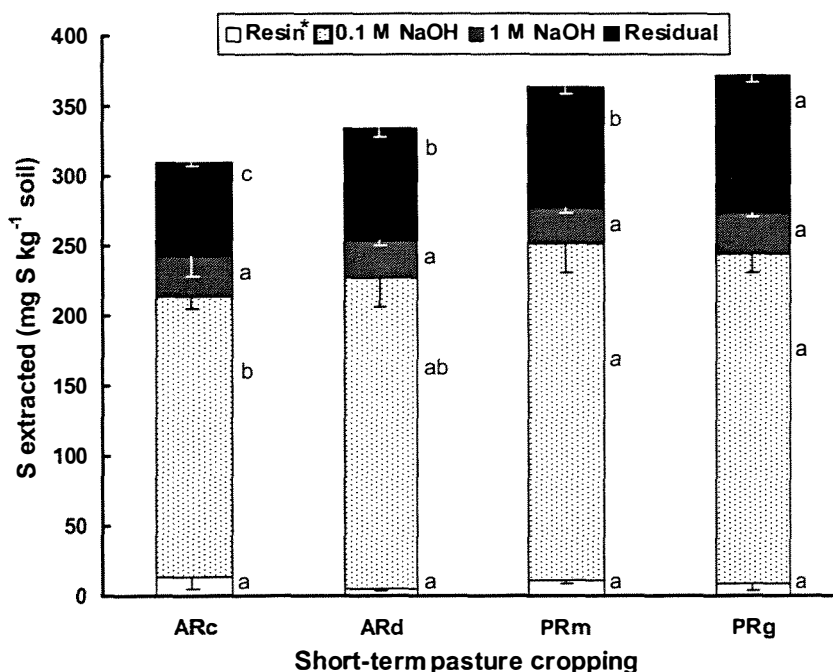
Figure 4.6: Effect of short–term pasture cropping on different S fractions in the 0–5 cm depth of Wakanui soil. *Treatment codes are given in Table 4.1. Error Bars: SE of treatment mean.*

Comparing across different pasture cropping treatments, fractions in the figure followed by same letter are not significantly different at P<0.05.

The amount of 0.1 M NaOH–S in the ARd treatment was not significantly different from all the pasture cropping treatments. The highest amount of 0.1 M NaOH–S was found in the PRm treatment. Residual–S differed significantly within various annual and perennial pasture treatments (Figure 4.6). When comparing between the annual and perennial

treatments, residual S was significantly lower in the ARc treatment compared to the PRm and PRg treatments. Residual–S in the ARd treatment was significantly lower than in the PRg treatment but was not significantly different from the PRm treatment. The greatest amount of residual–S was found in the PRg treatment. The rate of change in 0.1 M NaOH–S with the change in total soil S was significantly ($P < 0.01$) greater than that in residual–S and both were significantly greater ($P < 0.001$) than changes in resin–S and 1 M NaOH–S.

4.4 DISCUSSION

4.4.1 Organic matter accumulation and depletion

Fertiliser application to pasture soils (Mt. Thomas silt loam) over a 6–year period increased the accumulation of S in the soil. This accumulation was significantly higher than that in pastures with no fertiliser input for a similar period (Figures 4.1 and 4.3). There was an increase in the total soil C content as well (Figure 4.1). This result is similar to that reported by Nguyen and Goh (1990). They found significantly greater soil organic C in the irrigated, sheep grazed pastures receiving long–term application of superphosphate fertilisers than in the unfertilised pasture soil. This increase in soil C was associated with an increased biological N_2 fixation (Nguyen and Goh, 1990) as a result of providing S and removing the S constraint to legume growth, which in turn led to an increase in pasture dry–matter and hence greater organic C input to soil (Nguyen *et al.*, 1989; Goh and Nguyen, 1992; Murata *et al*; 1995). In the present Mt. Thomas field trial, S fertilisation markedly increased pasture dry–matter production (Craighead *et al.*, 1990) and this has resulted in most of the applied S accumulating in organic forms with greater accumulation occurring in 0.1 M NaOH–S (57–66%) followed by residual–S (17–27%) and 1 M NaOH–S (5–11%). Resin–S constituted between 9–13% of the total soil S (Section 4.3.1.1). The accumulation of total soil organic S upon application of S fertilisers, or S and P fertilisers, has also been reported by number of workers (Walker *et al.*, 1959; Russell and Williams, 1982; Lewis. et. al., 1987; Perrott and Sarathchandra, 1987; Nguyen *et al.*, 1989; Sakadevan *et al.*, 1993b). After many years of pasture improvement, the rate of accumulation of soil organic S decreases and the mineralisation of accumulated soil organic S becomes a significant source for pasture plants (Walker and Gregg, 1975; Sinclair, 1983; Ghani *et al.*, 1988; Nguyen *et al.*, 1989; Goh and Nguyen, 1992; Sakadevan

et al., 1993b). Accumulated soil organic S, therefore, improves the fertility and economic value of a pasture soil.

Higher amounts of organic S and organic C were present in the PP soils in the Kairanga. However, when the soil was cultivated, a large decrease in organic S and organic C occurred (Figures 4.1 and 4.4), due to enhanced soil organic matter decomposition and decreased organic matter inputs under cropping (Saggar *et al.*, 2000). The enhanced decomposition is considered to be due to mechanical stress breaking organic matter bound aggregates and exposing SOM to microbial decomposers and higher bare soil temperatures, which leads to higher rates of mineralisation. Higher rates of mineralisation invariably lead to increased loss of NO_3^- (McLenaghen *et al.*, 1996; Francis *et al.*, 1998) and $SO_4^=$ ions due to leaching when pasture soils are first cultivated. Murata and Goh (1997) also found that increased period of cultivation and cropping decreased total C and total N. But under PP phase, total C and total N, and aggregate stability of soils increased with time (Davies and Payne, 1988; Jenkinson, 1988; Paustian *et al.*, 1997b). Conversion of long–term arable soil (Wakanui silt loam) to perennial pasture management created a greater build up of total soil S and total soil C than in annually cultivated pasture. The depletion of soil organic matter (i.e., total C and N) under cultivation (or cropping) and its accumulation under pasture were also reported by Stout *et al.* (1981) and Stevenson (1982).

The comparison of unit change in total soil S and C at across all the three sites showed that changes in total soil S content in relation to unit changes in soil C were statistically (see methods Section 4.2.6) similar. This appears to suggest that although management practices change dramatically, change in soil C induces proportional change in soil S and perhaps vice–versa.

4.4.2   *Sulphur and carbon fractions sensitive to changes in management practices*

The fractionation procedure was designed to extract ionic forms of S i.e. inorganic $SO_4^=$–S and simple organic esters by the ion exchange resins (resin–S) and then extract remaining organic S by using reagents of varying strength with the assumption that milder reagent will extract the more labile form (0.1 M NaOH–S) and stronger reagent will extract

the less labile form of S (1 M NaOH–S). The initial concept viewed the unextracted residual–S as a stable organic matter fraction, which would be less susceptible to changes in management practices.

Resin S fraction constituted a small part of total soil S (1–13%). Resin–S was higher in the recently fertilised pasture soils (Mt. Thomas) compared to short– to long–term cultivated (Kairanga) and short–term pasture cropping (Wakanui) soils. This may reflect the fact that the Mt. Thomas (Yellow brown earth) soil has a higher anion exchange capacity (% phosphate retention, see Metson, 1979b) than the Kairanga (Gley recent) or Wakanui silt loam (Yellow grey earth) and more $SO_4^=$ can be retained on the greater hydrous oxide surfaces (Curtin and Syers, 1990).

In fertilised pasture soils (Mt. Thomas), soil S increased with an increase in fertiliser S applications, as expected. As total soil S increased, a greater rate of increase was found in 0.1 M NaOH–S (Figure 4.2; Section 4.3.1.1). Short– to long–term cultivated soils (Kairanga) showed that as soil S decreased, a greater rate of decrease was observed in 0.1 M NaOH–S and residual–S. When the cultivated soil was brought back to pasture after 11 year of arable phase, a greater rate of increase was again found in 0.1 M NaOH–S and residual–S (Figure 4.4a; Section 4.3.1.2). Furthermore, the result from the short–term pasture cropping trial (Wakanui) showed a greater rate of change in 0.1 M NaOH–S and residual–S (Figure 4.6; Section 4.3.1.3).

The results of these three studies suggest that residual–S, which represents the soil organic matter not extracted with alkali (humin fraction), also contains a relatively labile organic S fraction. These results support the findings of McLaren and Swift (1977) who found in their study that residual humin fraction, which is often regarded as being fairly inert, cannot in the long–term be neglected as a potential source of mineralisable S. A sizeable proportion of S mineralised from soils as a result of long–term cultivation was also derived from the residual humin fraction beside the humic and fulvic acid pools (McLaren and Swift, 1977).

Short– to long–term cultivation (Kairanga soil) caused the total soil C to decrease. As total soil C changed, 0.1 M NaOH–C and residual–C showed a greater rate of change (Figure 4.4b; Section 4.3.1.2).

The wide C:S ratio of the residual fraction compared with the 0.1 M NaOH fraction (Figure 4.5) suggests the presence of greater proportions of high molecular weight carbohydrates (probably undecomposed and/or partly decomposed roots and crop residues and dead microbial tissues) in the residual fraction (Hatcher *et al.*, 1985), which could be strongly complexed with mineral matter and were not extracted even with strong alkali reagent. The mild alkali reagent (0.1 M NaOH) could have hydrolysed a greater proportion of protein type S and therefore resulted in narrow C:S ratio of the 0.1 M NaOH fraction. Using "bomb" [14]C enrichment, the residual (humin) fraction has also been shown to contain both old (i.e., stable) and young (i.e., labile) forms of soil organic matter (Goh *et al.*, 1976). It has been demonstrated that the components of a residual fraction include a mixture of stable, high molecular weight soil organic matter forms associated strongly with soil mineral components (metal oxides) thus rendering them non–extractable by acids and alkalis (Goh, 1980; Stevenson, 1982). This fraction may also include cellulose and lignin rich remains of crop residues, old/dead roots and dead soil micro–organisms (Murata and Goh, 1997) that are not alkali soluble (Lowe, 1978). Furthermore, long–term incubation studies conducted on the decomposition of uniformly ^{14}C–labelled ryegrass plant material (over a period of 10 years) by Jenkinson (1977a) and on wheat straw (over a period of two years) by Sallih and Bottner (1988) have shown that about two–thirds of the relatively labile plant C (labelled C) was lost in the first few months (i.e. rapid decomposition as also noticed by Jenkinson (1977b) with different amounts of ryegrass roots and tops), after which decomposition became very much slower (i.e. one-eighth of the labelled C still remained in the soil after 10 years as reported by Jenkinson, 1977b). In this study, in the first four years of maize cultivation (short–term), it was found that residual–C decreased at a similar rate to 0.1 M NaOH–C. This suggests that, in the pasture soil, both fractions probably contained a proportion of material derived from pasture roots and litter materials that decompose in the short–term.

The S and C in 1 M NaOH fraction did not change much with change in management practices and only contributes very small proportions of the total soil S and C. It may represent the more recalcitrant, humified organic matter. The contribution of this fraction to plant available S pool may be small and therefore of little value in predicting S requirement of plants.

4.5   SUMMARY

Pasture phases, renowned for increasing soil organic matter and aggregate stability, increased both soil S and C content when S deficient permanent pasture was fertilised with S fertiliser or when previously cropped soils were returned to pasture.

Studies to characterise soil S and C fractions by the mode of their extraction and then observing the changes in those fractions induced by fertiliser application, and by short– to long–term cultivation and short–term pasture cropping practices, have demonstrated that the fractionation procedure produces chemically characterised soil S and C fractions that vary in their response to change. The 0.1 M NaOH and residual fractions constituted major portions of the total soil S and soil C. Under conditions of organic S and C build up or depletion, both the 0.1 M NaOH and the residual fractions showed significant changes and can be used as indicators of changes in SOM quantity and quality. Residual–S(C) appeared to contain both labile and stable SOM fractions. It was not possible, however, to separate soil organic S and C into fractions of decreasing lability by sequential extractions with alkali of increasing concentration. Further studies are required to investigate the nature of SOM in these two fractions. These studies to evaluate the 'functional nature' of SOM extracted in 0.1 M NaOH reagent and remaining in alkali–insoluble residue are summarised in Chapter 6.

CHAPTER 5   PRELIMINARY EVALUATION OF $^{14}CO_2$ PULSE LABELLING AND SOIL, ROOTS AND SOIL SOLUTION SAMPLING TECHNIQUES TO STUDY THE FATE OF RECENTLY FIXED C IN INTACT SOIL CORES GROWING PREDOMINANTLY RYEGRASS SWARDS

5.1   INTRODUCTION

The SOM fractionation conducted in Chapter 4 confirmed that the pasture renovation phase, at both the Kairanga (short– to long–term cultivation – Section 4.2.1.2) and Wakanui (short–term pasture cropping – Section 4.2.1.3) sites, was successful at raising SOM content. Similar results for New Zealand cropping soils have been reported by Sparling *et al.* (1992), Murata and Goh (1997), and Shepherd *et al.* (2001). Much of the increase in SOM is created by the increased rhizodeposition of C during the pasture phase. The allocation of C to plant roots and conversion to SOM is a major determinant of the size of terrestrial C pool in pastoral ecosystems (Schimel *et al.*, 1995; Paustian *et al.*, 1977a). The release of organic compounds in the rhizosphere of growing plants through exudation, secretion, sloughing and root death can account for up to 40% (or more) of the dry matter produced by plants (Lynch and Whipps, 1990).

In New Zealand, information on C fluxes from roots for legume based pastures is limited (Saggar *et al.*, 1997, 1999; Stewart and Metherell, 1999) and the impact this has on nutrient cycling and SOM dynamics is not well understood (Ross *et al.*, 1995, 1996). Organic matter formed from this C represents the major stores of soil N and S. Quantitative information is required on the rate at which C is translocated to roots and converted to SOM in order to accurately model C and other nutrient cycles. This chapter summarizes the results of a preliminary experiment designed to develop methods for measuring the extent of C rhizodeposition in undisturbed soil cores growing predominantly ryegrass/clover swards. The experiment involves techniques in which the growing plants are pulse–labeled with $^{14}CO_2$. ^{14}C activity is then tracked and monitored in the plant–soil system.

The partitioning of photosynthetically fixed C within the plant–soil system has been investigated by supplying $^{14}CO_2$ to the plant, either continuously (van Veen et al., 1989; Martin and Merckx, 1992; van Ginkel et al., 1997) or as a discrete pulse (Keith et al., 1986; Rattray et al., 1995). The use of ^{14}C pulse label technique is considered the most easily conducted method for determining C fluxes and root turnover in the plant–soil system. It has been used with either an in situ field grown 'single plant' (Keith et al., 1986; Martin and Kemp, 1986; Swinnen et al., 1994), pot grown plants (Davenport and Thomas, 1988; Todorovic et al., 1999), PVC constructed plant–soil chambers i.e. microcosms (Meharg and Killham, 1990a, 1990b; Rattray et al., 1995) or in situ field grown plants (Saggar and Searle, 1995; Saggar et al., 1997, 1999). Whilst studies in the past have estimated the total allocation of C to roots and soil using CO_2 pulse labeling with ^{14}C (Saggar et al., 1997, 1999) and ^{13}C (Stewart and Metherell, 1999), only a few studies have examined ^{14}C distribution between different layers in the soil profile (Swinnen et al., 1994). Root density decreases down the soil profile (Barker et al., 1988) and is reflected in decreasing SOM with soil depth. It is unclear, however, whether the turnover of root–derived C is the same per unit of root mass and therefore proportional to root density.

During the conduct of this current experiment recent rhizodeposited ^{14}C SOM was produced. Novel use was made of mini RSMS (Rhizon Soil Moisture Sampler) to obtain dynamic read out on the rhizosphere respiration rates with minimal disturbance of the soil profile. The second objective of the experiment was to use the ^{14}C labelled SOM to evaluate the 'functional nature' of fractions separated in the chemical and density SOM fractionation schemes (see Chapter 6 for more details).

The experimental objectives of this study were

a)   to conduct a $^{14}CO_2$ pulse labeling experiment and measure allocation of recently fixed ^{14}C to shoot, root and soil at different depths, and

b)   to evaluate the use of RSMS as non destructive technique for monitoring the turnover of root and root–derived ^{14}C at the plant/soil–water interface in intact soil cores growing pasture.

5.2   METHODS AND MATERIALS

5.2.1   *Soil*

The soil used was Tokomaru silt loam (Typic Perch–Gley Pallic soil, New Zealand soil classification; Typic Fragiaqualf, USDA soil classification – Hewitt, 1992) under ryegrass (*Lolium perenne* L.)/clover (*Trifolium repens* L.) based pastures. Soil pH, total C, total N, total P, total S and Olsen P values of different depths of the soil are given in Table 5.1.

Table 5.1: Soil pH, total C, N, P and S, and Olsen P values of different depths of Tokomaru silt loam soil.

Soil depth (cm)	pH	Total C $(g\,kg^{-1})$	Total N $(g\,kg^{-1})$	Total P $(g\,kg^{-1})$	Total S $(g\,kg^{-1})$	Olsen P $(mg\,kg^{-1})$
2	4.7	59.3	5.3	0.65	0.60	50.8
4	4.6	51.0	4.7	0.60	0.55	37.4
8	4.5	39.2	4.2	0.58	0.47	32.2
12	4.5	30.9	3.4	0.55	0.39	26.5
20	4.8	24.8	2.4	0.42	0.40	16.1

5.2.2   *Soil core collection*

Duplicate undisturbed soil cores (15 × 20 cm; volume 3.5 L) were collected in PVC storm water pipes (15 × 21 cm – 1 cm space was left at the top of the cylinder) from the soil growing predominantly ryegrass/clover swards. The lower side of the PVC sampling cylinder was sharpened to ensure smooth entry of the sampling cylinder into the soil. Core collection occurred when the field soil was sufficiently moist allowing easy collection of the core.

5.2.3   *Preparation and placement of RSMS in the soil cores*

Horizontal holes (4.2 mm diameter) were drilled into the PVC pipe and soil cores at 2, 4, 8 and 12 cm depths. The RSMS's (Eijkelkamp, Netherlands) were inserted horizontally through the holes adjacent to roots (Figure 5.1). The RSMS consists of a 10 cm porous
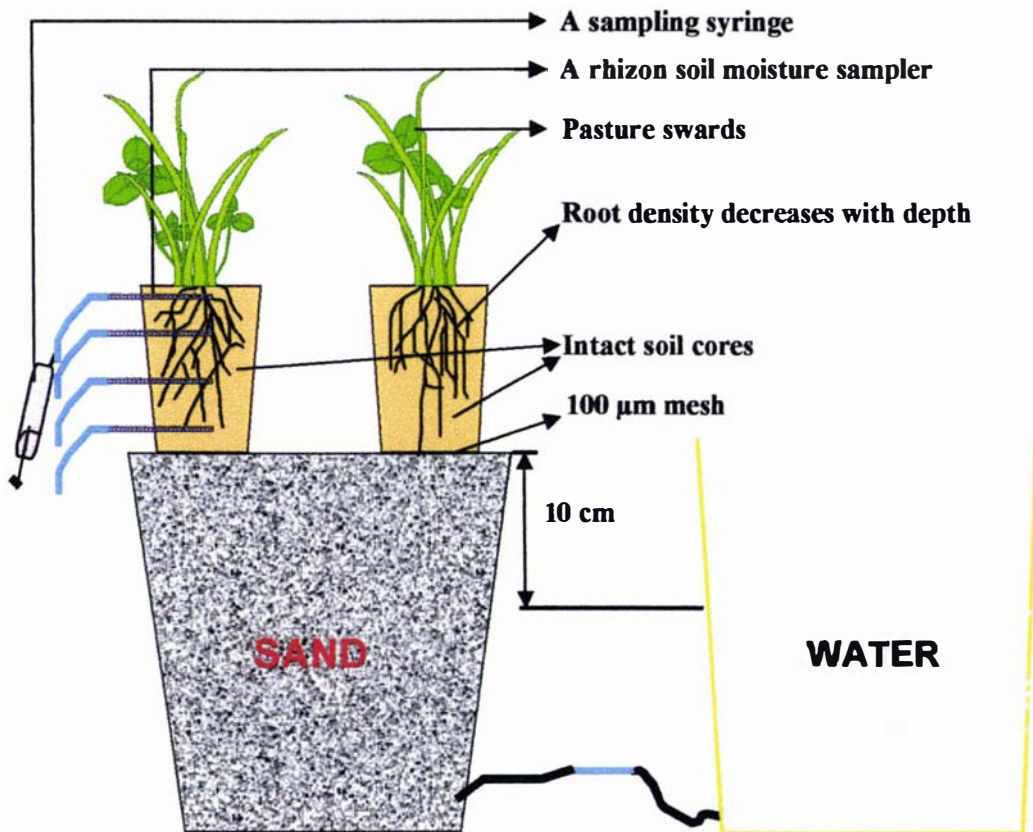
Figure 5.1: Rhizon soil moisture samplers placed at different depths of undisturbed soil cores growing pasture swards.

polymer tube connected to a 10 cm PVC tube (2.7 mm diameter) and a Luer–Lock (L–L) male connector and a cap that protects the L–L connector from contamination with soil (Figure 5.2). A 15–cm stainless steel wire strengthens the porous polymer (and 5 cm of the PVC tube). Before inserting the RSMS, a steel rod (4 mm diameter) was pushed through the hole (4.2 mm diameter) into the soil (about 13 cm) so that the RSMS can be easily pushed through the hole and into the soil. The PVC tube was crowded tightly onto the stainless steel wire and the sampler was pushed smoothly into the soil until 1 to 2 cm of the PVC tube was also inside the soil. In this way the micro–porous material was all surrounded by the soil. The access holes on the wall of cores were sealed for any leakage using silicon sealant. Both the cores were then placed in a glasshouse on a sand–bath

connected to the water source 10 cm below the surface of the sand. Plants were grown under natural light.

The Rhizon samplers (RSMS) were placed into the soil about 16 days before the pulse labelling. This allowed the RSMS to develop good contact with the soil before sampling of the soil solution. A cap at the L–L connector was replaced with a 3–way stopcock prior to
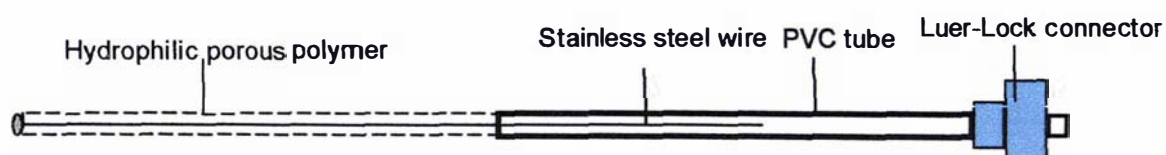
Figure 5.2: A rhizon soil moisture sampler (RSMS).

the pulse labelling experiment. The pasture plants in each core were cut to 2 cm height 6 days before $^{14}CO_2$ pulse labelling to remove the older parts of the plant leaf. Three days before $^{14}CO_2$ pulse labelling, the cores were watered from the top, with approximately 100 ml of distilled water added to each core.

5.2.4   *Pulse labelling technique*

The pasture plants were enclosed in a polyethylene bag ($300 \times 400 \times 0.07$ mm^3) with the neck taped firmly around the top of the PVC root lysimeter. Approximate volume of the air enclosed inside the plastic bag was measured as 5 L to the point where the bag mouth was taped to the PVC cylinder.

Volumes of $^{14}CO_2$ gas were produced in a 60 ml plastic syringe fitted with a 3–way stopcock (Plate 5.1). Seven and half ml of 0.2 M sodium carbonate ($Na_2^{14}CO_3$) was drawn into the syringe and reacted in the body of syringe with 10 ml of 1 M sulphuric acid (H_2SO_4) by manipulation of the stopcock (Saggar and Searle, 1995). On an average, 13.12 MBq of the 0.2 M $Na_2^{14}CO_3$ was reacted in the syringe with the sulphuric acid (Equation 5.1). Once evolution of CO_2 had ceased, the solution was decanted and stored to determine residual ^{14}C activity. The remaining 31.2 ml of $^{14}CO_2$ (12.17 MBq – calculated after deduction of the amount of ^{14}C in the decanted solution) was injected through the plastic bag, into the atmosphere above the plants. The hole in the plastic bag was sealed with

radioactive labelled tape after the injection of $^{14}CO_2$.

$$Na_2\,^{14}CO_3 + H_2SO_4 \rightarrow Na_2SO_4 + ^{14}CO_2 \uparrow + H_2O \qquad\qquad (5.1)$$
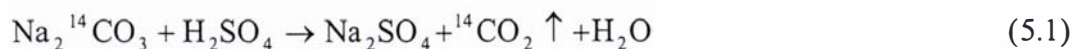
Plate 5.1: Dr. Surinder Saggar carrying out $^{14}CO_2$ production inside the body of 60–ml syringe fitted with a 3–way stopcock.

In the present study, it was assumed that there was no loss of $^{14}CO_2$ during recovery of the residual solution (although there is a possibility that some of $^{14}CO_2$ was lost by degassing as the residual solution was decanted from the syringe and before the storage container was sealed). Moreover, the residual solution of acid and $Na_2\,^{14}CO_3$ was not neutralised with NaOH (see Section 7.2.5.1), before the storage container was opened to obtain a sample for ^{14}C analysis. Therefore, any $^{14}CO_2$ in the headspace (formed due to the reaction of the acid with any unreacted $Na_2\,^{14}CO_3$) of the container was lost on opening the container.

The grass swards were pulse labelled at 0930 h when photosynthetically active radiation (PAR) (recorded using LI–185 LI–COR® light meter) varied between 310–800 μmoles s^{-1} m^{-2} during the ^{14}C assimilation period of 2 h and increased up to 1680 μmoles

during the daytime (Figure 5.3). The glasshouse temperature (recorded as average values using data–logger) varied between 18°C at the start to 29°C at the end of the pulse and increased up to 37.5°C during the day (Figure 5.3). After assimilation of $^{14}CO_2$ for 2 h, the $^{14}CO_2$ activity was monitored in the enclosed plastic atmosphere above the plant using a Geiger counter. The plastic bags were then removed after 2 h of $^{14}CO_2$ assimilation and the labelled plants were opened to the natural environment.

5.2.5   *Soil water potential (SWP) measurements at the soil solution sampling depths using RSMS's.*

Prior to pulse labelling each soil core, the RSMS at each soil depth was connected through a 3–way stopcock to a long PVC tube and a glass tube (internal diameter 2.2 mm), creating a 'U' bend, which was used as a manometer. A syringe (1 ml) was also attached to the 3–way stopcock for creating suction and taking soil solution samples through RSMS by manipulation of the 3–way stopcock. Immediately before sampling soil solution, the change in the manometer water level with reference to the sampling depths was recorded at 8, 12, 16, 20, 24, 30, 36 and 44 h after pulse labelling to monitor the diurnal change in the root activity (in terms of the soil water potential) at the root–soil water interface. The manometer readings recorded for all the depths were then adjusted for the rise of water level in the tube due to capillarity. Recording of manometer readings was stopped 44 h after pulse labelling because of the appearance of bubbles in the 3–way stopcock, which interfered with the measurements.

5.2.6   *Sampling and harvesting*

5.2.6.1   *Soil solution sampling*

Samples of the soil solution (0.5 ml) were taken from both the lysimeters through the RSMS placed at 2, 4, 8 and 12 cm soil depths by creating a vacuum produced by a 1 ml syringe attached to the 3–way stopcock on the L–L connector of the RSMS (see Plate 7.4). These samples were collected periodically at 1, 2, 4, 8, 12, 16, 20, 24, 30, 36, 44 and 48 h after exposing pastures to $^{14}CO_2$ atmosphere. The 0.5 ml samples of soil solution were added to polypropylene mini vials containing 1 ml of 0.17 M NaOH as a $^{14}CO_2$–trap solution. These vials were sealed immediately and held for scintillation counting.

5.2.6.2   Herbage, root and soil sampling and their preparation for analysis

Herbage samples (8 to 10 shoots/leaves per sampling) were taken randomly from the pasture swards growing on the lysimeter at 59, 79, 103.5, 129.5, 152.5 and 172.5 h after exposure to $^{14}CO_2$ pulse, dried at 70°C, finely chopped and stored for analyses of total C and ^{14}C. This enabled the specific activity (DPS mg^{-1} C) of herbage/shoots to be monitored during the one–week period of growth from the time of $^{14}CO_2$ exposure.

A week after pulse labelling, the lysimeters were removed from the sand bath (connected to water supply) and the RSMS's were gently pulled out of the lysimeters. The soil core was removed intact from the PVC cylinder. The shoots were excised at the soil surface and collected in paper bags, dried at 70°C in an oven, weighed and stored for total C and ^{14}C analysis. Also the clover/grass stems below the soil surface, along with some adhering soil, were removed from the soil and dried at 70°C in an oven for total C and ^{14}C analysis. The soil cores were sectioned at 2, 4, 8, 12, 16 cm depth using a sharp knife and circular sections of soil+roots were divided into eight equal sections. Two opposite sections (4 sections in all) were dried to a constant weight at 100°C for soil moisture content determination and then ground and stored for analysis of total C and ^{14}C in soil+roots. The soil+root samples were ground to <0.2 mm firstly with a pestle and mortar and then roots and soil were finely chopped using a domestic electric mini food processor in a fume cupboard. Any dead, brown/black material was discarded and a sub–sample (approximately 50 g) of the above processed soil samples were ring ground using a RockLabs ring grinder (for 10 seconds). A subsequent evaluation of the ring grinding procedure has been conducted in Section 6.2.5.1 (Figure 6.1b).

The roots were carefully separated from the remaining two halves of soil section by wet sieving (> 0.5 mm). The roots were dried at 70°C in an oven, weighed, finely chopped and stored for total C and ^{14}C determination. The root mass in each soil slice is calculated from the dry weight of the roots in the sub–sampled wet soil and the total net weight of wet soil in each slice.

All operations using ^{14}C labelled solutions, gas, herbage, roots and soil materials were conducted using appropriate safety clothing and equipment (Middleboe *et al.*, 1976).

5.2.7   Analysis

5.2.7.1   Basic chemical analysis of soil

Soil pH in distilled water (1:2.5 w/w) was determined using a glass electrode according to Blakemore et al. (1987). Olsen P was determined by extracting soil samples for 30 minutes with 0.5 M $NaHCO_3$ at pH 8.5 (Olsen et al., 1954) and measuring the phosphate concentration colorimetrically using the Murphy and Riley (1962) method as described by Blakemore et al. (1987). Total S in the soil determined using the $NaHCO_3$ fusion method of Steinberg et al. (1962) (see Section 3.2.4). Total N and P in the soil were determined by Kjeldahl digestion (McKenzie and Wallace, 1954). The Kjeldahl digests were analysed for ammonium and phosphate using a Technicon II autoanalyser.

5.2.7.2   Scintillation cocktail recipe

The scintillation cocktail used was prepared by mixing 8 g of PPO (2,5–diphenyloxazole) as a primary scintillant, 0.2 g of POPOP [1,4-Di-{2-(4-methyl-5-phenyl-oxazolyl)}-benzene] as a secondary scintillant, 2000 ml of toluene (solvent) and 1250 ml of surfactant triton–X 100 (Middleboe et al., 1976). The mixture was stirred overnight by a magnetic stirrer and kept in a dark glass bottle. The recipe for making the cocktail solution was slightly modified from the original recipe i.e. an additional 250 ml of the surfactant was added (surfactant volume used in the original recipe is 1000 ml) to ensure complete covering of salt and water molecules and provide a clear suspension of the scintillation cocktail and different soil extracts obtained in this study.

5.2.7.3 ^{14}C in soil solution

Scintillation cocktail (5 ml) was added to the soil solution (0.5 ml from RSMS), which was mixed with 1 ml of 0.17 M NaOH in the mini vials. Vials were shaken vigorously to produce a clear solution and placed in dark for 30–60 minutes to minimise the chemiluminescence before ^{14}C counting. The ^{14}C counts were measured using a Wallac 1414 WinSpectral™ liquid scintillation counter (LSC) with standard parameters for ^{14}C counting, and automatic quench correction.

To identify whether the appearance of ^{14}C in soil solution was due to: a) release of

$^{14}CO_2$ from root/microbial respiration and/or diffusion of ^{14}C–CO_2 into the soil through gaseous exchange process or b) the release of soluble ^{14}C–carbohydrate, amino acids etc. as root exudes, the suspension of the alkaline soil solution and cocktail solution was acidified to pH ≤4.5 with addition of 0.14 ml of 1M HCl. The vials were shaken well after the addition of the acid and placed open in the fume hood for three days so that any $^{14}CO_2$ formed upon acidification of the suspension could escape to the atmosphere. After three days, all the vials were placed in an ultrasonic bath (power rating – 200 watts) for about 20 minutes to remove any $^{14}CO_2$ entrapped in gas bubbles. The volume was readjusted to compensate the loss of toluene from the cocktail solution. The vials were capped and recounted for ^{14}C counts using LSC as mentioned above.

5.2.8   Total C and ^{14}C in shoot, root and soil samples

Roots and herbage were finely chopped using a domestic electric coffee grinder in a fume cupboard. Total C in shoot, root and soil (along with roots) samples (taken in duplicate) were determined by the dichromate digestion procedure (see Section 3.2.5). For total ^{14}C, the digestion procedure is same as described for total C determination. Another aliquot (0.2 ml) of the trapping solution (2 M NaOH and 0.2 M Na_2CO_3) was taken in the polypropylene vial and diluted to 2 ml with deionised water. The scintillation cocktail solution (4 ml) was added and the vial was shaken thoroughly to produce a clear solution and counted for ^{14}C activity using a Wallac 1414 WinSpectral™ LSC after one hour of keeping them in dark to minimise chemiluminescence.

The distribution of the ^{14}C in different components of the pasture shoot–root–soil system is expressed as a percentage of the total ^{14}C counts (12.17 MBq) injected above the swards at the start of the pulse labelling.

5.3   RESULTS AND DISCUSSION

5.3.1   Soil water potential (SWP) at the root–soil water interface

The SWP recorded using the U–bend manometer at 2 cm soil depth showed greater diurnal variation than the other depths, and the magnitude of variation decreased down the profile (Figure 5.3). This indicates that the root activity (in terms of SWP at the root–soil

interface) was greater in the top 2 cm soil depth where there was a greater proportion of roots (see Section 5.3.3.2 below). The diurnal variations in manometer readings at the soil depths were not induced by temperature fluctuations during the day (because all the manometers were subjected to same temperature errors, if any). The roots exerted tension on the soil water to meet the plant transpiration demand during the daytime when the temperature was high (Figure 5.3) and the photosynthetic activity of the sward was at its peak, as indicated by high PAR values (Figure 5.3). Therefore, the high tension exerted by the roots on the soil water in the 2 cm soil depth resulted in the greater decrease in SWP (i.e. a negative value increased; Figure 5.3) during the daytime compared to other soil depths. As the nighttime approached, the rate of decrease in SWP slowed down considerably (from 1530 to 2130 h) although there was a small lag in the increase of SWP. The occurrence of a lag phase could either be due to the slow response of the tensiometer system or due to plants needing to recharge vacuoles and other cells which are depleted of water (i.e. low water potential) during the daytime photosynthesis and transpiration activities. During the remainder of the dark period, at the 2 cm soil depth, SWP was increased (i.e. a negative value decreased; Figure 5.3) because of absence of PAR, temperature was low (Figure 5.3), and stomata were probably closed, therefore transpiration demand by the plants would be negligible. The increase in SWP at the 2 cm depth during this dark period and the steady decrease at the lower soil depths (i.e. 8 and 12 cm depths) (Figure 5.3) could be attributed to internal movement of soil water caused by capillary action to compensate for the loss of soil water in the layer closer to the soil surface.

Soil water potential measurements using mini RSMS provide a real time indicator of the root activity during pulse labelling experiments. Such readout would also be useful in nutrient uptake studies.

Campbell (1985) has shown similar simulated diurnal variations in SWP in relation to transpiration demand of plant over an 8 days period, with relatively more lag period. Using osmotic tensiometers, Seaton *et al.* (1977) found that when a wheat crop (grown in small lysimeters) was subjected to a drying cycle after the heading stage, the high root densities in the upper soil layers caused the SWP to drop much more rapidly compared to the lower soil layers. Warrick *et al.* (1998) examined SWP fluctuations in tensiometers in response to temperature and suggested that the hydraulic conductivity of the soil immediately around
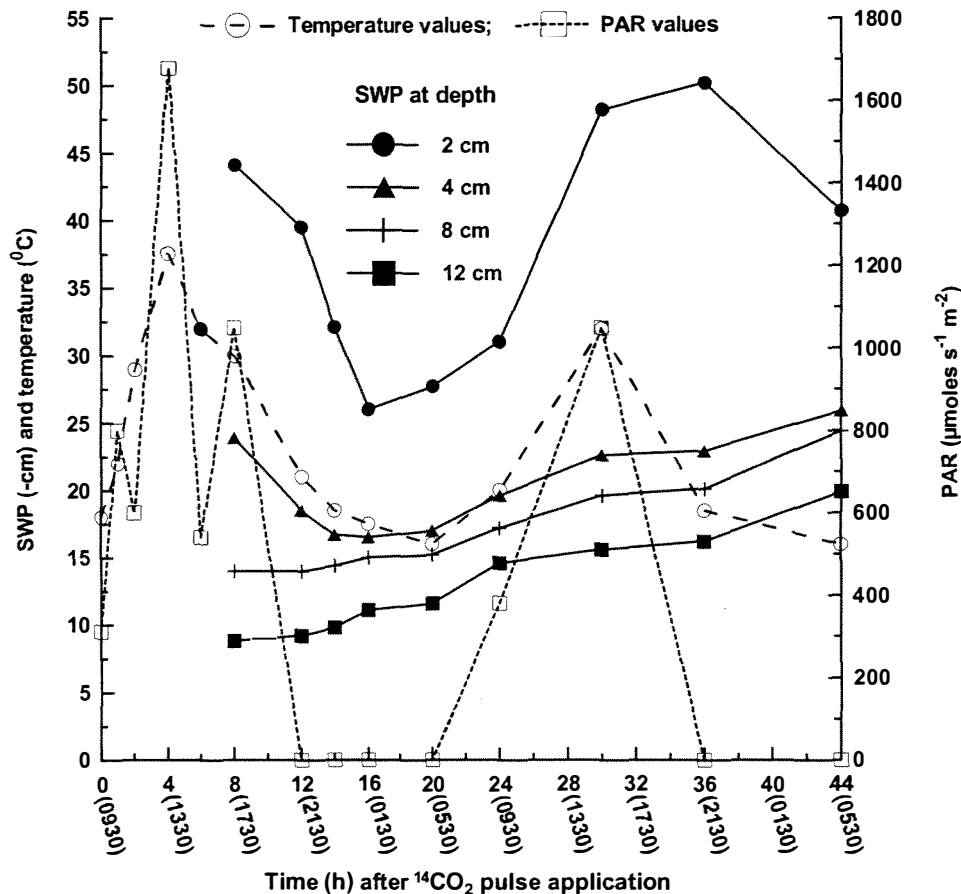
Figure 5.3: Changes in glasshouse temperature (°C), PAR (μmoles s^{-1} m^{-2}), and SWP (–cm) at the root–soil interface over time (h) at different soil depths. Pulse labelling was done at time 0 i.e. at 0930 h.

the cup was the main factor governing temperature induced SWP fluctuations. If the cup was not in proper contact with the soil, this could lead to very high fluctuations in SWP as air and soil temperature changed. In the present study, it was ensured that the mini RSMS established proper contact with the soil by placing them at least two weeks before recording the manometer readings (see Section 5.2.3).

5.3.2 ^{14}C in atmosphere

Two hours after the injection of 12.17 MBq $^{14}CO_2$, a Geiger counter detected negligible $^{14}CO_2$ activity in the enclosed air above the undisturbed soil core growing pasture swards. This indicates that almost all the $^{14}CO_2$ injected in the enclosed atmosphere above the pasture swards was either assimilated rapidly by the swards or some $^{14}CO_2$ exchanged with soil air within 2 h after injection of the pulse. Rattray *et al.* (1995) showed

that the ryegrass plants assimilated all the $^{14}CO_2$ pulse within 2 h of allocation period. Martin and Kemp (1986) estimated that shoots of wheat plant fixed the $^{14}CO_2$ produced in an enclosed atmosphere within 1 h in bright sunlight. Swinnen *et al.* (1994) reported an assimilation period of 1–2 h to allow for assimilation of *ca* 95% of the total ^{14}C label pulse by the wheat plant. These studies have monitored the rate of assimilation by sealing the surface of the soil so as to distinguish plant fixation of $^{14}CO_2$ from diffusion of $^{14}CO_2$ into the soil air and also to monitor shoot and root/soil respiration separately. Davenport and Thomas (1988) carried out the pulse labelling, to study the carbon partitioning and rhizodeposition in corn and bromegrass, by covering soil surface with plastic or a mat of moss and algae in an attempt to minimise $^{14}CO_2$ diffusion into soil. In this experiment with an undisturbed sward, it was not possible to seal the soil surface.

In the present study, the injection of 12.17 MBq of $^{14}CO_2$ (equal to 31.2 ml) into the enclosed atmosphere (5 L capacity) above the pasture swards raised the CO_2 partial pressure from $10^{-3.5}$ atm to approximately $10^{-2.18}$ atm. According to Dahlgren *et al.* (1997) and Parfitt *et al.* (1997), soil CO_2 concentration for pasture soils of NZ is about 70 to 140 times (2.5 to 5.0 %) the atmospheric level (0.035 %). Therefore, the partial pressure of CO_2 in the soil air in pasture soil would range between $10^{-1.6}$ and $10^{-1.3}$ atm. However, the combined partial pressure of $^{12}CO_2$ + $^{14}CO_2$ in the enclosed air above the lysimeter was $10^{-2.18}$ atm, therefore, movement of CO_2 by net diffusive flux of $^{12}CO_2$ + $^{14}CO_2$ into the soil would be minimal. As initially there were no $^{14}CO_2$ molecules in the soil air, it is the random Brownian movement of $^{14}CO_2$ molecules that tends to smooth out the concentration difference between the air above the soil and air in the soil (i.e. molecular diffusion). In the light conditions of this experiment, however, plant assimilation of $^{14}CO_2$ may dominate diffusion as a mechanism for atmospheric $^{14}CO_2$ transfer into the soil. Recently, Saggar *et al.* (1997, 1999) have shown; under *in situ* $^{14}CO_2$ pulse labelling conditions of typical New Zealand pasture soils (i.e. without separating the interface between the shoot and root/soil), that an allocation period of 2 h was sufficient for the disappearance (assimilation) of more than 98% of the $^{14}CO_2$ inside an airtight fish–bowl chamber enclosing pasture swards. However, Saggar and Searle (1995) have recorded about 5% of $^{14}CO_2$, even 3.5 h after injection of $^{14}CO_2$, in one of their experiments on *in situ* $^{14}CO_2$ pulse–labelling of pasture grasslands, and attributed this to less vigorous pasture swards.

5.3.3   *^{14}C in soil water*

The ^{14}C activity in the soil water samples reached maximum values at different times after injection of the pulse depending upon soil depth (Figure 5.4). Interestingly, the ^{14}C activity (MBq m^{-3} of soil water) reached approximately the same maximum values (i.e. 67 MBq m^{-3}) irrespective of soil depths. The deeper the sample was taken the longer the peak took to appear. At 1 h sampling time, the ^{14}C activity in the soil water from 2 cm depth of the core had almost reached (i.e. 65 MBq m^{-3}) the peak value. However, at 2 cm soil depth, the peak of ^{14}C activity in soil water was narrow, which indicates a rapid decline in soil solution ^{14}C concentration after reaching the peak value, whereas in lower soil depths, the peaks were broader and longer lasting. The rapid occurrence of peak at the 2 cm soil depth indicates that the rate of movement of $^{14}CO_2$ from atmospheric $^{14}CO_2$ to soil solution ^{14}C in the top 2 cm depth was very rapid, responding to the introduction of $^{14}CO_2$ pulse within one hour. Definition of the peak ^{14}C activity was poor. Soil solution sampling should have occurred earlier than one hour. The rapid appearance of ^{14}C in soil solution indicates that the recently assimilated ^{14}C is translocated and released very quickly from the metabolic pool of the roots. It is, however, unclear whether the rapid appearance of the ^{14}C in soil solution could be attributed to release of ^{14}C in soluble exudates, root–respired $^{14}CO_2$, microbial respired $^{14}CO_2$ or diffusion of $^{14}CO_2$ into soil air and water. Prosser and Farrar (1981) have indicated that an initial pulse of ^{14}C appears in soil from soluble organic root exudates followed by more constant release from labelled storage and structural material. Warembourg *et al.* (1982) has shown that when $^{14}CO_2$ is supplied to the leaves of soybean plants, it is readily taken up and, within 2 h, ^{14}C enters the metabolic pool of the roots and $^{14}CO_2$ is released into the soil atmosphere by respiration. A rapid $^{14}CO_2$ efflux from soil, i.e. within 1 h after labelling the shoots of *Lolium perenne* (Kuzyakov *et al.*, 1999) and within 30 minutes after pulse labelling of winter wheat and rye (Cheng *et al.*, 1993) is also reported. Kuzyakov *et al.* (1999) indicated that most rapid CO_2 efflux comes firstly from root respiration followed by microbial respiration of root exudates, as microbial respiration involves chain of successive processes such as exudation from roots, intake by microorganisms and respiration of microorganisms. Rattray *et al.* (1995) detected a small proportion (about 0.25%) of assimilated ^{14}C in the soil residue within 30 minutes after pulse labelling the shoots of *Lolium perenne*.
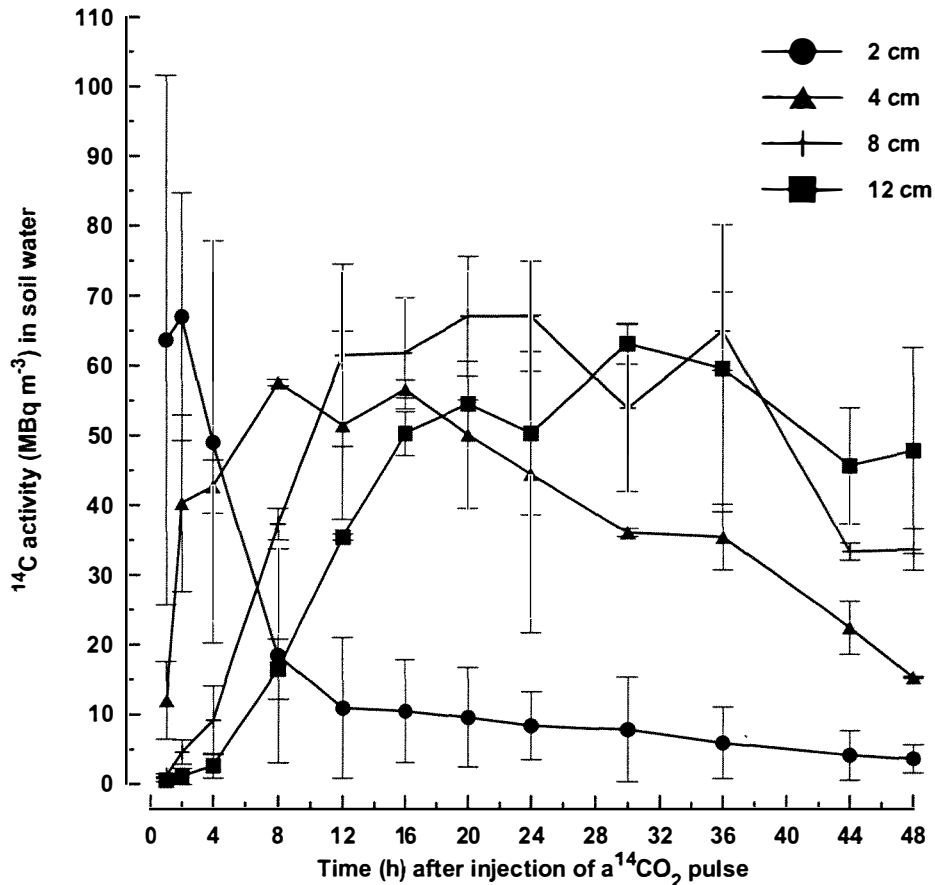
Figure 5.4: [14]C activity in soil water extracted from different depths (see legends) below ryegrass/clover swards at different times after injection of a [14]CO₂ pulse into the atmosphere above ground. *Error Bars: ±SE of mean of two cores.*

The rhizodeposition of C as root exudates (readily available microbial carbon source) greatly influences C turnover in soils and can lead to C accumulation or consumption due to influence on microbial activity in the rhizosphere (Kuzyakov *et al.*, 1999). Initially, following exposure to [14]CO₂ pulse, relatively simple compounds within the plant are labelled followed by increasing incorporation into bio–chemically more complex components with increasing length of chase period (Hodge *et al.*, 1997). Therefore, the initial root–released ^{14}C would be largely simple soluble compounds such as simple sugars, organic acids and amino acids, which are readily respired to CO₂ by microbial biomass. Rattray *et al.* (1995) indicated rapid incorporation of recently released root ^{14}C exudates (i.e. within 3 h after exposure of *Lolium perenne*) into the microbial biomass followed by concomitant release of [14]CO₂, indicating a rapid turnover of microbial biomass. With time, the contribution of biochemically more complex ^{14}C in rhizodeposits (i.e. complex polysaccharide secretions and structural compounds such as sloughed–off cells, root cell

lysate etc) to total ^{14}C released from root increases and therefore affects temporal microbial utilisation of ^{14}C (Rattray *et al.*, 1995). Merbach *et al.* (1999) separated the recent water extractable ^{14}C exudates by ion chromatography and found that these exudates consisted mainly of carbohydrates and organic acids, and a small proportion of amino acids. Schilling *et al.* (1998) also found greater proportions of carbohydrates and organic acids in the water–soluble portion of rhizodeposition.

5.3.3.1   Nature of ^{14}C in soil water

The disappearance of almost all the ^{14}C counts after acidification of the scintillation cocktail–soil solution suspension indicates that the ^{14}C activity was largely present as $^{14}CO_2$ in the soil water sample from all the depths (except the 12 cm depth) over 48 h after injection of the pulse (Table 5.2). Therefore, the $^{14}CO_2$ in the soil solution could be a function of either root respired $^{14}CO_2$, microbial respired $^{14}CO_2$ and/or net diffusion of $^{14}CO_2$. However, the temporal dynamics of $^{14}CO_2$ appearance in the root–soil column after a pulse labelling found in the present study were comparable with those reported by Cheng *et al.* (1993) and Kuzyakov *et al.* (1999), where the soil surface was sealed to prevent $^{14}CO_2$ diffusion into the soil. The similarity in the pattern of $^{14}CO_2$ activity in the soil with the work of others suggests that root and microbial respiration as the main pathways for $^{14}CO_2$ appearance in the soil water.

The soil solution from the 12 cm depth of one of the soil cores (as indicated by the larger errors; see Table 5.2) did show the accumulation of some ^{14}C exudates (as all the ^{14}C was not lost upon acidification) up to 24 h after $^{14}CO_2$ pulse labelling (Table 5.2). This small amount of ^{14}C counts became negligible by the time of final soil solution sampling (i.e. 48 h after the injection of ^{14}C pulse). The presence of ^{14}C counts even after acidification indicates that either the suspension of scintillation cocktail–soil solution from the 12 cm depth of one of the cores was not acidified appropriately or rhizosphere microbial activity was not sufficient to readily utilise the root–released ^{14}C exudates and convert them to microbial biomass ^{14}C, or respired as $^{14}CO_2$. Lower initial microbial activity could be expected at greater depth because, Tate (1979) showed that total C evolved from different layers of soil by the microbes oxidising SOM, and number of microbes, decreased with increasing depth of soil. Ahl *et al.* (1998) attributed the reduction in the microbial biomass to a reduced input of plant material i.e. energy deficiency and not

Table 5.2: ^{14}C activity in soil solution before and after acidification, and % ^{14}C lost upon acidification, at different depths and times after $^{14}CO_2$ pulse–labelling. The values of ^{14}C counts recovered after acidification (along with standard error values, shown in brackets) are given up to 2 decimal places to show the clear differences between two cores.

Soil depth	Time (h) of soil solution sampling after $^{14}CO_2$ pulse labelling											
	1	2	4	8	12	16	20	24	30	36	44	48
	^{14}C counts (Bq ml^{-1}) in soil solution before acidification											
2 cm	63.6	66.9	48.9	18.4	10.8	10.4	19.5	8.3	7.8	5.9	4.1	3.6
4 cm	11.9	40.2	42.6	57.6	51.4	56.6	50.0	44.4	36.0	35.4	22.4	15.2
8 cm	1.2	4.5	9.1	37.2	61.4	61.7	67.0	67.0	53.9	64.9	33.3	33.5
12 cm	0.5	1.0	2.6	16.4	35.4	50.2	54.4	50.3	63.0	59.6	45.6	47.7
	^{14}C counts (Bq ml^{-1}) in soil solution after acidification											
2 cm	0.41	0.26	0.24	0.09	0.09	0.20	0.13	0.09	0.09	0.02	0.00	0.07
	(0.51)*	(0.24)	(0.23)	(0.02)	(0.08)	(0.08)	(0.08)	(0.07)	(0.01)	(0.02)	(0.00)	(0.09)
4 cm	0.05	0.11	0.24	0.27	0.32	0.09	0.08	0.15	0.48	0.13	0.02	0.01
	(0.05)	(0.11)	(0.19)	(0.23)	(0.23)	(0.03)	(0.07)	(0.05)	(0.00)	(0.00)	(0.03)	(0.01)
8 cm	0.02	0.02	0.20	0.29	0.20	0.27	0.24	0.56	0.33	0.32	0.07	0.00
	(0.01)	(0.03)	(0.18)	(0.21)	(0.03)	(0.09)	(0.22)	(0.31)	(0.11)	(0.24)	(0.06)	(0.00)
12 cm	0.00	0.01	0.09	1.52	2.86	3.49	4.54	4.95	2.82	1.22	0.54	0.32
	(0.01)	(0.01)	(0.13)	(1.47)	(2.91)	(4.41)	(5.98)	(6.06)	(3.46)	(1.37)	(0.76)	(0.08)
	% ^{14}C lost from a soil-cocktail solution suspension after acidification											
2 cm	99.5	99.6	99.3	99.2	99.1	97.8	98.5	98.5	97.7	99.6	100.0	98.6
4 cm	99.7	99.7	99.4	99.2	99.3	99.8	99.8	99.6	98.7	99.6	99.9	99.9
8 cm	98.6	99.7	96.4	99.0	99.7	99.6	99.6	99.1	99.4	99.5	99.8	100.0
12 cm	99.4	95.0	97.6	89.2	92.0	92.8	91.6	88.4	95.4	97.4	98.6	99.3

*Values in brackets are SE of mean of two cores

to stress caused by reduced oxygen concentrations (Meyer *et al.*, 1996) in the lower compacted layer (i.e. high bulk density) of the soil profile. Lower turnover rates of root–released ^{14}C at depth would explain the similar peak heights and broader peaks for $^{14}CO_2$ activity (Figure 5.4) irrespective of greater travel time to deep sections of soil.

Saggar *et al.* (1997) found that between 2–3% of ^{14}C labelled assimilate appeared in the soil fraction of legume–based pasture soils (Fine loamy mix mesic Typic Dystrochrepts) within 4 h of ^{14}C pulse application. Rattray *et al.* (1995) also studying pastures found up to 2.5% of the assimilated ^{14}C in the soil residue (i.e. accumulation of root–released ^{14}C) ^{14}C between 3 and 24 h after the ^{14}C pulse. In these studies, although roots were carefully separated from soil, it was likely that some very minute root hair fragments were left unpicked from the soil. These unpicked root hair fragments could give the incorrect estimates for the amounts of the recent assimilate that was deposited in the rhizosphere soil, as root–released C. However, in the present study, negligible amounts of soluble organic exudates were found in the soil water up to 48 h after the initial pulse. Thus, the current results indicate that root exudates were either not released in measurable quantities or if released, they were readily degraded by the soil microbial biomass (Rattray *et al.* 1995; Jones *et al.*, 1996b) or sorbed to solid phase anion exchange sites (Jones *et al.*, 1996a).

Absence of $^{14}CO_2$ labelled soluble organics from soil solution is not a proof that they are absent from the rhizosphere. Recently, Jones and Brassington (1998) have found in acid soils that more than 80% of the organic acids entering soil solution will rapidly (within 10 minutes) become sorbed onto the soil's exchange complex in favour of remaining in the soil solution and therefore, may prevent rapid biodegradation as well (Gerke, 1992). Jones and Darrah (1993) provided experimental and modelled evidence of simultaneous exudation and resorption of soluble C compounds released by roots of *Zea mays* L., indicating the possible importance of resorption in a soil environment. Therefore resorption of released exudates, if any, could have also contributed to the disappearance of most of the soluble organic ^{14}C compounds in the soil solution.
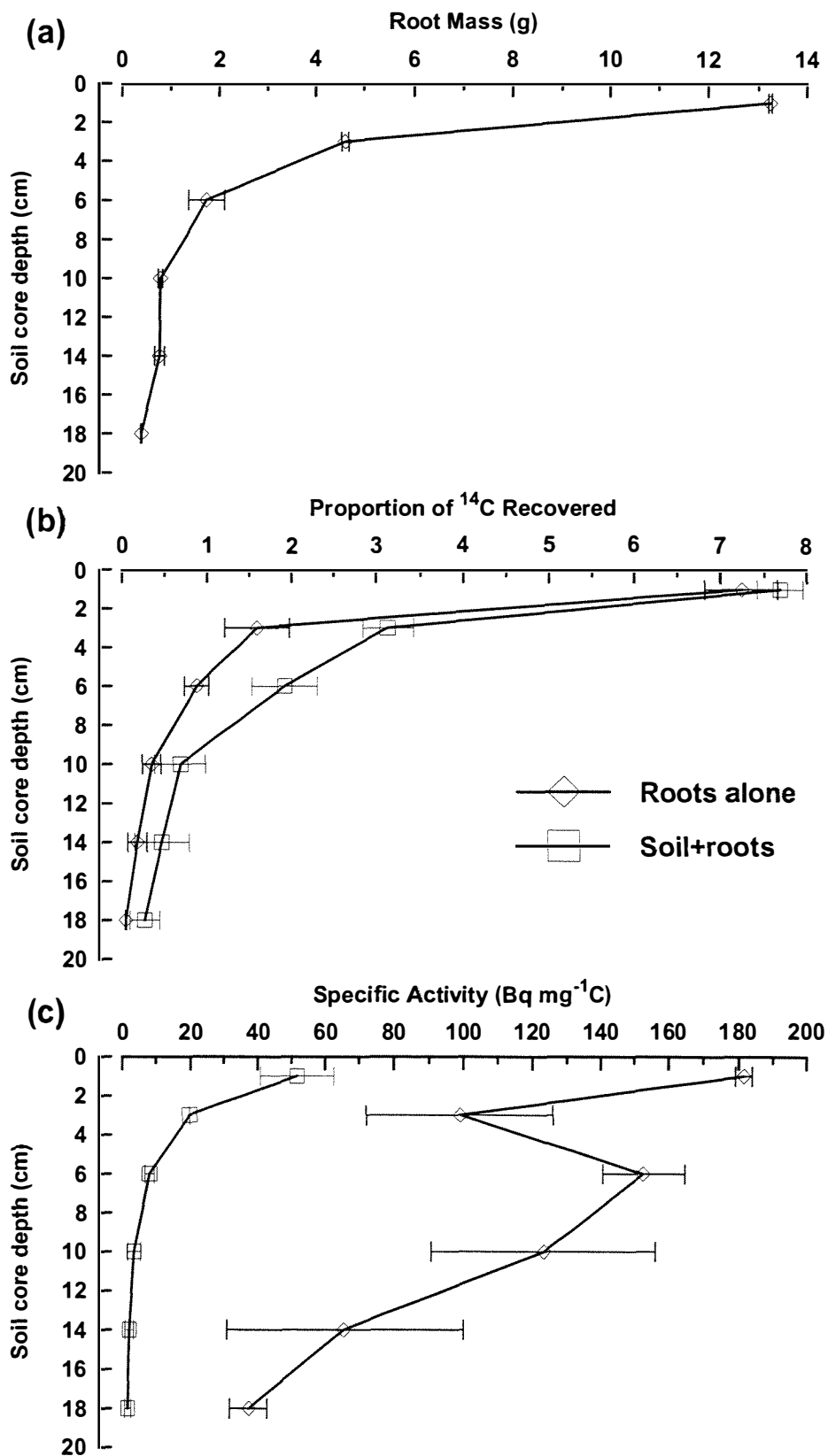
Figure 5.5: Root mass distribution (a), % of injected ^{14}C recovered in soil+roots and roots alone (b), and specific activity (Bq mg^{-1}C) of soil+roots and roots alone, at different soil depths. The legend points are plotted at the mean depth of various soil slices. *Error Bars: ± SE of mean of two cores.*

5.3.3.2   Soil solution $^{14}CO_2$ concentration and root mass distribution

The greater proportion of root mass was recovered in the top 0–2 cm soil depth (i.e. 61.5%) and decreased exponentially down the soil core (Figure 5.5a). The initial $^{14}CO_2$ counts per m^3 of soil water (i.e. up to 4 h of soil solution sampling time) was strongly associated with the root mass distribution with depth (Figure 5.6) indicating a greater contribution of roots to the soil solution ^{14}C than diffusion of $^{14}CO_2$ from the atmosphere
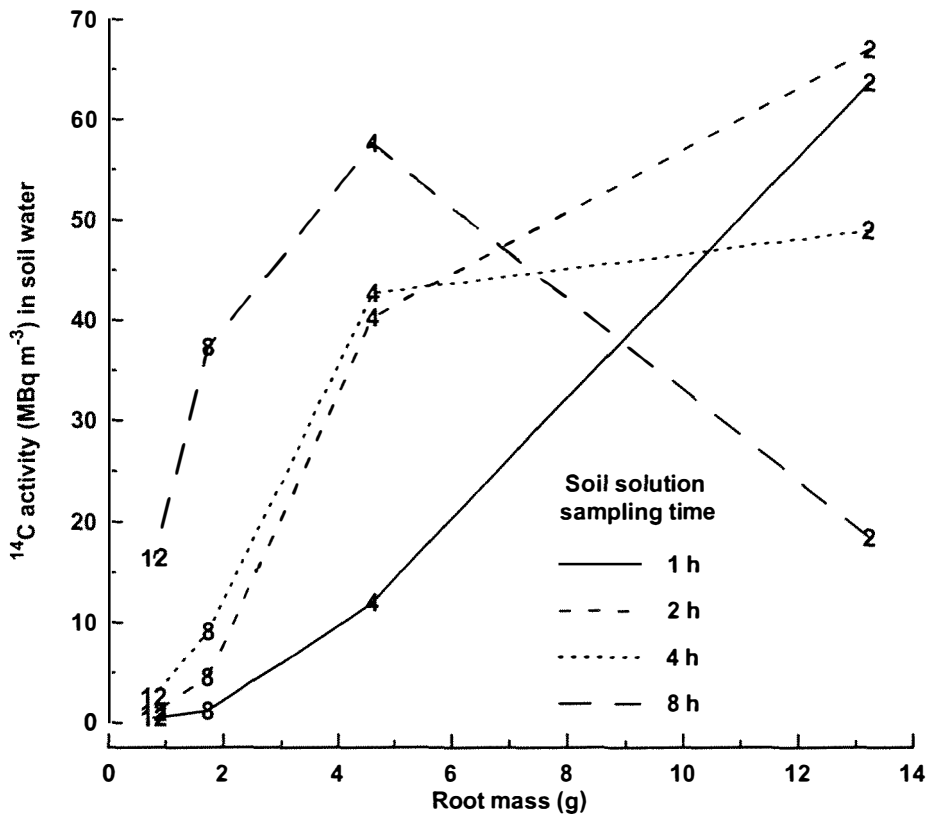
Figure 5.6: Initial ^{14}C activity in soil solution versus root mass distribution at different soil depths. The numbers shown are soil solution sampling depths.

into the soil. The earlier appearance of ^{14}C in soil solution in the topsoil depth followed by the increase in the lower depth (Figures 5.4 and 5.6) suggests that the recent ^{14}C assimilate from the shoot was transported via roots to lower depths with time (i.e. slower movement) and then released from root metabolisable pool as time passes (Figure 5.4). The decrease in soil solution ^{14}C concentration (after reaching the peak) occurred earlier in the top depth (Figure 5.4) suggesting the faster turnover (root and microbial metabolism) of the ^{14}C pulse, as unlabelled ^{12}C photo–assimilates continue to be transported to this zone of highest

root density and microbial activity (Tate, 1979; Ahl *et al.*, 1998). This upper zone also has enhanced aeration compared to lower soil zones allowing more rapid flushing of soil air. Slower turnover of ^{14}C and greater constraints on gaseous diffusion at lower soil depths causes the peak of soil solution ^{14}C concentration to be broader and longer lasting (Figure 5.4). The broadness of the peak also suggests that a greater proportion of ^{14}C assimilates translocated to lower soil depths was used in root and microbial metabolism i.e. for plant maintenance and less was used for plant growth (see discussion in Section 5.3.4.2 below).

The result from this study and other investigations (Cheng *et al.*, 1993; Kuzyakov *et al.*, 1999) suggests that $^{14}CO_2$ assimilation followed by downward transport in plants and $^{14}CO_2$ appearance and evolution from soil are very rapid processes. Others also reported rapid photo–assimilation and partitioning of ^{14}C–labelled assimilate below ground but monitored the pulse appearance either by harvesting soil and root samples from the soil–root chamber (Rattray *et al.*, 1995) or taking soil cores from field plots pulse labelled *in situ* (Saggar *et al.*, 1997). The *in situ* technique of sampling soil water using RSMS, with minimum disturbance of soil, reported in the present study was useful to monitor the rapid appearance of the recently assimilated ^{14}C in soil as root and microbial respired $^{14}CO_2$.

5.3.4   *^{14}C in shoots, roots and soil at harvest*

5.3.4.1   *Specific activity and ^{14}C recovery in plant shoot*

The specific activity (Bq mg^{-1}C) and ^{14}C activity (Bq mg^{-1}) of the ryegrass/clover shoots was highest at 59–100 h after exposure to $^{14}CO_2$ and then steadily declined. As $^{14}CO_2$ rich metabolites are respired and photo–assimilation continues to fix unlabelled CO_2 thereby simultaneously reducing and diluting the ^{14}C pulse within the plant. The high variability in shoot specific activity at the initial stages of the experiment is believed to result from the variation in leaf age and photosynthetic activity in the small sub–samples taken (Figure 5.7). Grass grows from a growing point, which remains below the grazing height. High specific activity and recently fixed ^{14}C will be associated with the growing points at the base of new tiller systems. When top shoots/leaves were picked randomly, there was a possibility that leaves of different age tillers were selected. Therefore, the low ^{14}C and specific activities at earlier sampling times (59 h and 79 h) compared to the later sampling (103 h) would be associated with the samplings of older leaves.
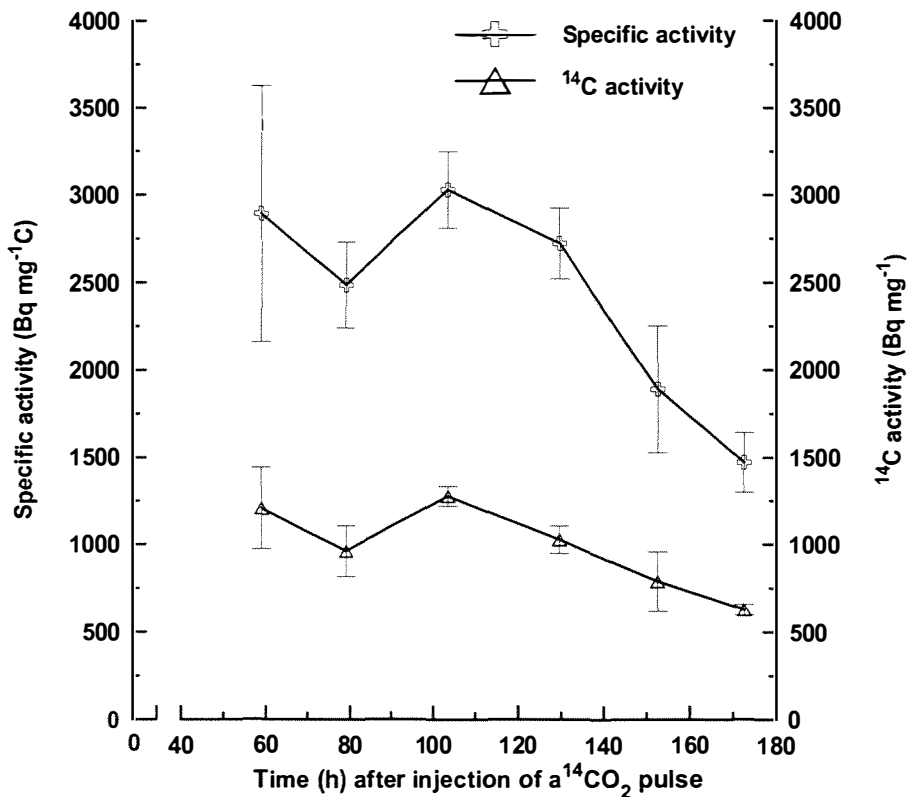
Figure 5.7: Change in specific activity (Bq mg⁻¹C) and ¹⁴C activity (Bq mg⁻¹) of ryegrass/clover sward shoots at different times after injection of a ¹⁴CO₂ pulse into the atmosphere above ground. *Error Bars: ±SE of mean of two cores.*

At harvest, 33.7% of the total ^{14}C counts injected into the enclosed canopy air above the pasture swards remained in the shoots (including ^{14}C counts in stems below ground) (Table 5.3). These rates of turnover of the ^{14}C pulse in the shoots are comparable to those measured by other workers. Saggar *et al.* (1997) reported that the proportion of net assimilated ^{14}C (i.e. sum of ^{14}C recovered in shoot, root and soil within 4 h after labelling) in shoots decreased from 58–74% at 4 h to 39–49% at 7 days and then to 26–31% at 35 days after labelling of permanent New Zealand grasslands. Similarly, Rattray *et al.* (1995) also reported that within a week of ^{14}C pulse labelling of *Lolium perenne*, about 50% of total assimilated ^{14}C was retained in the shoots.

5.3.4.2   Below ground recovery of ^{14}C

At harvest, the proportion of injected ^{14}C remaining below ground, as soil+roots, was 14.1% (i.e. sum total of all the depths of soil core) (Table 5.3). The greatest proportion of the total ^{14}C activity in soil+roots was recovered in the top 2 cm depth (54.6%) followed

by the 4 cm depth (22.2%) and only 23.2% remaining was present further down the soil core (Figure 5.5b). Furthermore, the specific activity of the soil+roots decreased with depth (Figure 5.5c), indicating a lower proportion of recently fixed ^{14}C against a background of 'native' soil+root C was allocated to lower soil depth.

The greater proportion of the total ^{14}C activity in roots (i.e. recovered from the whole soil core) was present in the top 2 cm depth (67.9%) followed by the 4 cm depth (16.8%) and only 15.3% was present further down the soil core, indicating relatively more new roots are formed and stored a greater proportion of the recently fixed ^{14}C in the top depth than in the lower depth. The root mass decreased (from 13.2 g to 0.4 g) down the soil core (Figure 5.5a), which again reflects in the lower proportions of the recently fixed ^{14}C activity that were recovered at lower soil core depth (Figure 5.5b).

Table 5.3 Distribution of total injected ^{14}C (12.17 MBq) in different components of pasture shoot–root–soil system a week after pulse labelling (i.e. harvest time).

Component	Amount (MBq) of ^{14}C	Proportion (%) of ^{14}C
^{14}C remaining in shoots (above–ground)	3.17 (0.55)[*]	26.0 (4.55)
^{14}C remaining in stems below ground plus adhering soil	0.93 (0.15)	7.7 (1.26)
^{14}C remaining in soil+roots (sum of all the depths)	1.72 (0.21)	14.1 (1.73)
^{14}C lost from the system by ^{14}CO$_2$ respiration (total injected ^{14}C minus ^{14}C in pasture–soil system)	6.35	52.2
^{14}C remaining in roots (sum of all the depths)	1.25 (0.14)	10.3 (1.17)

[*]*Values in brackets are standard errors of mean of two cores.*

Similarly, the proportion of ^{14}C remaining in roots alone, at harvest, was 10.3% of the injected ^{14}C (i.e. 73% of the total ^{14}C present in the soil plus roots together). More than 90% of the ^{14}C activity in the 2 cm depth were in the roots alone, while at the lower depths i.e. 4 cm and 8 cm slices, only up to 50% was present in roots and the remainder was

present in the soil (Figure 5.5b). Roots contained the greater proportion of ^{14}C in soil+root samples from 2 cm depth. This indicated a greater storage of the ^{14}C in the structural pool of the root. The greater proportion of ^{14}C activity present in the soil at lower depths may result from slower decomposition of root–released ^{14}C. The rate of decline in ^{14}C activity in soil water (see Section 5.3.3 above) also shows faster turnover of ^{14}C assimilates in the upper soil layer and slower turnover in the lower soil layers. Swinnen *et al.* (1994) attributed the loss of soil organic ^{14}C in the upper layer of the soil profile to the faster decomposition of recent root–released organic material, due to higher temperature and enhanced aeration. Lavahun *et al.* (1996) found in both grassland and arable sites that both microbial biomass C and mineralisation of soil organic C were higher in upper soil layer and decreased at lower depths.

The specific activity (Bq mg^{-1}C) of roots in the 0–2 cm soil layer was greater than the other soil layers (Figure 5.5c), indicating a greater proportion of ^{14}C activity were allocated to roots per unit proportion of C at the upper 0–2 cm layer. The specific activity decreased in the lower depth indicating that not many new roots are formed during the seven days of growth period after ^{14}CO$_2$ pulse labelling. However, the specific activity at the 8 cm soil depth showed an increase in the decreasing trend (Figure 5.5c), suggesting a greater allocation of labelled ^{14}C to the roots in the 4–8 cm soil layer.

In the present study, it was not possible to separate between shoot, root and soil respiration and back diffusion of soil ^{14}CO$_2$ into the atmosphere. Therefore, the unaccounted amount (52.2%) of the injected ^{14}CO$_2$ (Table 5.3), calculated as a difference between amount of ^{14}C injected and not recovered in shoots, roots or soil, was assumed to be lost through the system by shoot and root/soil respiration (Saggar *et al.*, 1997, 1999) and by back diffusion of soil ^{14}CO$_2$ into the atmosphere. The below ground allocation of ^{14}C, recorded in the present study, is within the range reported by Meharg and Killham (1990a) and Kuzyakov *et al.* (1999) (8–28%) for *Lolium perenne* at different stages of development. However, below ground allocation of ^{14}C reported by other workers (Rattray *et al.*, 1995; Saggar *et al.*, 1997, 1999) for similar plant material within a week after pulse labelling was greater than in the present study, and the ^{14}C recovered in the shoots (33.7%) within a week after pulse labelling was less in the present study. There could be a possibility that some of ^{14}CO$_2$ could have been lost during the process of sampling the residual reacting solution of Na$_2$^{14}CO$_3$ and acid (see methods Section 5.2.4 above). The

Geiger counter gives an imprecise estimate of the amount of $^{14}CO_2$ left unassimilated in the bag; therefore, when the bag was removed, small amounts of unassimilated $^{14}CO_2$ could have been lost into the atmosphere. These losses, if any, could lead to an overestimate of injected $^{14}CO_2$ and to lower estimates of the total ^{14}C allocated to shoots and below ground.

5.4   CONCLUSIONS

In situ sampling of soil solution with mini RSMS was an effective, simple and non–destructive method to monitor rapid (within 1 h) appearance of recently assimilated ^{14}C as root and microbial respired $^{14}CO_2$ in soil water and air. Normally all the ^{14}C in soil solution samples was $^{14}CO_2$. The contribution from diffusion of $^{14}CO_2$ in the soil water was uncertain and needs quantifying before statements can be made about the amounts of root–released ^{14}C. Similarly diffusion of atmospheric $^{14}CO_2$ into the soil may cause overestimate of photo–assimilation. The mini RSMS (suction cups) can also be used to monitor the diurnal variation in root activity at various soil depths in terms of SWP created at the root–soil water interface.

At harvest (one week after pulse labelling) little *de nouveau* synthesis of SOM had occurred. Most rhizodeposited ^{14}C had been respired. This observation supported the finding of the present study that initially soil water contained only $^{14}CO_2$ and not ^{14}C as soluble exudates. Either the root–released labile C is small or it is rapidly metabolised and respired by rhizosphere microorganisms. This technique (using RSMS in conjunction with ^{14}C pulse labelling) opens up new opportunities for researchers in improving their understanding of various rhizosphere processes.

The depth distribution of recently assimilated ^{14}C to pasture roots suggests that rhizodeposition occurs to a greater extent in the upper layer of the soil. However the expected increased rate of root–released ^{14}C cannot be observed probably because of faster decomposition of root–derived C, due to high temperature and enhanced aeration, and high microbial activity of the layer closer to the soil surface.

The $^{14}CO_2$ pulse labelling technique is very useful in monitoring the fate of recently assimilated ^{14}C to various components of pasture shoot–root–soil system, but using a Gieger counter to monitor the $^{14}CO_2$ activity in the enclosed air above pasture sward may

not give an accurate estimate of the extent of $^{14}CO_2$ assimilation. Taking air sample from the enclosed air at the end of the pulse labelling would give a better estimate of the extent of assimilation of a known volume of $^{14}CO_2$ by a plant, rather than using a Gieger counter. These improvements to the technique are implemented in Chapter 7.

CHAPTER 6   CHARACTERISATION OF RECENT ROOT AND ROOT–RELEASED CARBON BY SOIL ORGANIC MATTER FRACTIONATION

6.1   INTRODUCTION

In Chapter 4, the sequential chemical fractionation procedure of Hedley *et al.* (1982) identified two fractions of soil S and C (the 0.1 M NaOH and the residual) that showed most change with change in land management practices. Both fractions decreased significantly under continuous cultivation of maize and increased during a pasture renovation phase. The residual fraction reports on dynamic SOM but within the chemical fractionation is intended to report on a 'nominally' non–labile fraction.

In this chapter the 'functional' nature of these chemical fractions is investigated by examining the fate of recently fixed ^{14}C in a permanent pasture soil. Recent rhizodeposited C (including roots and root–released soil ^{14}C) is characterised by the chemical fractionation (Hedley *et al.*, 1982) described in Chapter 3 and density fractionation procedure similar to that used by Magid *et al.* (1996).

Soil organic matter is made up of a range of materials that degrade at variable rates due to their chemical composition as well as the degree of protection by absorption to minerals or entrapment within aggregates (Collins *et al.*, 1997). A wide variety of techniques have been applied to the measurement and characterisation of SOM in relation to its dynamics under various management conditions. Classical approaches have combined chemical extractions with identification of specific chemical compounds. Functional approaches have attempted to provide a description of SOM pool dynamics by incorporating radio– and stable–isotopes as tracers, or using ^{14}C–dating techniques, to identify specific fractions that are biologically active. Stevenson and Elliot (1989) stressed that methods used to evaluate SOM dynamics should be related to fractions or pools that have biological significance if they are to relate to the potential for soils to provide nutrients to plants.

It is generally accepted that a simple description of SOM dynamics can be achieved by using the concept that SOM contains fractions with a rapid turnover rate and fractions with a slower turnover rate (Cambardella and Elliott, 1992). The fractions with a rapid turnover (biologically active fractions) are assumed to play a dominant role in soil nutrient dynamics (Parton *et al.*, 1988; Janzen *et al.*, 1992).

Density fractionation techniques have been used by number of workers (Janzen *et al.*, 1992; Hassink, 1995; Barrios *et al.*, 1996; Magid *et al.*, 1996) to isolate the so-called light fraction. The size of the light fraction has been found to be more sensitive to differences in land management and input of residues than total organic matter (Dalal and Mayer, 1987; Janzen *et al.*, 1992; Hassink, 1995). A review on physical fractionation of soil organic matter by Christensen (1992) concluded that while density fractionation of soil has shown some promise for quantifying biologically meaningful fractions, there is a need to further identify the labile component of SOM with measurable and distinct turnover rates. Collins *et al.* (1997) suggested that physical fractionation followed by chemical, or biological, assays of lability is a useful approach to the study of SOM dynamics because of the unclear (labile or recalcitrant) nature of SOM being protected from microbial access within soil aggregates.

Earlier studies on the decomposition of ^{14}C labelled ryegrass tops and roots (Jenkinson, 1977a, 1977b) and blue grama grass herbage and roots (Nyhan, 1975), and a recent study on the decomposition of ryegrass roots (van Ginkel and Gorissen, 1998) have shown that the plant materials were attacked rapidly at first, but after a few months the rate slows substantially although considerable amounts of plant derived C remain in the soil. The decreased rate over time was due to the differences in the rate at which the various plant components decompose (e.g., lignin decomposes quite slowly) and to the resynthesis (during decomposition) of the more readily degraded constituents (e.g., soluble and cellulose) into microbial C components that are more resistant to decay than the original plant material (Stevenson and Cole, 1999). The components of SOM that undergo decay initially were the most labile components of SOM but less labile components may be created simultaneously.

In the present study, an *in situ* method of ^{14}C labelling of roots and soil (Chapter 5) was designed whereby plants were exposed to a $^{14}CO_2$ radioactive pulse and the recently

assimilated ^{14}C photosynthates (comprises largely of simple sugars and amino acids) were translocated from shoots to roots and subsequently released into undisturbed soil either as exudates, litter or respired as CO_2. Harvesting plants within a week of pulse labelling retained a considerable proportion of recently translocated ^{14}C photo–assimilate within the roots and soil (see Chapter 5). This ^{14}C labelled material can be considered more labile on average than the 'native' unlabelled C already present in the roots and soil.

The objectives of this study were:

a) To use "recent" ^{14}C labelled rhizodeposited soil C (including roots) to investigate the 'functional' value of fractions characterised by chemical and density SOM fractionation procedures with the specific purpose of characterising labile soil C fractions, and

b) To trace the redistribution and fate of recent rhizodeposited ^{14}C in different soil and root C fractions obtained from different depths of undisturbed pasture soil.

6.2   MATERIALS AND METHODS

6.2.1   *Soil*

Detailed description of the soil characteristics and soil site is given in Section 5.2.1.

6.2.2   *Soil core collection*

Detailed description of the collection of duplicate undisturbed soil cores is given in Section 5.2.2.

6.2.3   *Production of ^{14}C labelled rhizodeposited soil C*

Undisturbed soil cores growing pasture swards were pulse labelled with ^{14}C by enclosing the swards in a sealed plastic bag and introducing $^{14}CO_2$ (12.17 MBq per soil core) into the sealed environment. Plants were left enclosed for 2 h after $^{14}CO_2$ injection. The labelled rhizodeposited C (roots and root–released soil ^{14}C) and the total C in soil+root

samples were fractionated using the chemical and density separation procedures. A more detailed description of $^{14}CO_2$ pulse labelling technique is given in Section 5.2.4.

6.2.4 *Sampling and harvesting*

Plants were harvested and the soil cores dismantled for soil and root sampling one week after ^{14}C pulse labelling.

6.2.4.1 *Root and soil sampling and their preparation for analysis*

A detailed description of harvesting shoots and roots, and sampling soil cores is given in Section 5.2.6.2.

6.2.5 *Evaluation and modification of SOM fractionation procedures*

6.2.5.1 *Chemical fractionation of soil+roots and roots alone (Experiment 1)*

Duplicate samples (2 g) of oven dried, sieved soil+roots were taken from different soil core depth samples (i.e. 0–2, 2–4, 4–8, 8–12, 12–16 and 16–20 cm) and the "recent" rhizodeposited ^{14}C and total C in the soil+root samples were fractionated chemically by the procedure described in Section 3.2.1 for soil C. The anion exchange resin was used in the OH⁻ form instead of HCO_3^- form. Also, after soil extraction, both the anion and cation exchange resins were eluted in 0.5 M $NaNO_3$ instead of 0.5 M NaCl to avoid Cl⁻ interference (Quinn and Saloman, 1964) during the estimation of total C (Amato, 1983) (Section 3.2.5.1). The change in elutant to 0.5 M $NaNO_3$ from 0.5 M NaCl caused no change in the recovery of ^{14}C counts from the resin membranes. However, the change in anion resin form from HCO_3^- to OH⁻ increased the extraction of ^{14}C counts from soil (Figure 6.1a).

In the upper soil layers where root density was high (*cf.* Figure 5.5a), ring grinding soil and roots allowed greater recovery of ^{14}C activity by OH⁻ resin than simply sieving the sample to <2 mm (Figure 6.1b). Ring grinding obviously reduced sample particle size and mixed the sample more uniformly allowing sub–sampling to produce a lower standard error of the mean (Figure 6.1b). All soil+root samples were then subsequently ring ground prior to chemical fractionation studies, including acid digestion (sulphuric/nitric), strong
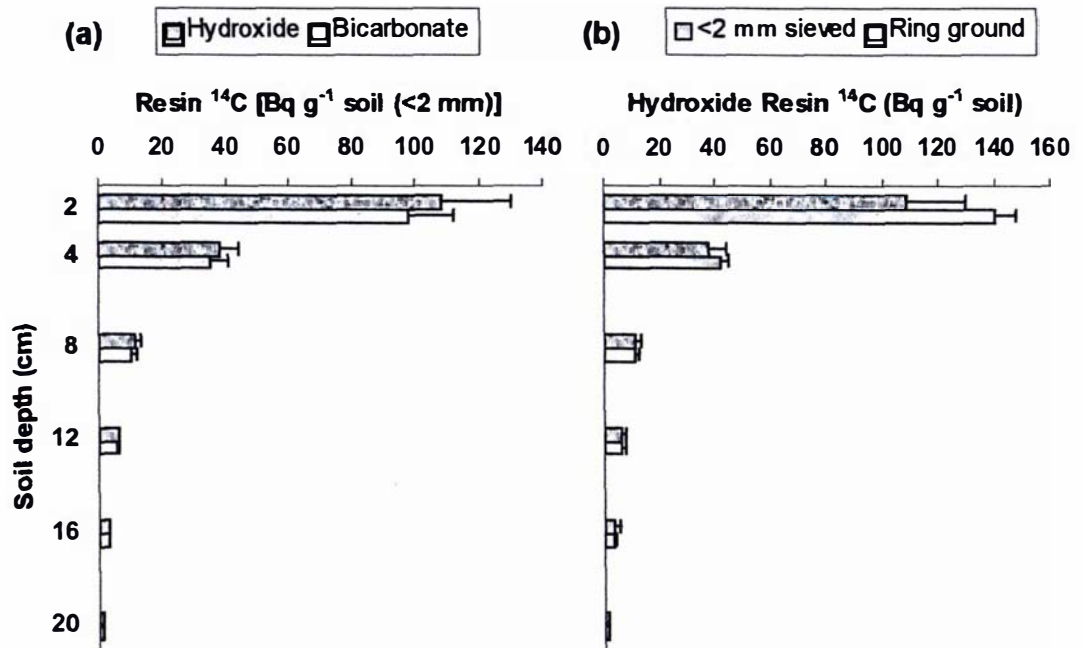
Figure 6.1: Comparison of ^{14}C counts recovered by ion exchange resin extraction from <2 mm sieved soil (a) with resin in the hydroxide and bicarbonate form and (b) ^{14}C counts recovered from either <2 mm soil or ring–ground soil. Soil samples were taken from different depths below a ^{14}CO$_2$ pulse labelled pasture sward. *Error Bars: SE of mean of two cores.*

oxidation (hydrogen peroxide/sodium perborate) or anaerobic incubation (see Sections 6.2.5.2, 6.2.6.1 and 6.2.6.2).

Chemical fractionation of roots alone

To determine the distribution of the recent root ^{14}C into the alkali soluble fractions (0.1 M NaOHand 1 M NaOH) and the alkali–insoluble residual fraction, a known weight (96 mg for core 1 and 75 mg for core 2 – taken in duplicate) of ^{14}C labelled roots, wet sieved from the duplicate segment of 2 cm soil depth, were fractionated chemically by the procedure described in Section 3.2.1 for soil C. The sequential extraction procedure for roots did not include the resin extraction step.

6.2.5.2   Modifications to the chemical fractionation of soil+roots (Experiment 2)

As most of the recent root and root–released ^{14}C was not extracted in alkali of increasing strength (Section 6.3.2.1), some modifications to the chemical fractionation technique (such as incorporating hot H_2SO_4 hydrolysis in the fractionation scheme) were attempted to recover root C in fewer fractions. These modifications were attempted as explained in the following subheading.

Hot H_2SO_4 (0.5 M) hydrolysis of whole soil+roots (Treatment a) and residual fraction (Treatment e)

The total C and ^{14}C activity in the whole soil+roots and the alkali–insoluble residual fraction of soil+roots were acid hydrolysed (Figure 6.2a,e) following the method of Cheshire *et al.* (1983) for sugar analysis. Dried, ring ground, duplicate samples (0.1 g) were firstly shaken with 1 ml of 12 M H_2SO_4 for 16 h and then diluted to 24 ml with distilled water (0.5 M H_2SO_4), and heated at 100°C for 8 h. The hydrolysates were neutralised to pH 7.0 with 6 M NaOH solution, centrifuged and filtered. The filtrates were analysed for total ^{14}C activity and total C. The residues obtained after the acid hydrolysed soil+roots were not subjected to further chemical fractionation because this procedure hydrolysed most of the recently fixed ^{14}C (Section 6.3.3.1). Similarly, the residues obtained after the acid hydrolysis of the residual fraction of soil+roots were not analysed for total C and ^{14}C activity (Section 6.3.3.2).

6.2.5.3   Density separation of soil+roots (Experiment 3)

The density fractionation procedure was similar to those described by Magid *et al.* (1996). The whole soil (including roots) without size separation was density fractionated. Sodium polytungstate solution (ρ = 1.4 g cm^{-3}) was used as a low viscosity density reagent. An ultrasonic bath was used to ensure proper dispersion of soil aggregates and to minimise the inclusion of soil micro–aggregates in light fraction. Without ultrasonic dispersion, entrapped air and adsorbed water would lower their effective density (Christensen, 1992).
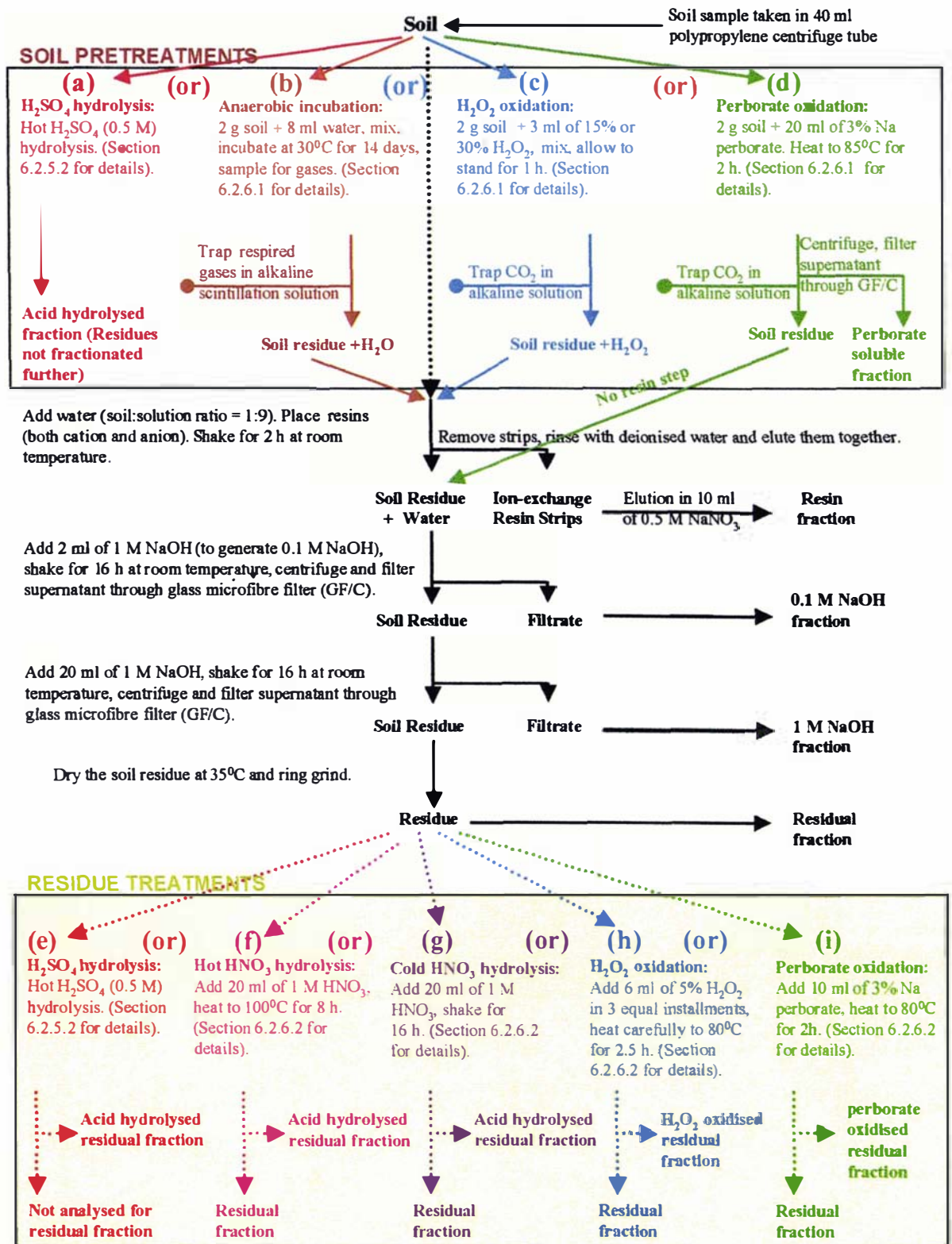
Figure 6.2: A range of modifications to the chemical fractionation technique that attempt to recover recently synthesised root carbon by one of four pre-alkali extraction treatments (a, b, c or d) or one of five treatments of the alkali–insoluble residue (e, f, g, h or i).

In soils with high calcium saturation there is a risk of insoluble calcium polytungstate forming during density separation. To facilitate dispersion and remove calcium, a NaCl extraction was conducted prior to the density separation.

Duplicate 10 g soil+root samples (<2 mm) were weighed into 40 ml polypropylene centrifuge tubes and 20 ml of 5% NaCl solution was added (Figure 6.3). The tubes were shaken end over end for 45 minutes and centrifuged thereafter at 2204 ×g (3500 rpm) for ten minutes. The supernatant was filtered through a Whatmann glass micro fibre filter (GF/C) into a container. Distilled water (15 ml) was added to the pellet of soil and roots left in the tube to wash off excess NaCl. The tube contents were shaken well, centrifuged again at the aforementioned gravity, and the supernatant filtered through the same GF/C filter into the same container. The accumulated supernatant (Figure 6.3) was stored for analysis of soluble C and ^{14}C.

The residual roots adhering to the filter paper were washed with 25 ml of sodium polytungstate ($\rho = 1.8$ g cm^{-3}) into a 50 ml beaker and then transferred back to the

Soil+roots

Dispersion in 5% NaCl → **Soluble fraction**

Soil+roots residue

Density separation in Sodium Polytungstate

Settled / *Floating*

Heavy fraction
(>1.40±0.05 g cm^{-3})

Light fraction
(≤1.40±0.05 g cm^{-3}; >54 μm)

Subjected to chemical fractionation
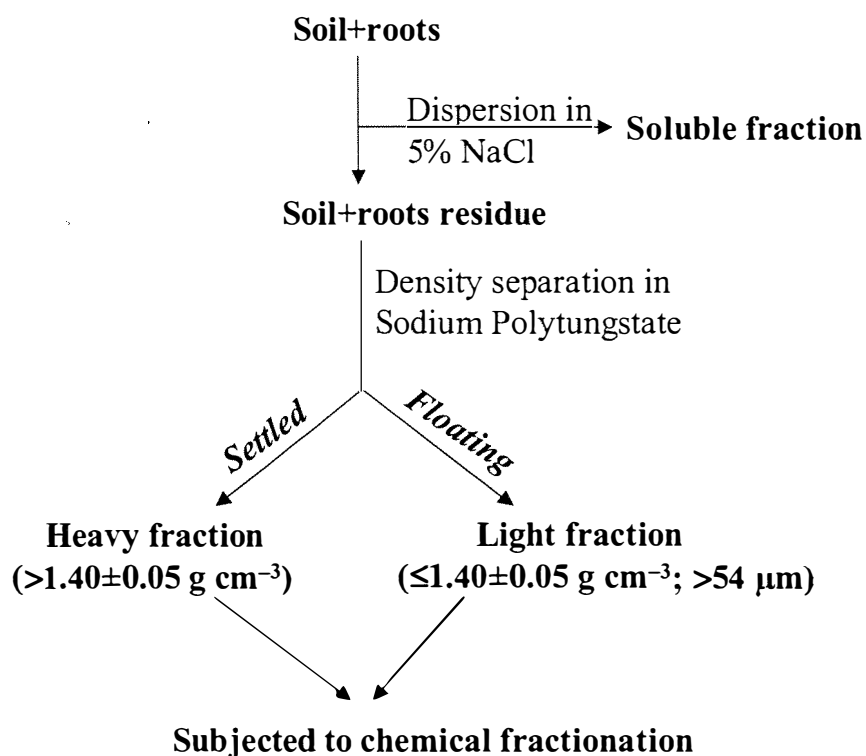
Figure 6.3: A soil organic matter density fractionation procedure followed by chemical fractionation

centrifuge tube, which contained the residual soil and roots. The amount of water with the residual soil and roots was adjusted so as to make the density of the polytungstate solution between 1.40 ± 0.05 g cm^{-3} (Figure 6.3). The tube contents were shaken well and the residual pellet redispersed. The tubes were then placed in an ultrasonic bath (power rating – 200 watts) for 30 minutes. After ultrasonic dispersion, tubes were left to stand overnight. The following morning, the top half of the suspended soil and floating roots was sucked out of the tube with a rubber hose attached to side arm flask (under vacuum). A glass tube with 54 μm nylon mesh fitted at the top was placed inside the flask for collection of the light fraction. The light material collected on the mesh (mostly particulate organic matter/roots) was washed thoroughly to remove any soil particles and polytungstate solution. This represented the light C fraction of >54 μm in size.

The suspended soil+roots (<54 μm) and the polytungstate solution collected in the glass tube were poured back into the centrifuge tube. The tube contents were shaken well, centrifuged and the supernatant decanted. The residual pellet (the heavy fraction) in the centrifuge tube was then washed three times with distilled water and centrifuged to remove excess sodium polytungstate and the supernatant (including the light floating fraction) from each washing decanted. Both heavy and light large (>54 μm) C fractions were dried at 70°C, and the whole heavy C fraction was ring ground as described for the whole soil samples (Section 5.2.6.2).

6.2.5.4   Density separation of roots, and roots remixed with non–radioactive soil (Experiment 4)

The density separation (Figure 6.3) of known weights (0.3 g and 0.12 g of roots from 2 and 4 cm soil depths, respectively) of radioactive ^{14}C roots (taken in duplicate from both cores) was also conducted. To examine the influence of the presence of soil on the distribution of root ^{14}C in soil C fractions, the same amounts of radioactive roots (i.e. 0.3 g and 0.12 g of roots from 2 and 4 cm soil depths, respectively) were remixed with a known amount (10 g) of non–radioactive Tokomaru soil (air–dried and <2 mm sieved) and then fractionated (in duplicate) by the density separation procedure described above (Section 6.2.5.3; Figure 6.3). The polytungstate solution (supernatant) obtained at the end of the density separation procedure was collected and stored for the analysis of ^{14}C. The

polytungstate supernatant would provide the soluble fraction of the root ^{14}C that remained in the polytungstate at the end of the density separation procedure.

6.2.5.5   Chemical fractionation of density fractions (Experiment 5)

Both the light (4.5–48.7 mg) and the heavy C fractions (2 g) were further subjected to the chemical fractionation by the procedure described in Section 3.2.1 for soil C. However, during the chemical fractionation of the light fraction, a technical problem was that the roots in the light fraction decanted with each extract. To overcome this problem, the entire light fraction was collected onto a GF/C filter while filtering supernatants during various steps of the fractionation procedure. Both the GF/C filter and the entire light fraction were processed through the various fractionation steps without any interference from the glass fibre in the analysis. The light fraction was not sub–sampled for analysis of total ^{14}C and total C, because the amount of the fraction recovered during the density separation was too small to be used for the analysis of total ^{14}C and total C. The ^{14}C activity and C content in all the chemical fractions of the light fraction were summed to determine the total ^{14}C activity and total C content in the light fraction. Duplicate samples of the heavy fraction were analysed for total C and ^{14}C (Sections 6.2.7.1 and 6.2.7.2).

6.2.6   Further attempts to modify the chemical fractionation procedure (Experiments 6 and 7)

6.2.6.1   Pre-treatments followed by chemical fractionation of soil+roots (Experiment 6)

Treatment b, anaerobic incubation of soil+roots followed by chemical fractionation

Anaerobic incubation of soil under waterlogged conditions for 14 days (Waring and Bremner, 1964) is used as a routine and rapid (i.e. mineralisation is more rapid under waterlogged than under aerobic conditions) method of assessing the potential ability of the soil to provide nitrogen to crop growth in many soil–testing laboratories. The anaerobic incubation was conducted, to determine the C fractions that were mineralised most during the incubation, in two ways: a) like the original method, soil+root samples were incubated anaerobically and sampled for microbial–respired gases at the end of incubation (i.e. full–

term incubation), and b) sampled for the gases at certain specific intervals (i.e. sampled during incubation). The detailed methodology is described below.

Four samples (2 g each) of soil+roots from the 2 cm depth of one of the cores were weighed into 40 ml polypropylene centrifuge tubes and distilled water (8 ml) was added. The tubes were then sealed with subaseal[TM] no. 37. The tube contents were mixed using a vortex mixer, incubated for 14 days at 30°C (Waring and Bremner, 1964), and sampled for respired gases (CO_2, CH_4 etc.) at the end of incubation period (i.e. full–term incubation) from two tubes. The gas samples were dissolved in alkaline scintillation (toluene based) solution placed in a sealed centrifuge tube and then counted for ^{14}C. Alkaline scintillation solution was prepared by mixing 1 ml of 0.2 M NaOH + 0.02 M Na_2CO_3 and 5 ml of scintillation solution (see the scintillation solution recipe in Section 5.2.7.2).

The remaining two soil+root samples were also incubated anaerobically for 14 days but sampled (i.e. 20 ml at each sampling time) for respired gases at 4, 8, 11 and 14 days of incubation period, and replaced with same volume of N_2 gas at each time (i.e. sampled incubation). The gas samples were dissolved in alkaline scintillation cocktail solution as described above. Controls were air–dried soil+root samples, without water, kept in sealed tubes (in duplicate) for both types of incubation (i.e. full–term and sampled incubations).

At the end of the incubation, the volume of the water in all the tubes was adjusted to 18 ml and the tube contents were then fractionated chemically (Figure 6.2b). All the fractions were analysed for ^{14}C activity and total C except the respired gases, which were only counted for ^{14}C activity.

Treatment c, H₂O₂ oxidation of soil+roots followed by chemical fractionation

The mild H_2O_2 treatment has been used either to oxidise a proportion of soil organic S (Surinder Saggar, Landcare Research, New Zealand Pers. Comm.), or to recover various soil/peat humic acid structural products under alkaline conditions at room temperatures (Almendros *et al.*, 1987), in contrast to drastic H_2O_2 oxidation at high temperatures, which yields more non–structural oxidation products like oxalic acid, CO_2 etc. (Griffith and Schnitzer, 1977). In the present study, the idea of the H_2O_2 treatment (at room temperature) was to oxidise some of the alkali–insoluble residual fraction to alkali soluble fractions.

Preliminary studies with standard soil samples showed that less than 1% of the total C in soil+root samples was oxidised as CO_2 after treatment with either 15% H_2O_2 or 30% H_2O_2 at room temperatures. This indicated that the hydroxyl radical, formed due to decomposition of the H_2O_2 (i.e. in contact with soil organic matter), is probably causing the abstraction of a hydrogen atom from the organic molecule and thereby breaking the complex polymers (Chin *et al.*, 1997), and may be releasing relatively simple organic ions, which could readsorb to soil surfaces. Therefore, the soil and H_2O_2 mixture was subsequently diluted either with dilute nitric acid (pH = 2) or dilute NaOH (pH = 8.5) solutions followed by shaking in the presence of ion–exchange resins. Resins were eluted as described earlier (Section 6.2.5.1). The detailed methodology is described below.

Ten dried, ring ground, samples (2 g each) of the soil+roots from the 2 cm depth of one of the soil cores were weighed into polypropylene centrifuge tubes, and the tubes sealed with a subaseal[TM] no. 37 bung. The CO_2 trapping solution (6 ml of 1 M NaOH + 0.01 M Na_2CO_3) was pipetted into 50 ml beakers and placed in 600 ml preserving jars, which were then sealed and evacuated. The sealed centrifuge tubes were then connected to the preserving jars through the connecting assembly, which is made up of PVC non–toxic tubing with 3–way stopcocks and hypodermic needles attached to both ends. Four samples of soil+roots were treated with a known volume (3 ml) of either 15% or 30% H_2O_2 by injecting the H_2O_2 into the sealed centrifuge tube with a syringe. The samples were then vortex mixed and allowed to react for 1 h at room temperature with regular mixing every 15 minutes interval. Any CO_2 produced was absorbed by the trapping solution placed in the jar. After one hour, the connecting assembly was removed and the volume of the H_2O_2 inside the centrifuge tube diluted to 18 ml with weak acid (HNO_3, pH = 2) and base (NaOH, pH = 8.5) solutions so as to give two samples for each treatment. These solutions were then subjected to resin extraction followed by alkali extraction (Figure 6.2c). Duplicate samples of soil+roots were chemically fractionated as control (i.e. without H_2O_2 and pH treatments). The jars were left sealed overnight for complete absorption of the CO_2 gas in the trapping solution. All the extracts and samples were analysed for ^{14}C and total C.

Hydrogen peroxide oxidation of soil+roots was also conducted at high temperature (50°C) in the centrifuge tube but the reaction was found to be too rapid and destructive and did not work due to too much frothing.

Treatment d, perborate oxidation of soil+root followed by chemical fractionation

The capacity of sodium perborate to produce H_2O_2 in contact with organic acids is well known (Almendros _et al._, 1987). Almendros _et al._ (1987) used 5% sodium perborate to oxidise soil/peat humic acids at 140°C that yielded high proportions of oligomeric products and the relatively low loss of C (about 10% of total humic acid C) by oxidation to CO_2. In the present study, pre-treatment of soil+roots with Na–perborate (3%) at 85°C was also tried to oxidise alkali–insoluble residual fraction to relatively simple alkali extractable organic compounds.

Duplicate, ring ground samples of soil+roots (2 g) from the 2 cm depth were weighed in 40 ml centrifuge tubes and 20 ml of 3% Na–perborate ($NaBO_2.H_2O_2.3H_2O$) solution was added and sealed immediately with a subaseal^{TM} no. 37 bung. The centrifuge tubes were then connected to the evacuated preserving jars that contained the CO_2 trapping solution in a beaker as described above for H_2O_2 oxidation procedure. The tube contents were heated carefully (i.e. the tubes were removed upon the formation of froth and cooled immediately by immersing in a cold water and reheated until the solution was clear) up to 85°C in a water bath for 2 h. The tube contents were centrifuged and the supernatant was filtered through a glass microfibre filter (GF/C) and the residues were then subjected to alkali extraction (Figure 6.2d). The resin fractionation step was omitted. All the samples were analysed for ^{14}C and total C.

6.2.6.2   Treatments of the residual fraction of soil+roots (Experiment 7)

Initially, duplicate, ring ground soil+root samples (2 g) from the 2 cm soil depth of both the soil cores were chemically fractionated (Section 3.2.1; Figure 3.1). At the end of the alkali extraction, the residual fraction was further subjected to various alternative treatments (Figure 6.2f,g,h,i). All the fractions were analysed for ^{14}C and total C except the residues obtained at the end of the hot 0.5 M H_2SO_4 hydrolysis of the residual fraction [(see Sections 6.2.5.2 (Treatment e) and 6.3.3.2)]. The further alternative treatments were:

Treatment f, hot HNO_3 (1 M) hydrolysis

The alkali–insoluble residual fraction of the soil+roots was further hydrolysed by adding 20 ml of 1 M HNO_3 and heating to 100°C in a water bath for 8 h (Figure 6.2f). The hydrolysates were adjusted to pH 6.5–7.0 with 6 N NaOH solution, centrifuged and filtered through a GF/C.

Treatment g, cold HNO_3 hydrolysis

Similar to hot 1 M HNO_3 hydrolysis, 20 ml of 1 M HNO_3 was added to the residual fraction of soil+roots (Figure 6.2g) and shaken on end–over–end shaker for 16 h. The hydrolysates were adjusted to pH 6.5–7.0 with 6 M NaOH solution, centrifuged and filtered through a GF/C.

Treatment h, H_2O_2 oxidation

At the end of the alkali (i.e. 1 M NaOH) extraction (Figure 6.3h), the centrifuge tubes were sealed with a subseal™ no. 37 bung and connected to evacuated preserving jars that contained the CO_2 trapping solution in a beaker (see Section 6.2.6.1; Treatment c). A known volume (2 ml) of 5% H_2O_2 was injected into the tube containing the residual fraction (and some leftover 1 M NaOH) of soil+roots and mixed using a vortex mixer. The tube contents were placed in a water bath (40°C) for 30 minutes until the frothing had subsided. The tubes were then placed in a water bath adjusted to 80°C for further 30 minutes, removed and allowed to cool. After 5 minutes of cooling, another 2 ml of 5% H_2O_2 added and allowed to react (froth) for 20 minutes and mixed occasionally during the frothing period using a vortex mixer. When the froth settled down, the tubes were heated again at 80°C for 20 minutes, removed and allowed to cool. Again, after 5 minutes of cooling, 2 ml of 5% H_2O_2 was added and allowed to froth for 20 minutes and mixed occasionally during the frothing period. The tubes were then heated again at 80°C for 30 minutes. The tubes were removed from the water bath, and 14 ml of 1 M NaOH was added and shaken for 1 h, centrifuged, and filtered through a GF/C.

Treatment i, perborate oxidation of soil+roots

Ten ml of 3% Na–perborate ($NaBO_2.H_2O_2.3H_2O$) solution was added to the alkaline residual fraction of soil+roots (Figure 6.2i) and sealed immediately with a subseal™ no.

37 bung. The centrifuge tubes were then connected to the evacuated preserving jars that contained the CO_2 trapping solution in a beaker (Section 6.2.6.1; Treatment c). The tube contents were carefully heated to 85°C in a water bath for 2 h. The tubes were centrifuged and the supernatant was filtered through a GF/C.

6.2.7 *Analysis*

This section describes the analysis of total C and ^{14}C activity of various samples (soil and roots, roots alone, various alkaline, acid and salt solution extracts, light and heavy density fractions, residual fractions etc.) obtained in this study.

6.2.7.1 *Total C analysis*

The total C content in all the samples except the acid (0.5 M H_2SO_4) hydrolysate was analysed by the modified dichromate digestion procedure (Section 3.2.5).

The total C in acid (0.5 M H_2SO_4) hydrolysate of the whole soil+roots and the residual fractions was determined by oxidation/refluxing method adapted from Vance *et al.* (1987). The method involves the use of two sets of six round bottom flasks (150 ml) so that one set can be titrated whilst the other is refluxing. A few anti–bumping granules were placed into the flask and 2 ml of 0.0667 M $K_2Cr_2O_7$ was added. Eight ml of the neutralised hydrolysate along with 16 ml of sulphuric – phosphoric acid mixture (2:1 ratio) were added into the flask and this flask was swirled to mix the content. The flask was fitted to the condenser and refluxed for at least 20 minutes. After 5 minutes of cooling, the top of the condenser was rinsed with 15–20 ml water and the flask was then removed from the hot plate and allowed to cool to <60°C. The content of the flask was titrated against acid ferrous ammonium sulfate (0.0333M in 0.4 M H_2SO_4) using phenylanthranillic indicator until the colour changes from purple to a clear green end point. The amount of the C in the hydrolysate was calculated assuming that 1 ml of 0.0667 M $K_2Cr_2O_7$ is equivalent to 1.2 mg C.

6.2.7.2 *Total ^{14}C analysis*

<u>*Scintillation cocktail recipe*</u>

The recipe for scintillation cocktail solution used for ^{14}C counting is given in Section 5.2.7.2

Standardisation of liquid scintillation counter for chemical and colour quench correction

The 0.1 M NaOH extract of soil was highly coloured because it was used before 1 M

Table 6.1: Change in ^{14}C counts and counting efficiency with the addition of different amounts of 0.1 M NaOH soil extract

Initial DPM of internal standard ^{14}C solution	Initial counting efficiency	Treatments (addition of 0.1M NaOH extract to soil)	Counting efficiency after the treatments (%)	% DPM recovered after treatment
561117	92.1	Blank (0.2 ml of water)	91.9	99.0
561387	92.1	0.05 ml	90.4	99.5
558500	92.1	0.1 ml	88.2	99.8
557788	92.1	0.15 ml	85.5	99.0
562508	92.0	0.20 ml	83.0	98.0
560478	92.1	0.25 ml	81.1	96.4
568013	92.1	0.30 ml	78.9	94.1
565000	92.1	0.35 ml	76.8	92.0

NaOH extraction in the fractionation procedure. Chemical and colour quench standards were prepared to standardise the liquid scintillation counting technique. Different volumes (blank, 0.05, 0.1, 0.15, 0.2, 0.25, 0.3 and 0.35 ml) of the 0.1 M NaOH extract of non–radioactive topsoil (0–5 cm depth) were added to the cocktail solution containing a standard amount of the ^{14}C counts as $Na_2{}^{14}CO_3$. Percent recovery of the ^{14}C counts and change in the counting efficiency were recorded to determine the optimum volume of NaOH soil extract required to give a better recovery of ^{14}C counts and maintain high counting efficiency. A comparison between the optimum amount of the 0.1 M NaOH soil extract volume and counting efficiency was made and a 0.2 ml aliquot (Table 6.1) was selected for the analysis of total ^{14}C using a Wallac 1414 WinSpectral^{TM} LSC (see Section 5.2.7.3).

^{14}C analytical procedure

The total ^{14}C activities in soil (with roots), roots alone, alkali–insoluble residual fractions of soil+roots, residues obtained at the end of all the fractionations (Figure 6.2b,c,d,f,g,h,i) (except the hot 0.5 M H_2SO_4 hydrolysed residues – Figure 6.2a,e), and the 'heavy' fraction (Figure 6.3) were determined by the modified dichromate digestion procedure (see Section 5.2.8).

In order to determine the ^{14}C activity in various extracts, a known volume of each extract [1 ml of the 0.5 M $NaNO_3$ resin elutant, 0.2 ml of the alkali extracts (0.1 M and 1 M NaOH), obtained during the chemical fractionation of the soil and the roots, 1 ml of 5% NaCl and Na–Polytungstate waste solutions obtained during density fractionation, 1 ml of resin elutant and 0.1 M NaOH, and 0.5 ml of 1 M NaOH obtained during chemical fractionation of the light and heavy density fractions; 1 ml of sulphuric acid hydrolysates; 1 ml of nitric acid hydrolysates; 0.2 ml of Na–perborate extract, 0.2 ml of 5% H_2O_2 + 1 M NaOH soil extract, 0.2 ml of 1 M NaOH + 0.1 M Na_2CO_3 trapping solution] was pipetted into polypropylene mini scintillation vials. The volume of the extracts, which were less than 1 ml, was adjusted to 1 ml with water. Five ml of scintillation cocktail solution (see Section 5.2.7.2) was added to vials. The alkaline cocktail solution obtained in the incubation studies (Section 6.2.6.1) was taken directly into the scintillation vials. These vials were capped and shaken well and counted for ^{14}C activity using a Wallac 1414 WinSpectral^{TM} LSC (see Section 5.2.7.3).

The counting efficiency of all the reagents/cocktail mixtures was checked using internal ^{14}C standards. Results were converted from counts (cpm) to dps based on counting efficiency.

Acidification of the resin elutant and cocktail solution suspension to remove ^{14}C labelled bicarbonate

After counting ^{14}C in the resin extracts, the suspension of the 0.5 M $NaNO_3$ resin elutant and cocktail solution was acidified to pH ≤ 4.5 as described in Section 5.2.6.1 for soil solution. This was done to remove $^{14}CO_2$ and $H^{14}CO_3^-$, and clarify the nature of C extracted onto resins. After shaking well, the vials were placed open in a fume hood for 3

days and then placed in a sonic bath for 20 minutes and measured for ^{14}C activity (see Section 5.2.7.3).

6.2.8 *Chloride ion interference in the 5% NaCl extract for total C determination*

There was no chloride interference observed during the dichromate digestion of the resin elutant (0.5 M $NaNO_3$) for C estimation from Tokomaru soil. This is in contrast to the chloride interference observed during the digestion of $NaNO_3$ resin elutant from Kairanga soil (see Section 4.2.5).

The total C in extracts having chloride in them (e.g. 5% NaCl reagent in the density fractionation) could not be analysed by the oxidation/refluxing technique (Vance *et al.*, 1987) because chloride reacts with potassium dichromate (Quinn and Saloman, 1964) interfering with the titration finish.

The total C in the 5% NaCl density fractionation extract was determined by the dichromate digestion procedure (as described in Sparling *et al.*, 1991) where CO_2 is evolved and trapped (see Section 3.2.5). The small amount of chloride ion interference (see Section 3.2.5.1) in the full C determination by the dichromate digestion and CO_2 trapping procedure was accounted by using the standard chloride correction curve (see Section 3.3.2.3).

6.3   RESULTS AND DISCUSSION

6.3.1   Total C and ^{14}C activity in samples of soil+roots and roots from different soil depths

The total C and ^{14}C activity in soil+root samples, per unit mass of soil+roots, were greatest in the 0–2 cm soil layer and decreased considerably with depth (Figure 6.4a,b). The pattern of decline of total C in soil+roots with depth was linear and not like the curvilinear (exponential) decline shown in both root C (Figure 6.4b) and ^{14}C activity in soil+roots and roots (Figure 6.4a). This exponential pattern matches root mass decline with soil depth (Figure 5.5a). The biological mixing of native soil C with time and cultivation could be the factors responsible for the decline of total C in soil and soil+root samples following neither the exponential decline in root C nor the recently fixed ^{14}C activity pattern. In a $^{14}CO_2$ pulse labelling experiment conducted on wheat, Swinnen *et al.* (1994) has reported an exponential decline in labelled soil organic ^{14}C with soil depth.

The pattern of decrease in total C and ^{14}C activity in the soil alone was irregular with an increase in the 2–4 cm soil layer (Figure 6.4a,b). The lower ^{14}C activity in the soil alone, in the 0–2 cm soil layer compared to in the 2–4 cm soil layer (Figure 6.4a) may indicate greater turnover of root–released exudates at this depth (for detailed discussion – see Sections 5.3.3.2 and 5.3.4.2).

At harvest (i.e. a week after pulse labelling), ^{14}C activity in roots makes up a substantial proportion of the ^{14}C activity (soil+roots) at each soil depth. More than 90% of the total ^{14}C activity in soil+roots in the 0–2 cm soil layer was recovered in the roots alone but at lower depths only up to 50% or less was present in the roots (Figure 6.4a). Root C, however, contributed to only 27% of soil+root C at the 2 cm soil depth and to less than 10% at depths below 4 cm (Figure 6.4b).

Given the variable and low contribution of root C to soil C at depth, but the high contribution of root ^{14}C activity to soil+roots ^{14}C activity, the calculation of soil+roots ^{14}C specific activity has little meaning. The specific activity (Bq mg^{-1}C) of roots, however, is high (Figure 6.4c) at all the soil depths and indicates storage of the ^{14}C in the structural pool of the root (Figure 6.4c). The specific activity of roots was greater in the 0–2 cm
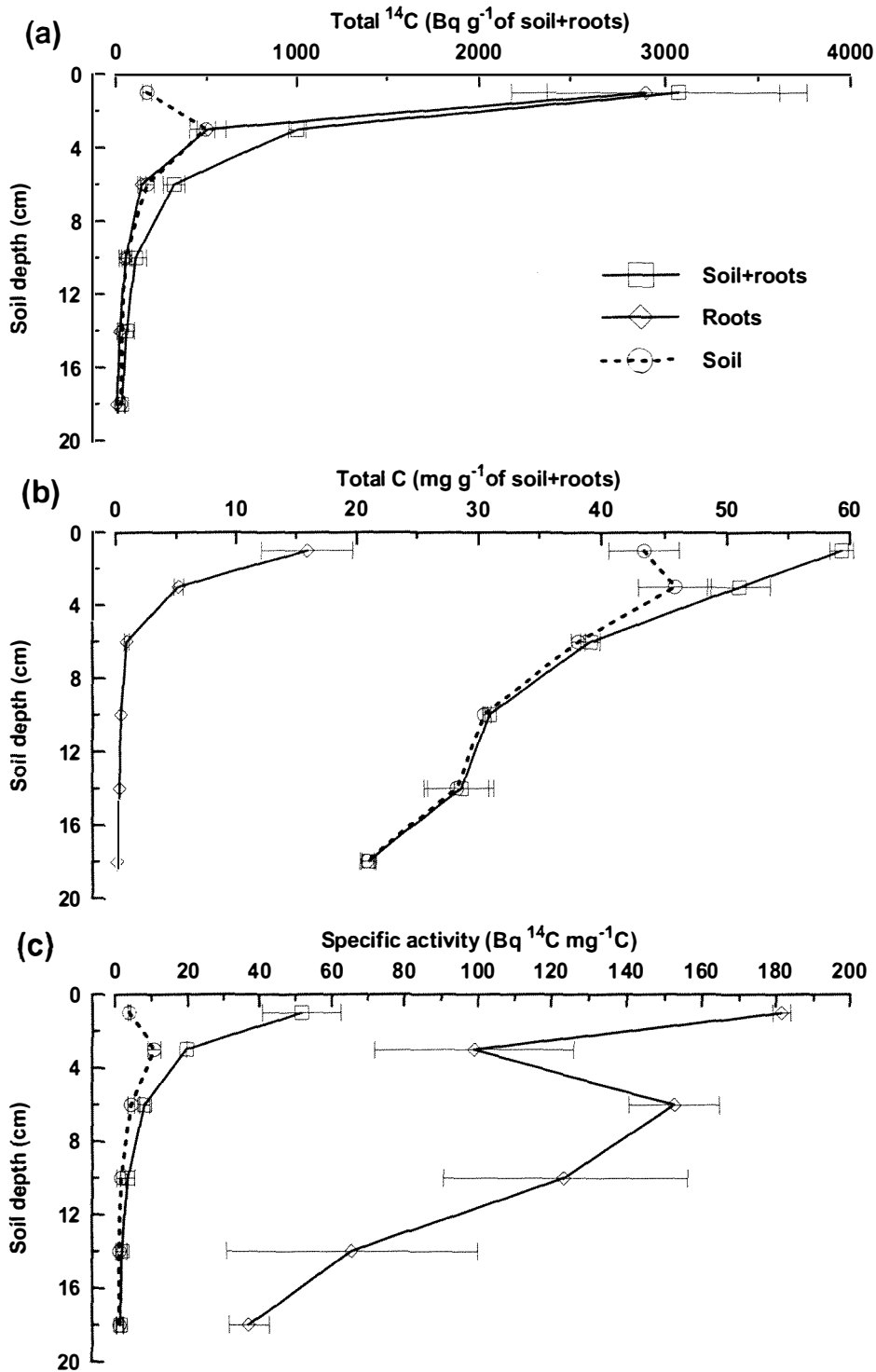
Figure 6.4: Distribution with soil depth of (a) total ^{14}C activity, (b) total C, and (c) specific activity (Bq ^{14}C mg^{-1} C) in whole soil+roots, roots and soil alone, a week after $^{14}CO_2$ pulse labelling of a pasture sward. The legend points are plotted at the mean depth of various soil slices. *Error Bars: ±SE of mean of two cores.*

soil layer than the other layers, which indicates proportionally more root growth and a greater storage of the recently fixed ^{14}C in the roots from the topsoil layer (Figure 6.4c). The specific activity of roots decreased in the 2–4 cm soil layer and increased again at in the 4–8 cm layer. This pattern may indicate a greater turnover of root C in the 2–4 cm soil layer since pulse labelling but this is not clear. The lower specific activity at depths below 8 cm may indicate slower root growth during the period of pulse labelling because $^{14}CO_2$ release from these depths was also slow (Section 5.3.3).

6.3.2   *Experiment 1: Chemical fractionation*

Roots contributed a considerable proportion of the ^{14}C in soil+roots but contributed little C towards the total C (Section 6.3.1). Roots and soil+roots were chemically fractionated to determine contribution of recent root and root–released ^{14}C to each chemically characterised fraction.

6.3.2.1   *Chemical fractionation of soil+roots*

The fractionation procedure (Section 3.2.1) recovered between 87–106% of the total ^{14}C activity and between 97–108% of the total C in soil+root samples from the range of soil depths. For comparison of the proportion of ^{14}C and C recovered in different chemical fractions at different soil depths, the recoveries were normalised to 100 percent (Figure 6.5a,b).

The distribution of ^{14}C activity among different chemical fractions from the range of soil depths was: 4–7% in the resin, 24–33% in the 0.1 M NaOH, 13–15% in the 1 M NaOH, and the remaining (50–56%) in the residual fraction (Figure 6.5a). The proportions of the ^{14}C activity in different chemical fractions did not change much with depth. This distribution of ^{14}C activity between all the chemical fractions indicates that recent root and released root C cannot be identified in a single chemical fraction. Furthermore, more than half of the total ^{14}C activity remained in the residual fraction.

Of the total C, about 2% was extracted onto ion–exchange resins, 39–45% in 0.1 M NaOH, 1–9% in 1 M NaOH and 48–54% remained in the alkali–insoluble residue for all the soil depths (Figure 6.5b). The proportion of the total C in different chemical fractions also changed little with depth, except the 1 M NaOH fraction, which recovered a lower proportion of the total C in the lower soil depths.

The distribution patterns of both ^{14}C activity (recent root and root–released C) and total C among different chemical fractions (Figure 6.5a,b) indicate that the resin and 1 M NaOH fractions recovered relatively more recent root and root–released ^{14}C and less unlabelled C. This resulted in high specific activity of these two fractions (Figure 6.6c and insert).

□ Resin ▣ 0.1 M NaOH ▓ 1 M NaOH ■ Residual
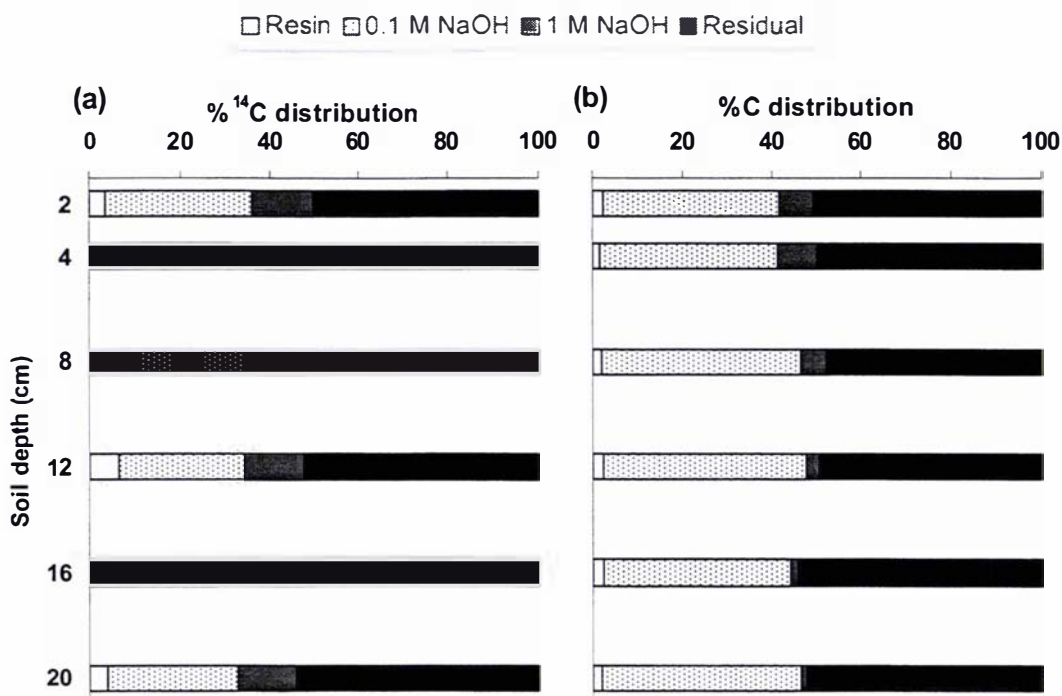
Figure 6.5: Percent distribution of (a) total ^{14}C activity and (b) total C in different fractions obtained during chemical fractionation of soil+root samples from different soil depths.

In contrast, although the residual and 0.1 M NaOH fractions together recovered a greater proportion (74–89%) of the soil+roots ^{14}C, these two fractions also recovered a greater proportion of the C (87–89%) in soil+root samples (Figure 6.5a,b). Therefore, the specific activities of these two fractions were lower.

A comparison of the specific activity of roots and those of the chemical fractions of soil+roots (see insert in Figure 6.6c) shows that ^{14}C specific activity of C in the resin and 1 M NaOH fractions are closer to root ^{14}C specific activity (1:1 line) than the 0.1 M NaOH and residual fractions. Compared to 0.1 M NaOH, the more strongly alkaline 1 M NaOH reagent hydrolysed a greater proportion of the recent root and root–released ^{14}C activity in soil+root samples and a lower proportion of unlabelled C.

The nature of ^{14}C in extracts

If it is also assumed that root C represent a dynamic soil C pool, then the resin and 1 M NaOH extracts with relatively high ^{14}C specific activities (Figure 6.6c) sample slightly more dynamic soil C fractions than the other fractions.

The resin, however, could also extract $^{14}CO_2$ and $H^{14}CO_3^-$ from soils. Acidification of the resin extract and scintillation solution suspension to pH ≤ 4.5 followed by recounting did not result in any loss of ^{14}C counts as $^{14}CO_2$. This confirmed that ^{14}C in organic anions and cations comprised the resin fraction. Although resin ^{14}C contributed little towards the total ^{14}C (4–7%), the soluble organic ion component could be an important SOM fraction from the point of view of nutrient availability in the short–term cycling of SOM.

6.3.2.2   Chemical fractionation of roots alone

The results show that during the chemical fractionation, ^{14}C in soil+root samples, which is predominantly in roots, is spread across all the chemical fractions. This distribution could be an artefact created by shaking roots and soil together in the presence of a weak extractant like resin. Although the resin extracted little C (Figure 6.5a,b), readsorption by the soil of C compounds leaking from roots may have occurred. This possibility was investigated by fractionating roots alone.

The chemical fractionation of the ^{14}C labelled roots (without resin extraction) recovered 31% of the total root ^{14}C activity in 0.1 M NaOH and 16% in 1 M NaOH, and the remaining 53% was recovered in the alkali–insoluble residual fraction (Table 6.2). This distribution of ^{14}C activity amongst fractions was similar to the fractionation of soil+roots. Therefore, fractionation in the presence of soil does not create soil fractions from root C.

The chemical fractionation of the root total C recovered proportionally more C (65%) in the residual and proportionally less in the two NaOH fractions (i.e. 26% in 0.1 M NaOH and 9% in 1 M NaOH extract) than the root ^{14}C. The majority of the ^{14}C remaining in the residual fraction is probably present in structural C (long–chain polysaccharides) associated with the root cell wall. These are not expected to be soluble in alkali (Lowe, 1978).
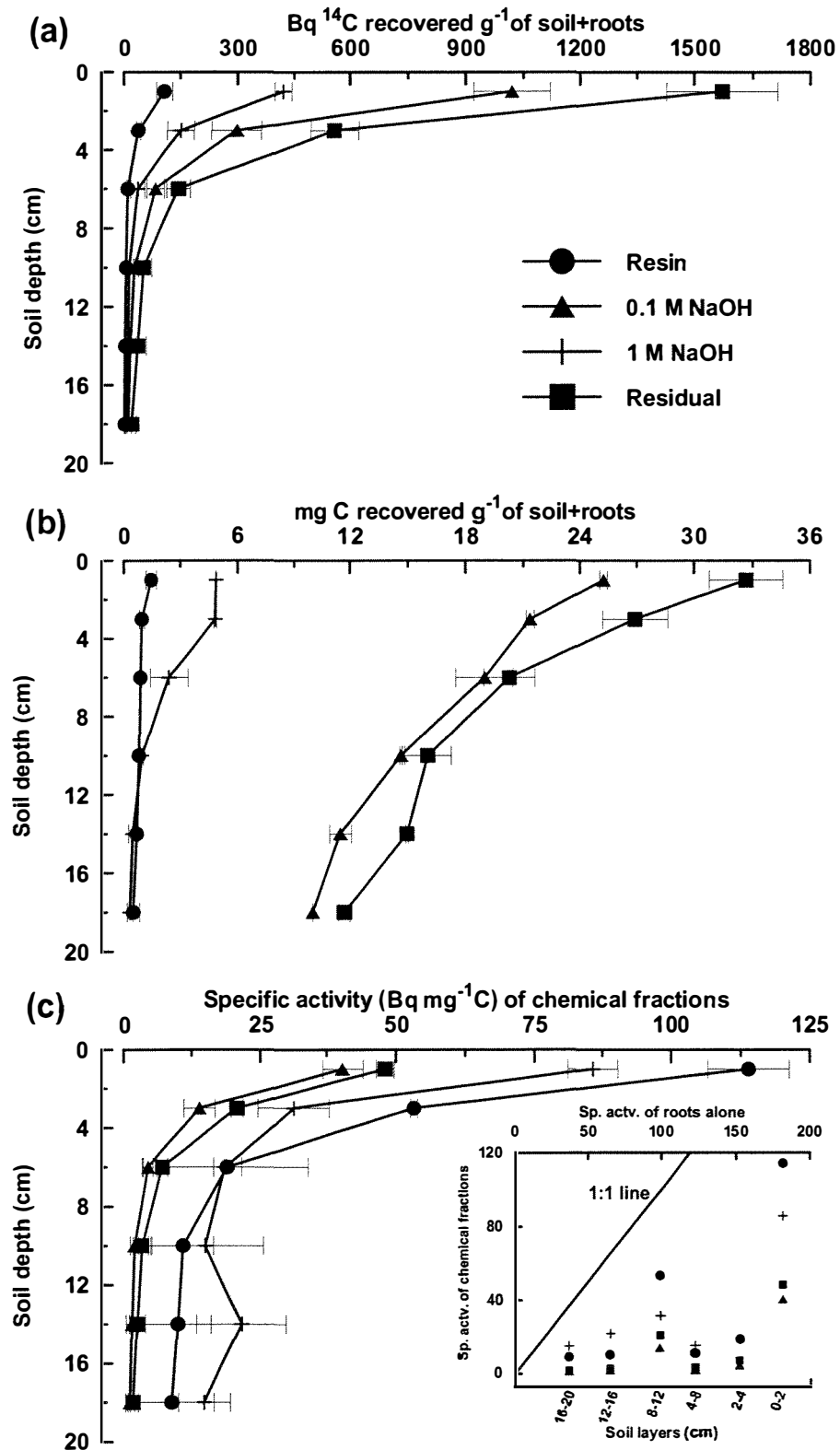
Figure 6.6: The amount of (a) total ^{14}C activity and (b) total C recovered from soil+roots in different chemical fractions, and (c) specific activity of these fractions at different soil depths. The insert in Figure (c) is comparing specific activity of chemical fractions with specific activity of roots at various soil layers. *Error Bars: ± SE of mean of two cores*

The specific activity of the 1 M NaOH fraction was higher than the 0.1 M NaOH and residual fractions (Table 6.2). One molar NaOH, therefore, extracts root C that has been more recently synthesised than that extracted by 0.1 M NaOH and that remaining in the alkali–insoluble residual fraction. The lower specific activity of the residual fraction of roots indicates that relatively less dynamic fractions of the root C (associated with the plant lignin and long–chain polysaccharides) remained in the residual fractions compared to the NaOH extracted C. Presumably more cell constituents and recent metabolites such as proteins, amino acids, simple carbohydrates (glucose, mannose, xylose, arabinose) etc. are soluble in NaOH. Why these are not extracted in 0.1 M NaOH is not clear but may relate to the disruption of disulphide bonds in more concentrated alkali such as 1 M NaOH.

Table 6.2: Percentage of total root ^{14}C and root C recovered by the chemical fractionation of roots (from 2 cm soil depth).

Chemical fractions	% of root ^{14}C recovered	% of root C recovered	Specific activity (Bq mg^{-1}C)
0.1 M NaOH	31	26	253 (11.6)[*]
1 M NaOH	16	9	380 (80.2)
Residual	53	65	167 (16.3)
Sum Total	100	100	207 (10.0)

[*]*Values in brackets are SE of means of two cores.*

6.3.2.3   Conclusions

a)   Chemical fractionation using alkali cannot identify recently formed root and root–released C as a single labile C fraction.

b)   The distribution of root C amongst resin, 0.1 M NaOH, 1 M NaOH and alkali–insoluble residual fractions is a function of the form of root C and not caused by the presence of soil. Modifications to SOM fractionation are required in an effort to characterise root C into fewer fractions.

c)   More recently synthesised root C (high specific activity) is preferentially extracted by resin and 1 M NaOH extracts.

6.3.3   Experiment 2: Attempts to recover root C in fewer fractions

With much of the root C not being soluble in alkali, it was decided to try to recover root C with acid hydrolysis. Acid hydrolysis was used in two ways: a) to extract most of the root ^{14}C from whole soil+roots prior to chemical fractionation with alkali, and b) to recover ^{14}C from the residue remaining after alkali extraction of soil+roots (Figure 6.2a,e). Acid hydrolysis (in this study – with cold 12 M sulphuric acid followed by hot 0.5 M sulphuric acid) has been shown to solubilise most polysaccharides C (Cheshire and Mundie, 1966; Martin et al., 1980; Rovira and Vallejo, 2000) and some lignified C (Martin et al., 1980) from soil organic and plant materials.

6.3.3.1   Part a, acid hydrolysis of soil+roots prior to alkali extraction

The acid hydrolysed fraction of whole soil+roots (Figure 6.7a) recovered most of the
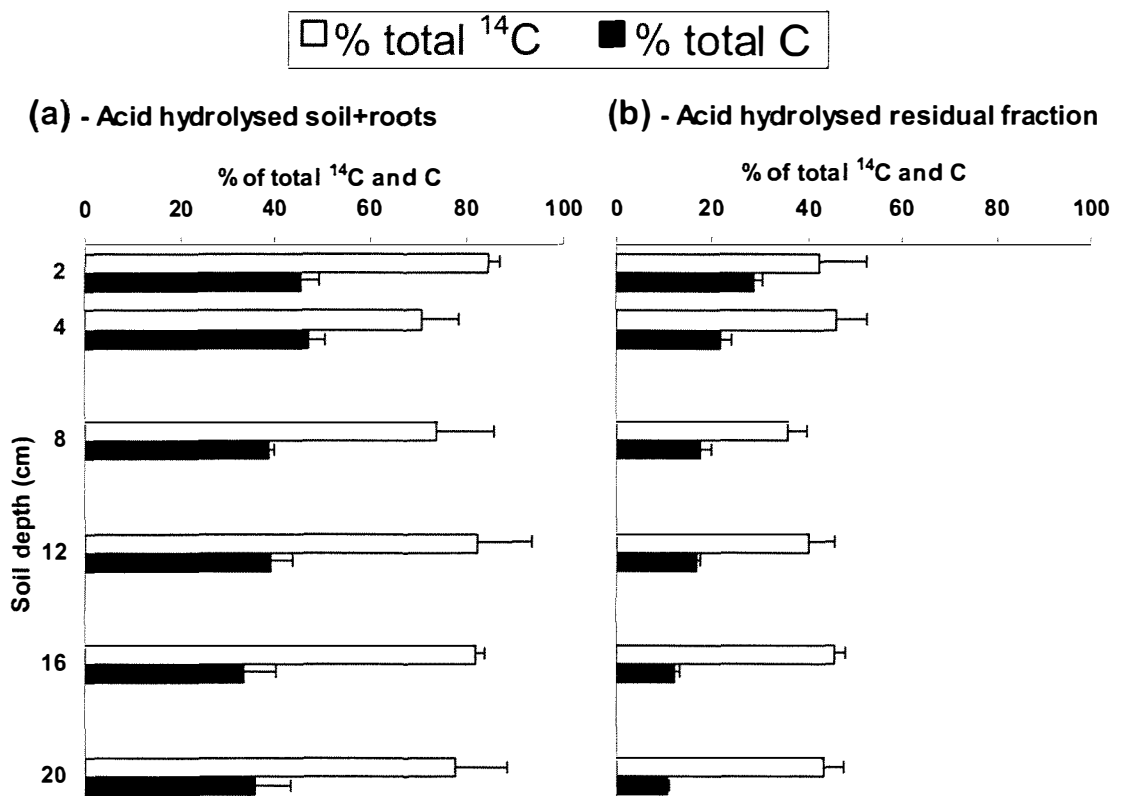
Figure 6.7: Percentage of total ^{14}C activity and total C recovered in acid hydrolysed fractions of (a) whole soil+roots and (b) residual fraction at different soil depths. *Error Bars: SE of mean of two cores.*

[14]C activity (71–85%) and between one–third to one–half of the total C from the range of soil depths. Acid–soluble fractions are reported to range from 25–60% of total soil C (Martel and Paul, 1974a; Martel and Lasalle, 1977; Jawson and Elliott, 1986; Xu *et al.*, 1997; Collins *et al.*, 2000).

The specific activity of the hot H_2SO_4 hydrolysed fraction of soil+roots was lower than that of the separated roots but higher than the specific activity of the original soil+roots (*cf.* Figures 6.8 and 6.4c) and the 0.1 M NaOH and residual fractions (*cf.* Figures 6.8 and 6.5c). This suggests that the acid has a tendency to hydrolyse proportionally more recent root and root–released ^{14}C and less total C than the other reagents.

The simple acid hydrolysis technique recovered 71–85% of the total ^{14}C activity, which was probably associated with long–chain polysaccharides like cellulose and hemicelluloses (comparatively resistant compounds) present in the root and microbial cell walls, besides the ^{14}C associated with proteins, simple carbohydrates and low molecular weight organic acids (easily decomposable materials) (Cheshire and Mundie, 1966; Martin *et al.*, 1980; Rovira and Vallejo, 2000). These compounds will differ in terms of lability. Long–term incubation studies conducted on the decomposition of ^{14}C enriched plant materials (Jenkinson 1977b; Jenkinson and Ayanaba, 1977; Sallih and Bottner, 1988) have shown that about two thirds of the relatively labile root C (labelled C) was lost in the first few months, after which decomposition became very much slower. This indicates that there are certain organic compounds (e.g. simple carbohydrates, proteins, amino acids etc.) in the plant material that would undergo decomposition readily (i.e. represent a most readily mineralisable fraction), followed by structural polysaccharides (cellulose, hemicelluloses) and then lignin (a phenolic substance), which decomposes at a much slower rate. Acid hydrolysis of the soil+root samples thus appears to extract a combination of labile and non–labile ^{14}C fractions plus has a high (almost half – Figure 6.7a) recovery of soil+root C. Recovery of almost half of the soil+root C in the first extraction is not suitable in a sequential fractionation scheme.

6.3.3.2 *Part b, acid hydrolysis of the alkali–insoluble residual fraction*

The hot H_2SO_4 (0.5 M) hydrolysed between 78–83% of the ^{14}C and 19–53% of the C remaining in the residual fraction of the soil+root samples from the range of soil depths
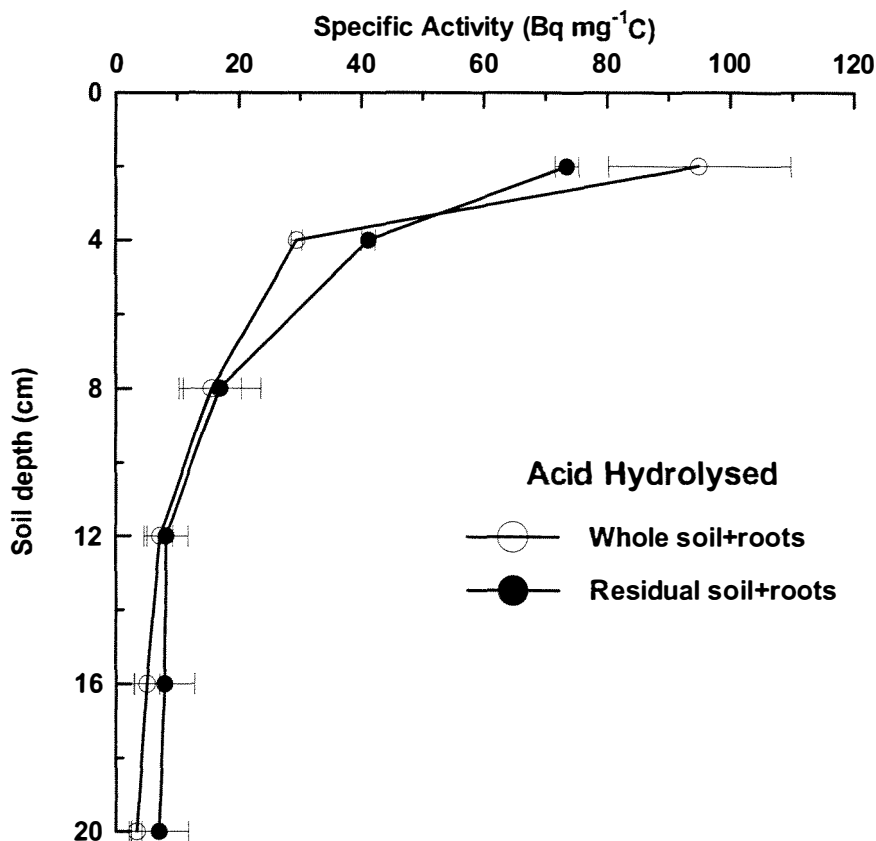
Figure 6.8: Specific activity of the acid hydrolysed fractions from whole soil+roots and residual fraction at different soil depths. *Error Bars: ±SE of mean of two cores.*

(Figure 6.7b). These equate to 36–46% of the total ^{14}C and 11–29% of the total C in soil+roots. The proportion of the acid hydrolysed C in the residual fraction decreased with depth indicating a decrease in polysaccharide type C with depth (*cf.* Figure 6.7a and 6.7b).

The specific activity of the acid hydrolysed residual fraction of soil+roots was mostly similar to the acid hydrolysed fraction derived directly from soil+roots (Figure 6.8). This similarity suggests a similar source of C was extracted despite the fact that only 36–46% of the initial ^{14}C activity was acid hydrolysed from the residue after alkali pre-extraction, whereas 71–85% was acid hydrolysed from the whole soil+roots. The decline in ^{14}C specific activity of both acid hydrolysed fractions with soil depth indicates that less new root cell wall material is being synthesised per unit root in the lower soil layers.

6.3.3.3   Conclusions

The conclusions from experiment 2 can be drawn as below:

a) Whilst hot H_2SO_4 hydrolysis recovered most of the recently synthesised root and root–released ^{14}C, it is not a suitable pre-extraction because it recovers about half of the total soil C fraction.

b) Hot H_2SO_4 hydrolysis recovers most of the remaining root ^{14}C from the alkali–insoluble residue whilst extracting 53% or less of the C from the residue. Acid hydrolysis of the residue should be investigated further as a method of identifying the contribution made by dynamic C to the residual fraction.

6.3.4   *Experiment 3: Density separation of soil+roots*

Chemical fractionation failed to recover recently synthesised root C in a single fraction that could be designated 'labile' C. After reviewing density separation techniques (Section 2.1.2.4), it was proposed that most recent root C could be recovered in a light fraction. Therefore, density separation of the whole soil+root samples was undertaken (Section 6.2.5.3).

The primary function of the density fractionation procedure reported in this study was to isolate recent root material in a light fraction. The light fraction (mostly large undecomposed roots and soil organic matter, and any floating micro–aggregates not dispersed by sonication and shaking) was retained on the 54 μm mesh. The material <54 μm in size (suspended silt and clay, small roots and soil organic matter particles) was poured back into the 'heavy fraction' (sand, silt and clay particles, and relatively decomposed roots with high specific density and organic residues attached to soil particles). Therefore, some of the recent ^{14}C associated with the <54 μm roots and soil organic matter fragments would get included in the 'heavy fraction'.

The density separation procedure recovered between 91–105% of the total ^{14}C, and between 91–99% of the total C (total of NaCl soluble, light and heavy fractions), at various soil depths. However, the recoveries were adjusted to 100% for comparison of the proportions of ^{14}C activity and total C recovered in these fractions (Figure 6.9a,b).

Using a soil sample from the top 2 cm depth of soil, this procedure allocated 13%, 42% and 45% of the total ^{14}C activity into dispersing reagent (5% NaCl) soluble, light and heavy fractions, respectively (Figure 6.9a). Although the amount of ^{14}C activity in both the light and heavy fractions decreased with soil depth (Figure 6.10a), the proportion of total ^{14}C activity in the light fraction decreased (from 42% to 5%) whilst the heavy fraction increased (from 45% to 87%) with soil depth (Figure 6.9a). The proportion of 5% NaCl soluble ^{14}C (ranged from 7% to 13%) was greatest at the 2–cm depth and decreased with soil depth.

It had been assumed that the density separation procedure would allocate most of the new root ^{14}C to the light fraction, but the heavy fraction recovered relatively greater

proportions of the ^{14}C than the light and soluble fractions, irrespective of soil depth. The 5% NaCl soluble fraction probably included ^{14}C present in the roots, and microbial biomass killed during chemical pre-treatment and dispersion of soil samples. Magid *et al.* (1996) also found that up to 13% of the total ^{14}C was solubilised by the chemical dispersing agent.
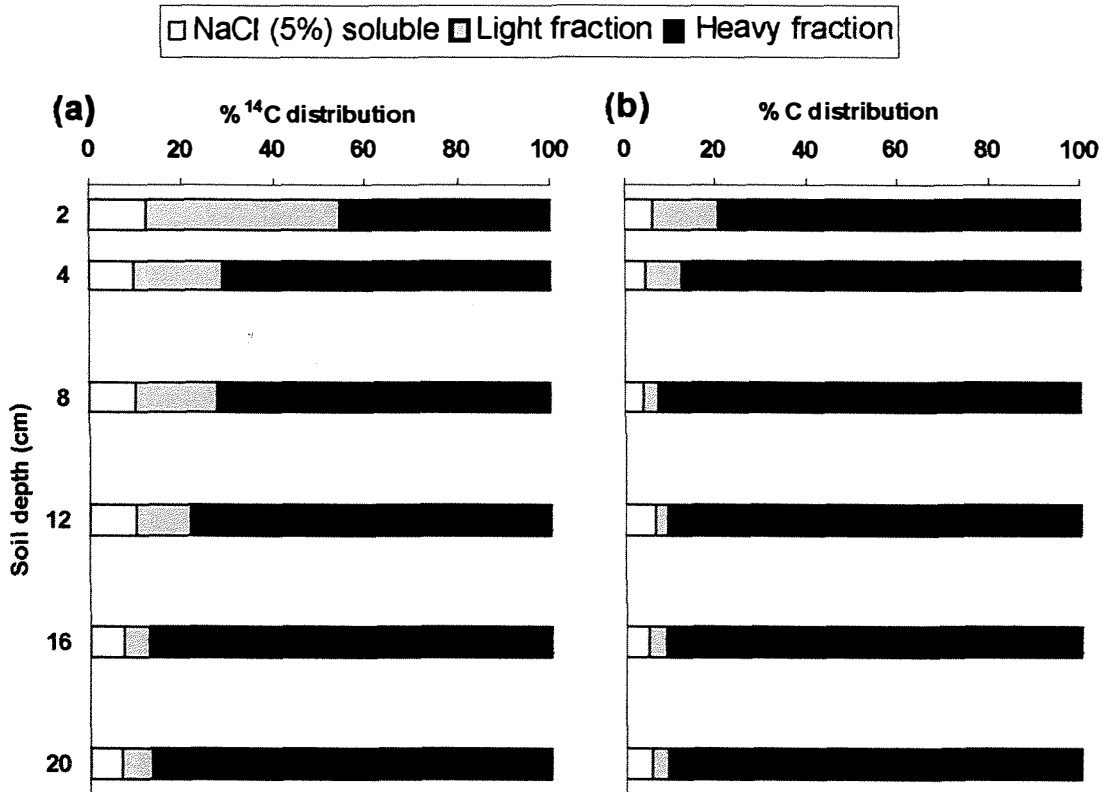
Figure 6.9: Percent distribution of (a) total ^{14}C activity and (b) total C in different fractions obtained during density separation of soil+root samples from different soil depths.

The heavy fraction recovered by far the greatest percentage (79–93%) of the total C followed by smaller percentages in the light (3–15%) and soluble (4–6%) fractions from the range of soil depths (Figure 6.9b). Like ^{14}C activity, the proportion of the total C recovered in the light fraction decreased and the heavy fraction increased at lower depths. The proportion of soluble C fraction (5% NaCl) did not change much with soil depth. Density separation results similar to this study have been reported by Janzen *et al.* (1992). They found that light fraction comprised of 2–17% of the soil organic C and was highest

where soils were in perennial forages or continuously cropped and lowest where bare fallow was part of the management. Similarly, Boone (1994) found that the light fraction varied under different landuse and constituted between 5–14% of mineral SOM.

Strickland and Sollins (1987) reported that the light fraction is comprised of mineral-free organic matter of partly decomposed and/or undecomposed plant debris, and the heavy fraction is composed of organic matter adsorbed or deposited by microorganisms on aggregate surfaces and sequestered within organo-mineral aggregates. Cheshire and Mundie (1981) found that soils incubated with plant materials had substantial proportions of ^{14}C labelled glucose, xylose, and arabinose (the dominant sugars in plant materials) in particulate organic matter accompanying the sand size separates (50–2000 µm) (Tiessen and Stewart, 1983; Cambardella and Elliott, 1992; Christensen, 1992; Buyanovsky *et al.*, 1994). This sand sized particulate decomposition product could be expected to appear in the light fraction. The light fraction, which mainly contained root debris and soil organic matter of >54 µm size has been reported, using ^{13}C nuclear magnetic resonance, to contain high proportions of carbohydrates, including cellulose (Oades *et al.*, 1987).

Elliott (1986) and Golchin *et al.* (1994) have demonstrated that the organic matter associated with or occluded within micro–aggregates was relatively resistant or more decomposed than free organic materials. These occluded organic materials were small plant fragments in the 10–100 µm size classes (Golchin *et al.*, 1994). Larger occluded material will appear in the light fraction.

Forty five to eighty seven percent of ^{14}C activity from root and root–released C was recovered in the heavy fraction. In addition to including <54 µm root fragments, and possibly extraradicle mycorrhizal hyphae (Harris and Paul, 1987) and root–released C, the heavy fraction can include a significant component of aggregate–protected, active organic matter [perhaps charged metabolites adsorbed to clay and silt (<50 µm), microbial biomass and microbial–derived material] (Boone, 1994). Silt and clay fractions can contain recent decomposition products (Magid *et al.*, 1996) and some of the oldest soil C (Anderson and Paul, 1983), which may be highly aromatic (Oades *et al.*, 1987). Skjemstad *et al.* (1986) found that the heavy fraction contains relatively labile long–chained alkyl group that are decay resistant because of physical stabilisation and protection by the inorganic component of the soil.

During the density separation, the chemical dispersant (5% NaCl) was used in combination with shaking and centrifugation followed by sonication in sodium polytungstate solution to ensure maximum dispersion of the soil aggregates. Whitbread (1995) considered that the greatest problem with the use of sonication is the redistribution of organic matter among size/density fractions, which depends on the energy output (Gregorich *et al.*, 1988) and duration of sonication (Christensen, 1992). Therefore, some of the ^{14}C activity from roots may have been transferred from the light to the heavy fraction during the density separation process (also see later discussion in Section 6.3.5).

The distribution of the soluble, light and heavy ^{14}C fractions with depth was similar to that of root ^{14}C or soil+roots ^{14}C (Figures 6.10a and 6.4a). The depth distribution of C in heavy fraction was comparable with the soil+root C, and the light fraction C similar to root C (Figures 6.10b and 6.4b). Declining amounts of organic C in the light and heavy fractions with increasing depth have also been observed by Janzen *et al.* (1992) and Hassink (1995).

The specific activity of the light and soluble (NaCl) fractions was higher than the heavy fraction (Figure 6.10c). The lower specific activity of the heavy fraction indicates that although the amount of soil+root ^{14}C activity recovered in the heavy fraction was greater than the light and soluble fractions at all the soil depths (Figure 6.10a), it has also retained considerable proportions of relatively old/lignified organic material and resistant organo-mineral complexes (Figures 6.9b and 6.10b). The higher specific activity of the light and NaCl soluble fractions indicates that they are more labile in nature (relatively more ^{14}C activity and less unlabelled C). Magid *et al.* (1996) found that the dispersion reagent soluble fraction included material that had fast initial decomposition rates. This may expected to be charged metabolites in soil solution and those desorbed from soil surfaces by 5% NaCl.
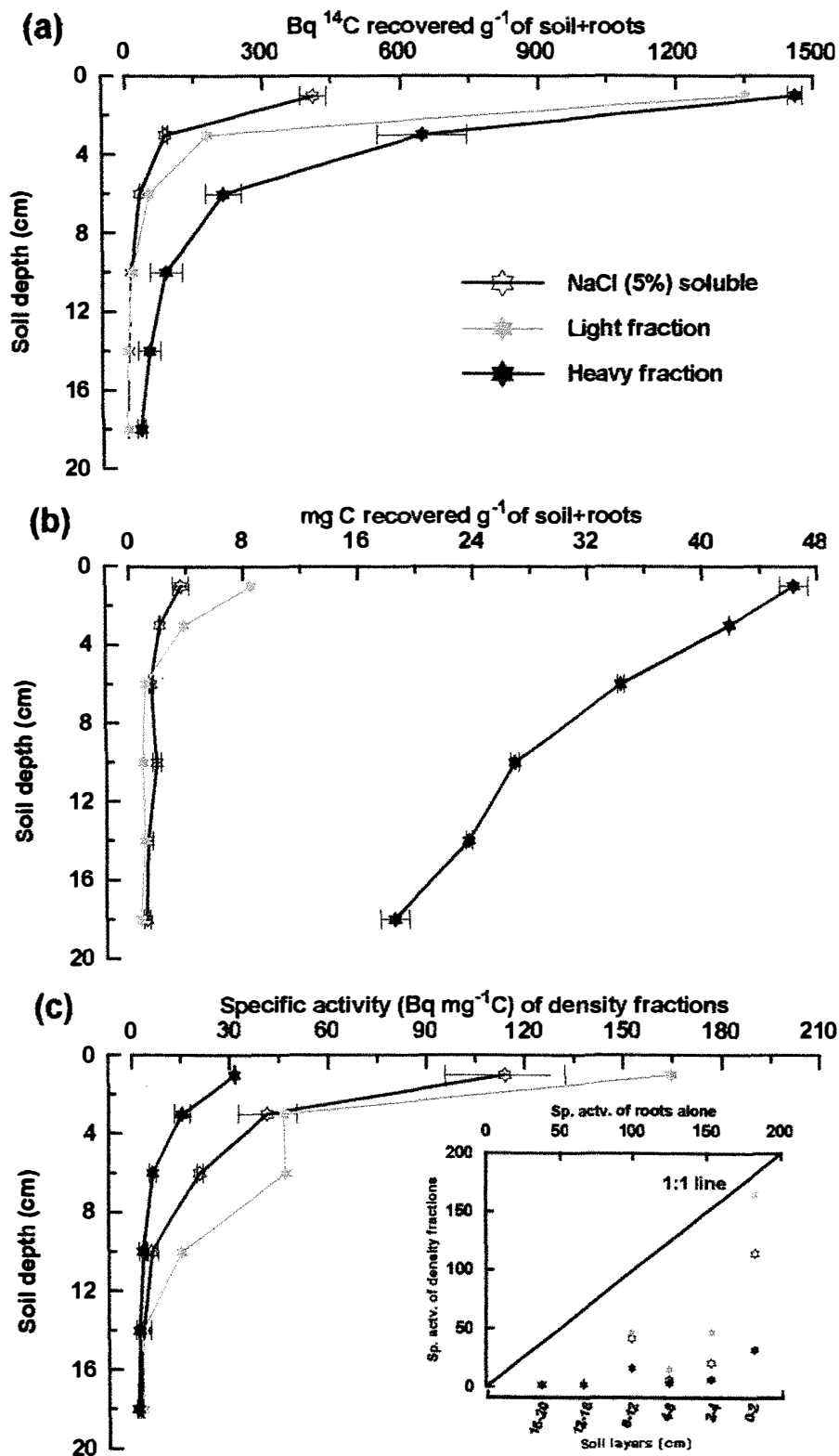
Figure 6.10: The amount of (a) total ^{14}C activity and (b) total C recovered from soil+roots in soluble (NaCl) and density (light and heavy) fractions, and (c) specific activity of these fractions at different soil depths. The insert in Figure (c) is comparing specific activity of soluble and density fractions with specific activity of roots at various soil layers. *Error Bars: ± SE of mean of two cores*

The importance of the light fraction as a biologically active fraction, with a rapid turnover rate, has been recognised previously (Janzen, 1987; Christensen, 1992; Barrios *et al.*, 1996; Magid *et al.*, 1997) due to the labile nature of its constituents, such as easily decomposable carbohydrates (Skjemstad *et al.*, 1986; Golchin *et al.*, 1994), and the lack of protection by soil colloids (Meijboom *et al.*, 1995). It must be concluded from a comparison of light fraction and root ^{14}C specific activity (see insert in Figure 6.10c) however that the light fraction is also a mixture of C of different lability. For example, Magid *et al.* (1997) amended the soil with ^{14}C labelled plant residues (rape straw) and separated the residues remaining in the soil, over the 20 month decomposition period, into light and heavy fractions. They found that in the unamended treatment, the light fraction decayed at a slower rate than the rape straw amended treatments, which was due to the high lignin content of the 'native' light fraction. In the amended treatment, the light fraction closely resembled the characteristic decomposition pattern of fresh plant materials i.e. initially rapid followed by a slower decay rate. Cellulose was preferentially utilised in the early stages of decomposition and the proportion of lignin increased in the later decomposition stage of the light fraction. Furthermore, the heavy fraction, which was unaffected by the application of residues, consisted mainly of 'native' organic matter with a lignin content of 30–45% (which was not very much different from the later decomposition stage light fraction) and a low rate of decay.

6.3.4.1   Conclusions

The main conclusions from the experiment 3 can be drawn as below:

a)   The light C fraction contains a major contribution from fresh roots.

b)   However, recently synthesised root C cannot be recovered solely in the light fraction. This may result partly from the redistribution of root ^{14}C during the initial NaCl dispersion.

6.3.5   *Experiment 4: Density separation of roots alone, and roots remixed with non–radioactive soil*

In order to check whether the redistribution of ^{14}C among density (light and heavy) fractions occurs during dispersion, and the fractions are artefacts of the density separation procedure (dispersion, centrifugation etc.); density separation of roots alone, and in the presence of non–radioactive soil was conducted.

The density fractionation of roots alone accounted for 105% of the total root ^{14}C separated from the 2 and 4 cm soil depths. When roots were remixed with the non-radioactive soil, the density separation procedure recovered 94–96% of the total root ^{14}C. Again, the sum total of all the fractions recovered by the fractionation procedure was adjusted to 100% to enable comparison between the proportions of total ^{14}C activity recovered in the different fractions (Figure 6.11).

The light fraction recovered 59% and 81% of the root ^{14}C from the 2 and 4 cm soil depths, respectively. The heavy fraction recovered 32% of the total root ^{14}C from the 2 cm depth roots and 12% from the 4 cm depth roots (Figure 6.11). The appearance of a proportion of the root ^{14}C (i.e. associated with recent roots) in the heavy fraction could be due to the high gravity field (2204 ×g) created during centrifugation, which could have liberated entrapped air from some of the young root tissues (Magid *et al.*, 1996), causing them to become part of the heavy fraction due to their increased specific density. The difference in the recovery of ^{14}C from roots sampled from different soil depths may be attributed to the greater proportion of fine (recent) roots at the 2 cm depth.

When the radioactive roots were remixed with the non–radioactive soil and fractionated again by the density separation procedure, the light fraction recovered only 35% and 22% of the total root ^{14}C, compared to 50% and 64% retained in the heavy fraction from 2 and 4 cm depths, respectively (Figure 6.11). There was a shift of ^{14}C counts from the light fraction to the heavy fraction when radioactive (^{14}C) roots were remixed with the non–radioactive soil and fractionated by the density separation procedure. The shift was greater with roots and soil from the 4 cm soil depth. The shift of ^{14}C counts indicates that either the soil particles remaining in the heavy fraction held some of the recent ^{14}C roots and SOM, or some soluble organic anions released from roots on
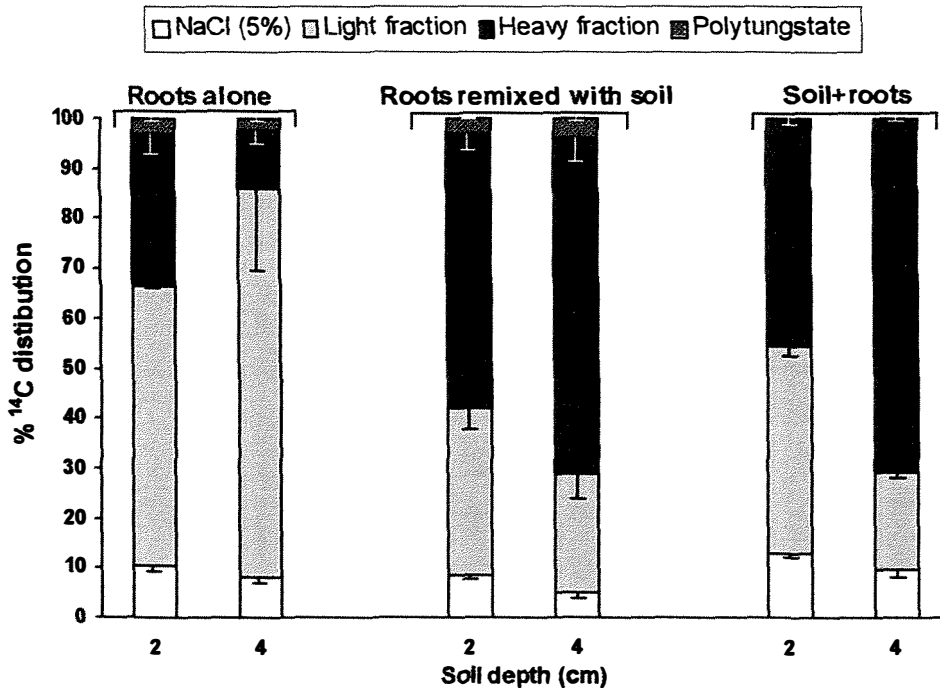
Figure 6.11: Percent distribution of ^{14}C activity, recovered from roots alone and roots remixed with non–radioactive soil, in different density fractions at 2 and 4 cm soil depths. *Error Bars: SE of mean of two cores.*

dispersion are adsorbed by clay and silt, which contributed to more ^{14}C counts in the heavy fraction, in addition to the gravity effect. A comparison of the ^{14}C in soluble (5% NaCl), light and heavy fractions of roots remixed with non–radioactive soil, and that of ^{14}C labelled soil+roots (Figure 6.11) confirms that there was definitely an artefact of soil that caused the redistribution of root or soil ^{14}C among fractions separated by the density separation procedure. A small proportion (<12%) of soluble root ^{14}C appeared in the dispersing reagent. This amount was little affected by the presence of soil. Therefore, redistribution of ^{14}C counts involves loss of the light fraction ^{14}C to the heavy fraction. This was likely caused by adsorption of fine root particle onto soil material during the density separation.

6.3.5.1 Conclusions

The adhesion of soil particles to the young ^{14}C roots during the density separation procedure was one of the major factors that caused root ^{14}C activity to appear in greater amounts in the heavy fraction.

6.3.6 Experiment 5: Chemical fractionation of density (heavy and light) fractions

As recent root and root–released ^{14}C was distributed between the light and heavy fractions, density separation of soil+roots prior to chemical fractionation was unlikely to
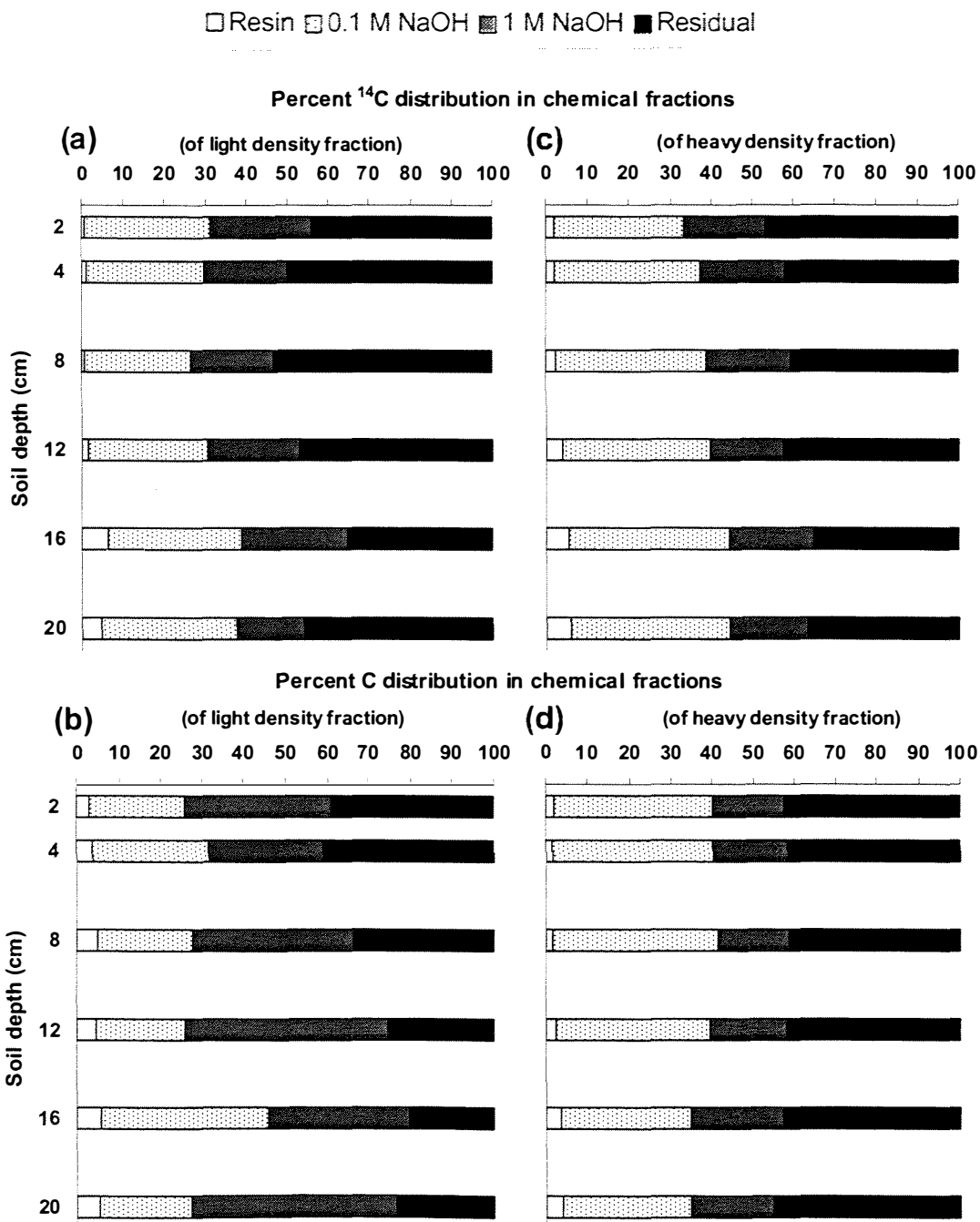
Figure 6.12: Percentage of (top – a,c) total ^{14}C activity and (bottom – b,d) total C recovered in each chemical fraction of the light and heavy density fractions of soil+root samples from different soil depths.

assist in clarifying the nature of SOM, particularly as root and root–released ^{14}C were also distributed among all the chemical fractions (Section 6.3.2; Experiment 1). To confirm, however, that density separation followed by chemical fractionation would not assist but was more likely to complicate SOM characterisation, the light and heavy fractions that had been separated in Experiment 3 (Section 6.3.4) were chemically fractionated.

The greatest proportion of the light fraction ^{14}C was recovered in the residual fraction (35–53%) followed by the 0.1 M NaOH (26–33%), 1 M NaOH (16–26%) and resin fractions (1–6%) over the range of soil depths (Figure 6.12a). The greatest proportion of the light fraction C was recovered in the 1 M NaOH fraction (27–49%) followed by the residual (20–41%), 0.1 M NaOH (21–40%) and resin (3–6%) fractions (Figure 6.12b). The pattern of extraction of ^{14}C from the light fraction was similar to the extraction of roots alone (Section 6.3.2.2; Table 6.2). The pattern of C extraction, however, differed slightly between the roots (Table 6.2) and the light fraction, in that a greater percentage of the light fraction C was extracted as 1 M NaOH–C (27–49% against 9%) rather than as residual–C (20–41% against 65%) (Figure 6.12b; Table 6.2).

The greatest proportion of the heavy fraction ^{14}C was recovered in the residual fraction (36–47%) followed by the 0.1 M NaOH (31–39%), 1 M NaOH (18–21%) and resin (2–6%) fractions from the range of soil depths (Figure 6.12c). Similarly, the greatest proportion of heavy fraction C was recovered in the residual fraction (41–45%) followed by the 0.1 M NaOH (31–40%), 1 M NaOH (17–22%) and resin (2–4%) fractions over the range of soil depths (Figure 6.12d). The distribution of ^{14}C and C in chemical fractions of 'heavy' material (Figure 6.12c,d) was similar in pattern to when soil+roots were chemically fractionated (Figure 6.5). However, the percentages of ^{14}C and C recovered in the 1 M NaOH fraction were greater, and the residual slightly less, than in the fractionation of soil+roots (Figure 6.5).

6.3.6.1   Conclusions

The chemical fractionation of density fractions confirms that pre-treatment of soil+root samples with a density separation procedure will not assist in reducing the distribution of recent root and root–released C amongst chemical fractions. Rather, the density separation step causes the redistribution of root ^{14}C to the other fractions.

6.3.7 *Experiments 6 and 7: Further attempts to modify the chemical fractionation procedure*

With the unsuccessful attempt to use a combined density and chemical fractionation procedure to identify recent root and root–released ^{14}C in a more contiguous fraction, attention returned to improved chemical characterisation of the more labile C in the 'residual' fraction of the chemical separation. Further modifications to the chemical fractionation procedure were attempted to recover recently synthesised root C either by (i) pre-treating soil+root samples prior to the chemical fractionation with the aim of extraction of root C or conversion of root C to alkali soluble material (see Section 6.2.6.1; Figure 6.2b,c,d), or (ii) treating the residual fraction of soil+roots (see Section 6.2.6.2; Figure 6.2f,g,h,i) so as to reduce the size of the residual fraction.

6.3.7.1 *Pre-treatments followed by chemical fractionation of soil+roots (Experiment 6)*

Anaerobic incubation of soil+roots followed by chemical fractionation

The amount of ^{14}C labelled gases (Bq g^{-1} soil) that were evolved during anaerobic incubation of soil+roots increased between subsequent sampling times until 11 days of incubation, and then decreased again in the final sampling period (i.e. 11–14 days) (Figure 6.13 – Sampled incubation). In the incubation, sampled over 14 days (Section 6.2.6.1; Treatment b), the total ^{14}C labelled gases evolved was greater than the full–term incubation. This may be because each sampling for gases was followed by replacement with N_2 gas, which ensured complete anaerobic conditions after 4 days and perhaps allowing the rapid mineralisation of soil+roots. Alternatively, flushing out the gaseous products of fermentation may have accelerated decomposition.

In full–term incubation, the ^{14}C gases evolved appeared to consume ^{14}C from the resin fraction of ^{14}C [compare full incubation and full incubation (control)] whereas in sampled incubation, the respired fraction was mainly drawn from the resin and residual fractions [compare sampled incubation and sampled incubation (control)] (Table 6.3). However, the % of total ^{14}C recovered in the respired fraction in both types of incubation was small (i.e. 2% in the full incubation and 5% in the sampled incubation).
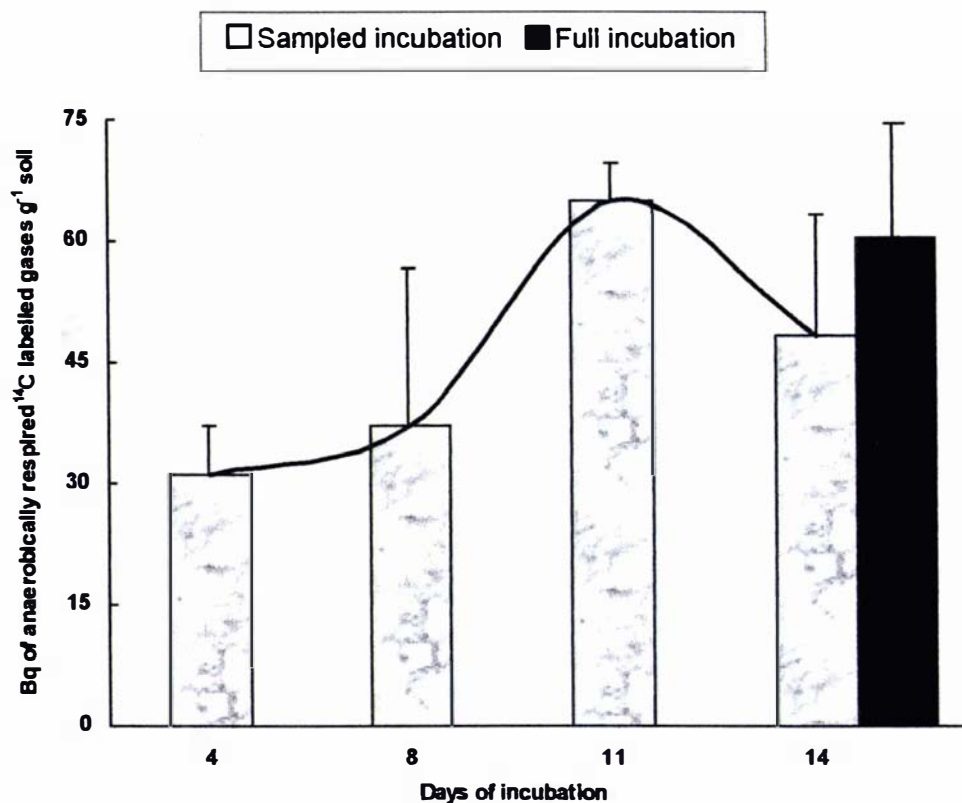
Figure 6.13: The amount of ^{14}C labelled gases evolved during sampled (i.e. measured between different sampling periods) and full (i.e. measured at the end of incubation) anaerobic incubations of soil+roots. *Error Bars: SE (sampling errors).*

The total C in the respired fraction was not measured. However, on estimating the percentage of total C recovered in different fractions (i.e. by dividing the amount of C recovered in each fraction by the sum total of the C in the respective controls), it was revealed that approximately 2% of the C was lost by anaerobic microbial respiration in full incubation experiment and about 8% was lost in sampled incubation experiment. There were not any significant changes in the specific activity of different fractions caused by incubation (Table 6.3), which indicates that about same proportions of total ^{14}C and total C were utilised by soil microbes during anaerobic incubation.

Table 6.3: Effect of anaerobic incubation of soil+roots on the recovery of ^{14}C and C, and the specific activity of different fractions with the specific purpose of looking at the fractions used by soil anaerobic microbes during 14 days of incubation period.

Treatments for anaerobic Incubation	Fractions	% of total ^{14}C	% of total C	Specific activity (Bq mg^{-1}C)
Full incubation[#]	Respired gases	1.7(0.4)[*]	–	–
	Resin	1.4 (0.0)	0.7 (0.2)	130.9 (45.1)
	0.1 M NaOH	33.5 (1.0)	41.3 (1.0)	48.3 (0.2)
	1 M NaOH	14.5 (0.9)	7.3 (0.0)	118.1 (7.2)
	Residual	48.9 (2.7)	49.0 (0.6)	59.5 (4.0)
	Sum total	100.0	98.3	–
Full incubation (control)	Respired gases	0.0 (0.0)	–	–
	Resin	3.5 (0.0)	1.8 (0.1)	120.0 (8.4)
	0.1 M NaOH	33.4 (0.5)	41.3 (0.2)	49.7 (0.5)
	1 M NaOH	14.8 (1.1)	8.2 (0.7)	110.1 (1.3)
	Residual	48.3 (0.6)	48.7 (1.3)	60.8 (0.9)
	Sum total	100.0	100.0	–
Sampled incubation[#]	Respired gases	5.3[+] (1.3)	–	–
	Resin	1.8 (0.1)	0.8 (0.0)	120.4 (0.4)
	0.1 M NaOH	34.1 (1.3)	39.9 (0.4)	48.8 (2.3)
	1 M NaOH	14.9 (1.8)	7.3 (1.8)	119.8 (16.0)
	Residual	43.9 (0.9)	43.6 (2.8)	57.7 (2.5)
	Sum total	100.0	91.6	–
Sampled incubation (control)	Respired gases	0.0 (0.0)	–	–
	Resin	3.4 (0.2)	1.9 (0.0)	110.3 (6.3)
	0.1 M NaOH	33.3 (3.3)	41.0 (0.6)	49.2 (5.6)
	1 M NaOH	15.5 (4.1)	6.7 (0.2)	138.5 (33.2)
	Residual	47.8 (7.5)	50.4 (2.0)	57.8 (11.3)
	Sum total	100.0	100.0	–

*Values in brackets are SE (sampling errors).
+Sum total of all the 14C labelled gases respired during 14 days of incubation
#As the respired unlabelled C gases were not measured, therefore, % of total C in the full and sampled incubations were obtained by dividing the amount of total C recovered in different fractions by the sum total of C in the respective control.

The anaerobic incubation was intended to rapidly degrade recently synthesised C associated with the plant components (e.g. cellulose, hemicelluloses) in the residual fraction, by fermentative organisms, into relatively short–chain organic acids, alcohols, and reduced gases, etc. (Stevenson and Cole, 1999). However, microorganisms grew on recent

^{14}C and total C (i.e. both recent and old) associated with both the resin and the residual fractions, but the proportion of the C they utilised was small. Therefore, this approach of making alkali–insoluble fraction into alkali soluble fraction was not successful. The incubation does confirm, however, that the residual fraction contains some easily decomposable C as indicated by its dynamic change during the cultivation studies (Section 4.3.1.2; Figure 4.4).

Hydrogen peroxide oxidation of soil+roots followed by chemical fractionation

As the anaerobic incubation of soil+roots did not work, chemical oxidation of soil+roots material in the presence of H_2O_2 at room temperature (see Section 6.2.6.1; Treatment c) was undertaken to try to maximise the recovery of the residual ^{14}C in resin, 0.1 M NaOH and 1 M NaOH fractions.

In 15% or 30% H_2O_2 treated soil+root samples, the proportion of the total ^{14}C and total C recovered in the resin fraction was not more than in control (Table 6.4). Adjusting the pH of the resin (soil suspension) to either pH 2 or pH 8.5 indicated that more negatively charged organic ions (e.g. $R\text{–}COO^-$), present at pH 8.5, were recovered by the resin compared to positively charged organic ions (e.g. $R\text{–}NH_3^+$). Only pre-treatment with 30% H_2O_2 created the desired effect of converting more ^{14}C and C from the residual fraction to the 0.1 M NaOH and 1 M NaOH fractions. Most of this converted C was recent root and root–released ^{14}C because the specific activity of the residual fraction decreased, whereas specific activities of 0.1 M NaOH and 1 M NaOH fractions increased (Table 6.4).

These results indicate that H_2O_2 treatment has actually reduced the size of the recently fixed ^{14}C in the residual fraction probably by breaking the hydrogen bond between the complex polymers and making them alkali soluble but still a considerable proportion of the C was present in the residual fraction.

Table 6.4: Effect of different H$_2$O$_2$ and Na–perborate pre-alkali extraction treatments of soil+roots on the recovery of ^{14}C and C in different chemical fractions, and the specific activity (Bq mg^{-1}C) of these fractions with the specific purpose of looking at the treatments that reduce the size of the alkali–insoluble residual fraction having a greater proportion of root (structural) C.

Pre-treatments of soil+roots	Fractions	% of total ^{14}C	% of total C	Specific activity (Bq mg^{-1}C)
---	No pretreament	-------	---------------------------------------	
Control	Resin	3.2 (0.2)*	1.6 (0.1)	104.4 (14.1)
	0.1 M NaOH	34.0 (1.1)	39.0 (2.4)	43.6 (4.1)
	1 M NaOH	14.9 (0.9)	8.5 (1.9)	90.1 (25.4)
	Residual	47.9 (2.0)	50.9 (4.5)	47.0 (2.1)
----Pre-treatments with different conc. H$_2$O$_2$ and resin extraction in different pH water----				
15% H$_2$O$_2$	H$_2$O$_2$ oxidised CO$_2$	0.1 (0.0)	0.8 (0.1)	4.7 (0.9)
(pH = 2)	Resin	1.3 (0.2)	0.8 (0.1)	82.6 (3.6)
	0.1 M NaOH	37.5 (0.1)	40.4 (3.1)	50.9 (4.1)
	1 M NaOH	20.9 (0.3)	10.9 (0.6)	106.0 (4.2)
	Residual	40.2 (2.6)	47.0 (1.9)	46.8 (1.1)
15% H$_2$O$_2$	H$_2$O$_2$ oxidised CO$_2$	0.1 (0.0)	0.8 (0.2)	7.4 (3.4)
(pH = 8.5)	Resin	2.7 (0.1)	1.5 (0.2)	98.6 (21.6)
	0.1 M NaOH	37.0 (3.8)	40.6 (4.7)	48.0 (10.4)
	1 M NaOH	21.7 (0.1)	10.3 (0.7)	109.5 (7.2)
	Residual	38.6 (0.8)	46.8 (0.7)	42.9 (1.5)
30% H$_2$O$_2$	H$_2$O$_2$ oxidised CO$_2$	0.2 (0.0)	1.0 (0.0)	9.3 (1.7)
(pH = 2)	Resin	1.3 (0.1)	0.8 (0.2)	76.2 (17.4)
	0.1 M NaOH	41.8 (0.5)	42.4 (1.9)	52.2 (3.0)
	1 M NaOH	22.6 (3.1)	10.9 (0.5)	109.6 (19.9)
	Residual	34.1 (4.0)	44.8 (4.3)	40.7 (8.7)
30% H$_2$O$_2$	H$_2$O$_2$ oxidised CO$_2$	0.2 (0.0)	1.0 (0.2)	12.1 (0.4)
(pH = 8.5)	Resin	3.0 (0.1)	1.5 (0.4)	105.5 (23.0)
	0.1 M NaOH	43.0 (0.7)	42.1 (2.8)	54.9 (2.7)
	1 M NaOH	20.8 (3.1)	10.6 (0.2)	104.7 (13.1)
	Residual	33.0 (5.4)	44.9 (3.0)	39.2 (3.8)
----------------Pre-treatment with sodium perborate (NaBO$_2$.H$_2$O$_2$.3H$_2$O)----------------				
3% Na-perborate	Perborate oxidised CO$_2$	0.7 (0.0)	1.6 (0.0)	23.5 (0.2)
	Perborate soluble	36.7 (1.2)	45.2 (1.0)	46.3 (4.4)
	0.1 M NaOH	7.9 (0.3)	5.6 (0.1)	80.5 (7.4)
	1 M NaOH	10.8 (0.3)	5.2 (0.9)	120.2 (12.8)
	Residual	44.7 (1.7)	42.4 (0.2)	60.1 (0.1)

Values in brackets are SE (sampling errors).

Perborate oxidation of soil+roots followed by chemical fractionation

Pre-treatment of the soil+root samples with Na–perborate oxidsed only 0.7% of the ^{14}C and 1.6% of the total C as CO_2. The organic compounds soluble in Na–perboarte after heating constituted a considerable proportion of the total ^{14}C (37%) and the total C (45%) (Table 6.4). However, these originated mainly from the 0.1 M NaOH fraction. Some were solubilised from the 1 M NaOH fraction. The specific activity of perborate fraction was similar to the 0.1 M NaOH and residual fractions. Therefore, pre-treament with Na–perborate offers little advantage.

6.3.7.2   Treatments of the residual fraction of soil+roots (Experiment 7)

The various mild pre-treatments, such as anaerobic incubation and chemical (H_2O_2) oxidation of soil+roots, were either not very effective or moderately effective at extracting root C or converting root C to alkali soluble fractions. Moreover, harsher pre-treatments (hot H_2SO_4 and hot Na–perborate) have either recovered most of the recent root and root–released ^{14}C along with almost half of the total C (e.g. hot H_2SO_4 hydrolysis – see Section 6.3.3.1; Figure 6.2a), or caused the shift of NaOH–soluble C fractions to a perborate soluble fraction (Section 6.3.7.1; Figure 6.2d), but did not improve the conversion of the residual fraction to more easily alkali soluble material. Also, treating the alkali–insoluble residue with hot H_2SO_4 hydrolysis solubilised most of the residual fraction ^{14}C and about half of the C (Section 6.3.3.2). Taking all these points into consideration, some further attempts were made to chemically treat the alkali–insoluble residual fraction (Section 6.2.6.2; Treatments f,g,h,i) to recover a dynamic C fraction (Table 6.5). The results are discussed below.

A comparison of treatments applied to the alkali–insoluble residue is shown in Table 6.5. The results indicate that shaking of the alkali–insoluble residual fraction with cold HNO_3 (1 M) recovered only 7% of the residual ^{14}C (i.e. 3.2% of the total ^{14}C in soil+roots) and a slightly more proportion of the residual C (i.e. 9% – which is 3.6% of the total C in soil+roots), thereby leaving the residual fraction with relatively high specific activity. Hot HNO_3 (1 M) treatment, however, recovered 35% of the residual fraction ^{14}C (i.e. 14% of the total ^{14}C in soil+roots) compared to 25% (9% of the total C in soil+roots) of the residual fraction C. This caused the specific activity of the hot 1 M HNO_3 hydrolysed

fraction to be reasonably higher than the remaining residue, which suggests that the hot 1 M HNO$_3$ hydrolysis was more successful in recovering a greater proportion of the ^{14}C associated with recent root and root–released C in the residual fraction. Therefore' the hot 1 M HNO$_3$ hydrolysis could be ranked as a useful step in measuring dynamic C in the alkali–insoluble residual fraction.

Table 6.5: Different treatments of the alkali–insoluble residual fraction of soil+roots to recover a fraction with greater proportions of the recently synthesized root ^{14}C and a high specific activity.

Treatments of the residual fraction	Fractions	% of total ^{14}C	% of total C	Specific activity (Bq mg^{-1}C)
--------------------------------No treatment of the alkali–insoluble residue--------------------------				
Control	Resin	3.2 (0.2)*	1.6 (0.1)	104.4 (14.1)
	0.1 M NaOH	34.0 (1.1)	39.0 (2.4)	43.6 (4.1)
	1 M NaOH	14.9 (0.9)	8.5 (1.9)	90.1 (25.4)
	Residual	47.9 (2.0)	50.9 (4.5)	47.0 (2.1)
--------------------------------Treatments of the alkali–insoluble residue--------------------------				
Cold (C) HNO$_3$ hydrolysis	Resin	3.1 (0.0)*	1.6 (0.2)	130.6 (2.8)
	0.1 M NaOH	37.1 (0.2)	49.9 (2.7)	49.6 (8.8)
	1 M NaOH	16.1 (0.3)	8.6 (0.1)	124.8 (17.7)
	1 M HNO3 (C)	3.2 (0.3)	3.6 (0.3)	60.4 (9.3)
	Residual	40.4 (0.5)	36.4 (2.1)	73.6 (4.4)
Hot (H) HNO$_3$ hydrolysis	Resin	3.3 (0.2)	1.4 (0.1)	151.8 (12.2)
	0.1 M NaOH	38.6 (0.4)	52.8 (3.3)	48.1 (8.3)
	1 M NaOH	17.2 (0.1)	8.5 (1.4)	132.2 (8.9)
	1 M HNO3 (H)	14.4 (0.0)	9.4 (0.4)	100.5 (6.1)
	Residual	26.5 (0.5)	27.9 (1.5)	62.2 (1.8)
5% H$_2$O$_2$ oxidation	Resin	3.2 (0.3)	1.4 (0.1)	138.2 (18.9)
	0.1 M NaOH	37.1 (1.7)	47.8 (3.7)	47.9 (9.4)
	1 M NaOH	16.8 (0.1)	9.3 (1.4)	110.8 (9.6)
	5% H2O2	21.2 (0.3)	21.0 (1.7)	61.6 (0.6)
	Residual	21.6 (2.2)	20.5 (0.5)	64.3 (3.3)
3% Na-perborate oxidation	Resin	3.2 (0.3)	1.6 (0.1)	124.4 (13.2)
	0.1 M NaOH	37.0 (1.6)	48.4 (3.1)	47.2 (9.1)
	1 M NaOH	17.0 (0.2)	9.6 (1.4)	109.7 (5.8)
	3% Na-perboarte	21.2 (0.3)	20.9 (1.6)	62.2 (1.4)
	Residual	21.6 (2.3)	19.6 (0.0)	67.4 (1.6)

Values in brackets are SE of mean of two soil cores

The treatment of the alkali–insoluble residue with 5% H_2O_2 or 3% Na–perborate recovered a greater proportion (about 50%) of the residual ^{14}C but has oxidised approximately the same proportion of the residual C (Table 6.5). This caused almost same specific activity of the acid hydrolysed fraction and the leftover fraction, which suggests that these treatments are not very successful in oxidising the relatively labile C fraction associated with the structural component of the roots.

6.4   GENERAL CONCLUSIONS

The information obtained on the distribution and amount of both labile and passive SOM pools using chemical and density fractionation procedures and chemical fractionation of density fractions, in combination with the functional approach (i.e. the use of relatively labile ^{14}C labelled SOM) indicates that these procedures are not able to singly characterise the "labile" C. The residual fraction of soil+roots, which is intended to be a "nominally" non–labile fraction, retained considerable proportions of active SOM associated with the root and microbial cell walls. However, the lower specific activity of the residual fraction of soil+roots indicates that it also contains much older soil C, which is relatively resistant in nature. The resin fraction of the soil+roots, which recovered soluble and adsorbed organic ions, had a high specific activity and can be considered as a labile fraction in the short–term cycling of SOM and this was confirmed by its ready decomposition in anaerobic incubations.

The light density fraction, even though it possesses a higher specific activity (i.e. of relatively recent origin) compared to the heavy fraction, may not constitute the majority of the active SOM pool as the heavy fraction includes a considerable proportion of the recent root and root–released [14]C. This conclusion is supported by number of other workers (Skjemstad et al., 1986; Christensen, 1992; Boone, 1994; Magid et al., 1996).

The density separation procedure is not capable of separating the SOM into active and stable pools, as indicated by the appearance of labile ^{14}C in the heavy fraction and recalcitrant C in the light fraction. The density separation procedure needs to undergo rigorous standardisation measures in terms of sonication, centrifugation and the dispersion reagents to be used in conjunction with the density separation procedure. Resolving these issues would help to clarify the relative significance of the density separation technique in

terms of defining SOM pools that are important in the cycling and release of plant nutrients.

Density separation followed by chemical fractionation, which has been promoted by some researchers, would seem to be an overly complicated procedure – offering no improved categorisation of dynamic SOM C into pools of distinct lability. The most promising avenue to follow appears to be chemical fractionation by sequentially using ion exchange resins, 0.1 M NaOH, 1 M NaOH and hot 1 M HNO_3. Besides the hot 1 M HNO_3 treatment of the alkali–insoluble residue, the 30% H_2O_2 pre-treatment of soil+roots may also offer an opportunity to better characterise changes in SOM with changes in management and landuse practices. This needs further investigation.

CHAPTER 7   A STUDY OF PROCESSES (DIFFUSION AND PHOTO–ASSIMILATION) INVOLVED IN THE TRANSLOCATION OF $^{14}CO_2$ BELOW GROUND IN PASTORAL SOIL SYSTEM

7.1   INTRODUCTION

Soil organic matter (SOM) represents an important reserve of essential macro–nutrients and maintains essential soil structure. Recently the role of SOM as a large sink for global C has been recognised as important in strategies to control atmospheric CO_2 levels and the 'greenhouse effect' (Paustian *et al.*, 1997a). A number of long–term experiments show that the most efficient method of SOM maintenance in continuously cultivated soil is through the inclusion of fine–rooted cereals and pasture species (including N–fixing species) in crop rotations because of their higher C input rates to the soil (Sparling *et al.*, 1992; Paustian *et al.*, 1997b; Shepherd *et al.*, 2001). Photosynthetic assimilation of atmospheric CO_2 is the primary route for organic C input to soils. To improve the accuracy of predicting the effectiveness of pasture rotations at raising SOM status, more information on the rate of transport, distribution and fate of assimilated C in the soil is required, rather than information solely on the 'net' accumulation of SOM. Plant C enters the soil as dead roots and litter material, but also from actively growing roots as exudates, mucilage's, secretions, sloughed off cells, decomposition of roots as well as respiratory CO_2. It can be argued that C allocated to growing roots more directly influences soil structural improvement (Davenport and Thomas, 1988) than that returned to the soil surface as litter.

Perennial pasture plants maintain roots throughout the year (Caradus and Evans, 1977) and rhizodeposition is cyclical depending on periods of active growth (Singh and Coleman, 1974; Warembourg and Paul, 1977; Meharg and Killham, 1989). Rhizodeposition of C increases with an increase in the quantity of root produced regardless of the crop (annual or perennial) grown (Davenport and Thomas 1988). In annual plants (e.g., wheat) 15% of total fixed C was transferred to roots and 50% of the C transferred to roots was respired and 25% was rhizodeposited into the soil (Keith *et al.*, 1986). However, in perennial plants (e.g., Agropyron–Koeleria), between 30 and 50% of the net fixed C was transferred to

roots and only between 20 and 28% of the C moved to roots was respired. No figures were reported for rhizodeposited C in the soil (Warembourg and Paul, 1977). In general, perennial plants invest more of their productivity in root materials than annuals (Lynch and Whipps, 1990). Paustian *et al.* (1990) reported that perennial plants allocated twice as much C below ground (to root production and root respiration) than annuals. However, the relative proportions of assimilate allocated below ground were fairly similar between annual and perennial crops.

Up to 40% (or more) of the C fixed above ground is subsequently released by roots as respired CO_2 and organic compounds depending on atmospheric CO_2 (van Ginkel *et al.*, 1997; Cheng and Johnson, 1998), soil fertility status (Saggar *et al.*, 1997), slope category (Saggar *et al.*, 1999), water stress (Martin, 1977), temperature (Martin and Kemp, 1980; Meharg and Killham, 1988, 1989) and pH of the soil (Meharg and Killham, 1990b). Little information is available on the quantitative allocation of C to roots at different soil depths depending on root density and root activity. As well as root density this allocation is affected by a number of other factors like soil moisture, bulk density, soil aeration etc. This kind of information is important for optimising the management of pastures to renovate low organic matter soils.

The fate of much of the fixed C allocated to roots is either respiration by roots to CO_2 (Kuzyakov *et al.*, 1999) or root–released C being utilised by soil microbes with concomitant release of microbial respired CO_2 (Rattray *et al.*, 1995). To monitor root and soil (microbial) respiration, $^{14}CO_2$ pulse labelling of crop plants has often been conducted by sealing the soil surface so as to avoid interference from diffusion of $^{14}CO_2$ (Cheng *et al.*, 1993; Rattrays *et al.*, 1995; Kuzyakov *et al.*, 1999). Subsequent evolution of $^{14}CO_2$ from soil is then measured. Soil surface sealing is not feasible under pasture field conditions. Therefore, pulse labelling of different grasslands *in situ* have been done with $^{14}CO_2$ (Saggar and Searle, 1995; Saggar *et al.*, 1997, 1999) and $^{13}CO_2$ (Stewart and Metherell, 1999) without sealing the soil surface. The latter studies (without sealing) did not account for diffusion of $^{14}CO_2/^{13}CO_2$ into the soil assuming that diffusion of isotopically labelled $^{•}CO_2$ into the soil and resultant bicarbonate in soil water contribute little to CO_2 assimilation by plants. Little information is available regarding CO_2 diffusion under these conditions.

The development of mini RSMS allowed the ^{14}C species in the soil water to be sampled with minimum disturbance of the undisturbed cores of permanent pasture soil. This allowed the measurement of respiratory activity at different times and soil depths (see Chapter 5). As the $^{14}CO_2$ gas in the enclosed chamber was also free to diffuse into the soil through gaseous exchange, the measurement of $^{14}CO_2$ activity in soil water may overestimate root and soil (microbial) respiration. Similarly, reductions in enclosed air $^{14}CO_2$ activity caused by diffusion of $^{14}CO_2$ may cause overestimation of photo–assimilation rates. There is, therefore, a need to account for the amount of $^{14}CO_2$ moving into the soil via diffusion. The present study reports experimental work using $^{14}CO_2$ pulse labelling of undisturbed soil cores growing pasture swards under both dark and light conditions. The aim of this was to assess the contribution that $^{14}CO_2$ diffusion into soils makes to below ground allocation of tracer in pulse labelling experiments on pasture. The hypothesis is that there would be little assimilation by the plants during dark conditions and the rate of decline of $^{14}CO_2$ activity in the air would be governed by diffusion of atmospheric $^{14}CO_2$ into the soil. In the light lysimeters, however, plant assimilation of CO_2 may dominate diffusion as a mechanism for atmospheric CO_2 to be transferred to the soil.

The experimental objectives of this study were:

a)   to measure allocation of recently assimilated ^{14}C to shoots, roots and soil, and root–released ^{14}C and diffused $^{14}CO_2$ to soil solution using RSMS at different soil depths below a typical New Zealand Pasture sward under both dark and light conditions, and subsequently in Chapter 8

b)   to model the diffusion of $^{14}CO_2$ and account for the contributions made by $^{14}CO_2$ diffusion, and root respiration and root C release to the appearance of ^{14}C at soil depth.

7.2   METHODS AND MATERIALS

7.2.1   Soil

The soil used was Tokomaru silt loam (Typic Perch–Gley Pallic soil – New Zealand soil classification; Typic Fragiaqualf – USDA soil classification) under ryegrass (*Lolium*

perenne L.)/clover (*Trifolium repens* L.) based pastures. Soil pH, total C, total N, total P, total S and Olsen P at different depths of soil are given in Table 5.1

7.2.2   *Soil core collection*

Four undisturbed soil cores (15 × 21 cm; volume 3.7 L) were collected in PVC cylinders (15 × 21 cm) from soil growing predominantly ryegrass/clover swards as described in Section 5.2.2. However, initially the soil was not sufficiently moist to insert the PVC sampling cylinders easily. The site, from where cores were taken, was watered by ponding for about 5 h using infiltration rings to ensure that the soil was sufficiently moist. Any gap between the moist soil and the wall of the PVC cylinder was packed with soil to prevent the preferential flow of the gas between cylinder wall and soil. Two replicate cores were used for the light and dark experiments, respectively.

7.2.3   *Preparation and placement of RSMS in the soil cores*

The detailed description of preparation, pre-treatment and installation of RSMS in the soil cores is given in Section 5.2.3. Any differences in the setting up procedure for RSMS on these soil cores are mentioned below.

Horizontal holes (4.2 mm diameter) were drilled into the PVC and soil cores at 3, 5, 9 and 13 cm depth below the soil surface. The RSMS were placed in the soil 12 days before pulse labelling to allow contact to develop with the soil. One day before pulse labelling with $^{14}CO_2$, all the lysimeters were watered carefully from the top with 200 ml of distilled water. Two lysimeters were covered with black polyethylene bags during the night, 12 h before the pulse labelling.

7.2.4   *Pulse labelling canopy*

7.2.4.1   For dark lysimeters

A special 'dark canopy' made of a transparent polyethylene bag (300 × 400 × 0.07

Plate 7.1: The $^{14}CO_2$ pulse labelling system. Dark (A) and light (B) lysimeters placed in sand baths (on the left hand side) and connected to a water bath (on the right hand side). Also showing (C) thermometer for noting glasshouse temperature, (D) quantum sensor for PAR measurements, and (E) 3–way stopcock on the dark bag for sampling and injecting $^{14}CO_2$.

mm^3) covered with a black polyethylene bag and final aluminium foil cover (for temperature control) was prepared to provide an enclosed environment and dark conditions (absence of light) for the pasture plants of two lysimeters (dark treatment) and to make injection and sampling of gas easy (Plates 7.1; 7.2). Two bicycle puncture repair patches were affixed to the outside of the transparent polyethylene bags (one for sampling gas and the other for injecting gas) and their edges reinforced using insulating tape. A drop of silicon gel was dried on each patch to ensure air seal that would support a syringe needle (Plate 7.1). For 'dark' cores, the transparent polyethylene bag was inserted inside a black polyethylene bag and the injection and sampling needles were inserted through the dark bag and into the patch on the transparent bag. A subaseal rubber bung on the inside of the transparent bag was used to retain the needle/transparent bag/black bag sandwich in position. A three–way stopcock was attached to the outer end of the needle (Plate 7.1). Early (0830 h) on the day of pulse labelling, the dark polyethylene bags, which had been

loosely placed overnight, were removed and the above–mentioned 'dark canopy' was quickly (< 15 seconds) placed over the dark lysimeters. The mouth of the transparent polyethylene bag was taped firmly around the top of the PVC cylinder to make it air tight and provide an enclosed environment required for pulse labelling. Then the dark polyethylene bag was taped around the top of the PVC lysimeter to ensure no light entered the system. Finally the aluminium foil cover was placed above the dark core canopy to reflect the sunlight and minimise temperature increases inside the canopy (Plate 7.2).

The temperature variations inside the 'dark canopy' were recorded, using a

Plate 7.2: The $^{14}CO_2$ pulse labelling system, after injection of $^{14}CO_2$ pulse. Aluminium foil cover is in place over the dark polyethylene bags on 'dark' soil cores to control temperature rise inside the bag.

thermometer, by setting up one additional soil core covered with the dark canopy (Plate 7.3). It was found that before covering the dark bag with the aluminium foil, the temperature inside the dark bag was 30°C and outside was 23°C. But within half an hour of covering the dark bag with aluminium foil, the temperature inside was 27°C and outside 25°C and did not vary much (±2°C) inside the canopy compared to outside during the 6 h pulse–labelling period.

Plate 7.3: Monitoring temperature fluctuations inside of the dark canopy placed over another soil core (i.e. not used for pulse labelling study) in the open environment.

7.2.4.2   For light lysimeters

The pulse labelling canopy for the remaining two 'light' lysimeters was the transparent polyethylene bag only (as mentioned above and in Section 5.2.4).

7.2.5 $^{14}CO_2$ generation and injection for pulse labelling

7.2.5.1   Dark lysimeters

Volumes of $^{14}CO_2$ gas were produced in a 60 ml plastic syringe as described in Section 5.2.4 (Plate 5.1). Ten ml of 0.2 M $Na_2^{14}CO_3$ was drawn into the syringe and reacted in the body of syringe with 5 ml of 2 M H_2SO_4 by manipulation of the stopcock (Saggar and Searle, 1995). On an average, 15.4 MBq of the $Na_2^{14}CO_3$ was reacted in the syringe with the sulphuric acid. (Equation 5.1).

Once evolution of $^{14}CO_2$ had ceased, the residual solution (Na_2SO_4+H_2O) was injected into sealed Vacutainer™ tubes and stored to determine the residual ^{14}C activity. The ^{14}C–

CO_2 gas remaining (between 30 and 35 ml) was injected into the enclosed air inside the transparent polyethylene bag. This injection was made through the 3–way stopcock attached to one end of the needle and the other sharp end was already inside the polyethylene bags (Plate 7.1).

The residual solution in the Vacutainer^{TM} tube was reacted with NaOH before removing the subaseal cap to neutralise excess acid and ensure trapping of the residual $^{14}CO_2$ and unreacted $Na_2^{14}CO_3$. The ^{14}C activity of the residual solution was determined by scintillation counting. The residual ^{14}C activity was subtracted from the original $Na_2^{14}CO_3$ activity to calculate the activity of $^{14}CO_2$ injected. It was calculated that 11.7 MBq (equal to 34 ml) and 11.8 MBq (equal to 34 ml) of $^{14}CO_2$ were injected into the dark1 and dark2 lysimeters enclosures, respectively. The volume of the enclosed air was calculated by dividing the total $^{14}CO_2$ injected in the enclosure (e.g. 11.7 MBq in dark1 enclosure) by ^{14}C counts ml^{-1} of the air in the enclosure at the start of the pulse (i.e. determined by extrapolating the curve fitted to the declining trend of $^{14}CO_2$ activity inside the 'dark canopy' – see Section 7.3.1; Figure 7.1). However, in the dark2 lysimeter, some of $^{14}CO_2$ activity was lost during injection process from sealing joints between the 3–way stopcock and the syringe because of a blockage in the needle at injection point. Therefore a calculated volume of the enclosure for the dark1 lysimeter was used as a standard to determine the total counts injected in the dark2 lysimeter (i.e. by multiplying the volume with ^{14}C counts ml^{-1} of the enclosed air at the start of the pulse). The calculated volume of the enclosed air for dark1 lysimeter was 2.7 L. It is estimated (by multiplying 2.7 L volume with initial ^{14}C counts per ml – see Section 7.3.1; Figure 7.1) that only 2.7 MBq (equals to 8.9 ml of $^{14}CO_2$) were injected in the enclosed air above the dark2 lysimeter and the remaining 9.1 MBq were lost during injection.

The pasture swards inside the dark canopy were exposed to the $^{14}CO_2$ for 6 h. After pulse labelling for 6 h, the sealed dark canopy was removed and plants were opened up to the natural environment. Even after 6 h, ^{14}C activity in the enclosure, as determined by a Geiger counter, remained high (*cf.* light lysimeters).

7.2.5.2 Light lysimeters

The $^{14}CO_2$ generation procedure was same as described above for the dark lysimeters.

Injection of $^{14}CO_2$ was made as described in Section 5.2.4. Enclosures above the light1 and light2 lysimeters were injected with 11.3 MBq (equal to 33 ml) and 12.0 MBq (equal to 35 ml), respectively. The calculated volumes inside the enclosures placed over the light lysimeters, as described in the previous section for the dark1 lysimeter, were 3.2 L for the light1 and 2.3 L for the light2 lysimeters.

Light lysimeters were pulse labelled for 3 h. After assimilation of $^{14}CO_2$ for 3 h, the activity of $^{14}CO_2$ appeared negligible in the atmosphere above the plants as examined with Geiger counter. The sealed transparent polyethylene bags were removed 3 h after labelling and labelled plants were opened to the natural environment.

Photosynthetically active radiation (PAR) varied approximately between 200 μmoles $s^{-1}m^{-2}$ at the beginning of the pulse (0830 h) to 1050 μmoles $s^{-1}m^{-2}$ maximum at the end of the 3 h pulse–labelling period (1130 h). The atmospheric temperature varied between 20°C at the beginning to 28°C at the completion of the pulse labelling (i.e. after 3 h).

7.2.6   Sampling

7.2.6.1   Air sampling

In the dark lysimeters, gas samples (5 ml) were taken through the 3–way stopcock attached to the dark canopy, using 5 ml syringes, at regular intervals up to 6 h after injection of the $^{14}CO_2$ pulse label. Whereas, in the light lysimeters, 5 ml gas samples were taken from within the transparent polyethylene bag at intervals up to 3 h. The point of sampling was the rubber patch that was affixed on to the polyethylene bags (Plate 7.1). Gas samples from both dark and light enclosures were injected into 10 ml Vacutainer[TM] tubes containing one ml of 2 M NaOH + 0.02 M Na_2CO_3 as a CO_2 trap solution.

7.2.6.2   Soil solution sampling

Samples of soil solution (approximately 0.5 ml) were taken from both the dark and the light lysimeters through the RSMS's placed at 3, 5, 9 and 13 cm soil depths by creating a vacuum in 1 ml syringes attached to the 3–way stop cock on each RSMS (Plate 7.4). These samples were collected periodically up to 28 h after pulse labelling from the dark

lysimeters and up to 168 h from the light lysimeters. The soil solution samples were added to polypropylene mini scintillation vials (LABSERV Cat No.LBS2403JN) containing 1 ml of CO_2 trap solution (0.2M NaOH+ 0.02M Na_2CO_3). These vials were capped immediately and held for scintillation counting. The exact volume of the soil solution (V_s) sampled at different times was calculated after deducting the standard weight of the vial plus 1 ml of trap solution (V_t) from the weight of the vial containing 1 ml of the trap solution and soil solution sampled at each time (V_{ts}) i.e. $V_{ts} - V_t = V_s$.

Plate 7.4: The author sampling of soil solution from one of the light lysimeters by creating vacuum using 1 ml syringe. Transparent polyethylene bag isolates $^{14}CO_2$–enriched atmosphere above soil core.

7.2.6.3   Herbage, soil and root sampling and their preparation for analysis

Samples of the plant shoots/leaves were taken randomly (5 to 6 shoots and leaves) from the light lysimeters sward at regular intervals up to 190 h after $^{14}CO_2$ pulse labelling. These were oven dried (70°C), chopped and stored for total C and ^{14}C analysis (see Section 5.2.6.2). Dark lysimeters were sampled at 30 h only.

At harvest (30 h and 190 h for dark and light lysimeters, respectively), lysimeters were removed from the sand bath (connected to water supply). The shoots were cut at the soil surface, and soil cores were carefully sliced at the soil solution sampling depths (i.e. 3, 5, 9 and 13 cm). Root and soil samples from both the light and dark lysimeters were taken, dried, weighed, ground and stored for C and ^{14}C analysis as described in Section 5.2.6.2. In contrast to the ^{14}C pulse labelling study described in Chapter 5, the clover/grass stems below the soil surface were not removed from the soil (see Section 5.2.6.2) but included in the root and soil samples collected from the upper soil layer i.e. 3 cm. Also, some white roots were separated from the whole roots to determine the ^{14}C activity of recently translocated and stored ^{14}C in new roots. The selected white roots were also dried at 70°C, weighed, and stored for ^{14}C analysis. Total root ^{14}C counts were calculated as the sum of white and older root samples.

The chopping, grinding and sieving of soil+roots and root samples were described in detail in Section 5.2.6.2.

7.2.7 *Analysis*

7.2.7.1 ^{14}C in air samples

Duplicate 0.2 ml aliquots of the trapping solution were transferred to the polypropylene mini vials and diluted to 1.5 ml with deionised water. Scintillation cocktail solution (see Section 5.2.7.2 for the recipe) (4.5 ml) was added to these vials and shaken vigorously to produce a clear emulsion and stored overnight in the dark prior to measuring ^{14}C activity using a Wallac 1414 WinSpectral^{TM} LSC, with standard parameters for ^{14}C counting, and automatic quench correction. The results were reported as ^{14}C activity in MBq m^{-3}. For comparison of between lysimeters, the ^{14}C activity (MBq m^{-3}) data in the enclosed atmosphere was normalised to the percent of the initial activity (see Figure 7.2) because of the differences in the initial activity of the ^{14}C injected in each core.

7.2.7.2 ^{14}C in soil solution

Scintillation cocktail solution (4.5 ml) was added to soil solution (approximately 0.5 ml from RSMS samplers) mixed with 1 ml of 0.2 M NaOH + 0.02 M Na$_2$CO$_3$ trapping

solution and shaken vigorously to produce a clear solution and counted for ^{14}C after storing overnight in the dark.

7.2.7.3 *Total C and ^{14}C in plant and soil samples*

Total C in the shoot, root, and soil+root samples were determined by the modified dichromate digestion procedure (see Section 3.2.5). For total ^{14}C in the shoot, root, separate white root and soil+root samples, the procedure is same as described in Section 5.2.8.

7.2.8 *Methods for estimating possible plant uptake of $H^{14}CO_3^-$ by transpiration*

It was assumed that daily water uptake by the pasture roots via transpiration at $\sim 25°C$ in the glasshouse was 5 mm. A smooth curve of ^{14}C activity in soil water at intervals of 1 cm depth and various times were interpolated from measured values by using Visual basic for Applications and Solver in MS Excel 97 (see Chapter 8 on modelling). The total predicted ^{14}C activity (Bq ml^{-1}) in soil water over a period of 30 h and 8 days for the dark and light lysimeters, respectively, averaged for each soil layer (i.e. 0–3, 3–5, 5–9, 9–13 and 13–21 cm), was obtained. The activity of $H^{14}CO_3^-$ taken up by plants was calculated from the volume of total water removed due to the transpiration demand from each soil layer (assuming the volume of water removed by plants from each layer is proportional to root mass distribution in that layer) and the total predicted ^{14}C activity per volume of soil solution at the same layer.

7.3   RESULTS AND DISCUSSION

7.3.1 ^{14}C activity in air above the pasture swards

The ^{14}C activity [$C_g(t_M)$ as MBq m^{-3}] in the enclosed atmosphere above the pasture swards (i.e. at the surface, z = 0 cm) of both the dark and light lysimeters decreased with time (t_M as minutes) after the injection of $^{14}CO_2$ pulse. For the dark lysimeters, the decline in the ^{14}C activity was modelled by the logarithmic curve (Figures 7.1 and 7.2). For the light lysimeters, the ^{14}C activity decline in the enclosed air was better explained by an exponential decay curve (Figures 7.1 and 7.2).
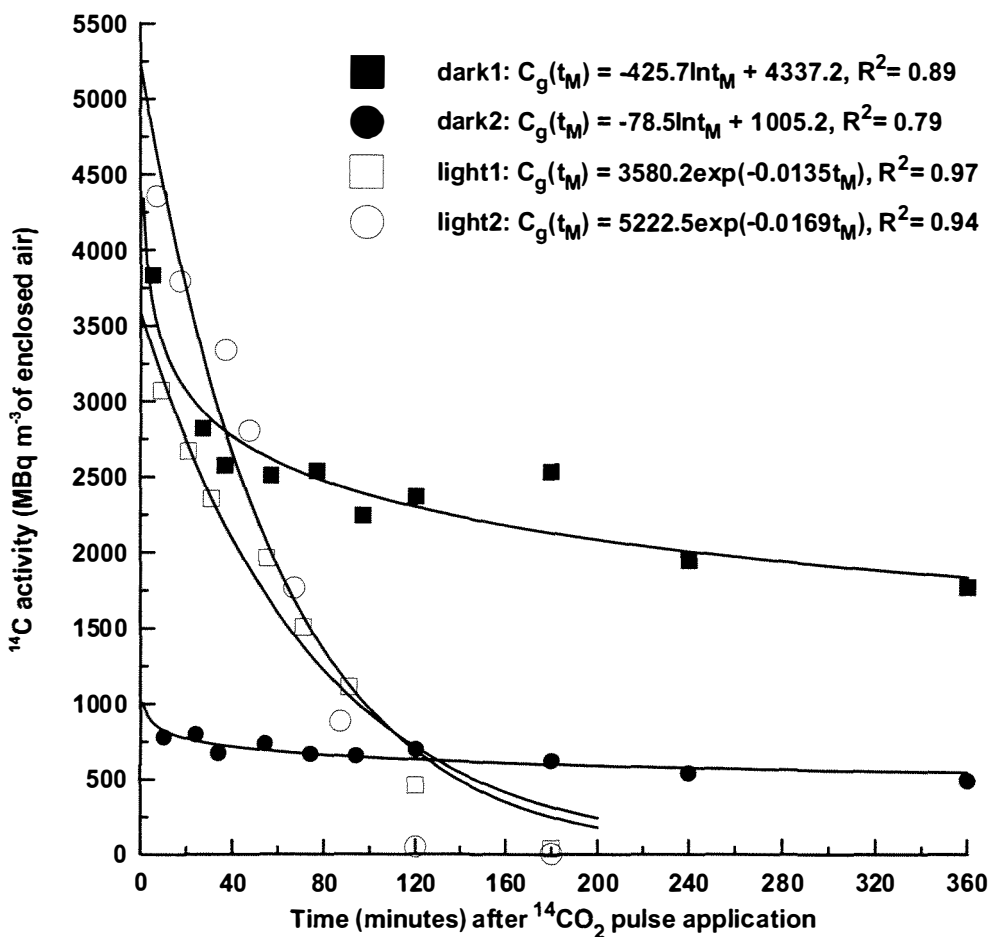
Figure 7.1: The fitted curves represent the ^{14}C activity (MBq m^{-3}) in the enclosed surface air (surface boundary conditions for the diffusion model) above the pasture swards of both dark and light lysimeters as a function of time after the $^{14}CO_2$ pulse was first introduced.

The ^{14}C activity in the enclosed air above the pasture swards reduced to 41% of the initial injected activity $[C_g(t_M = 1)^{\dagger}]$ in the dark1 lysimeter and 49% in the dark2 lysimeter 6 h after injection (Figure 7.2). In light conditions, the ^{14}C activity in the enclosed air above the pasture swards reduced rapidly (within 3 h of pulse application) to $\leq 1\%$ of $C_g(t_M$

dark1: $C_g(t_M) = -9.8\ln t_M + 100$, $R^2 = 0.95$

dark2: $C_g(t_M) = -7.8\ln t_M + 100$, $R^2 = 0.90$

light1: $C_g(t_M) = 100\exp(-0.0135t_M)$, $R^2 = 0.98$

light2: $C_g(t_M) = 100\exp(-0.0169t_M)$, $R^2 = 0.95$
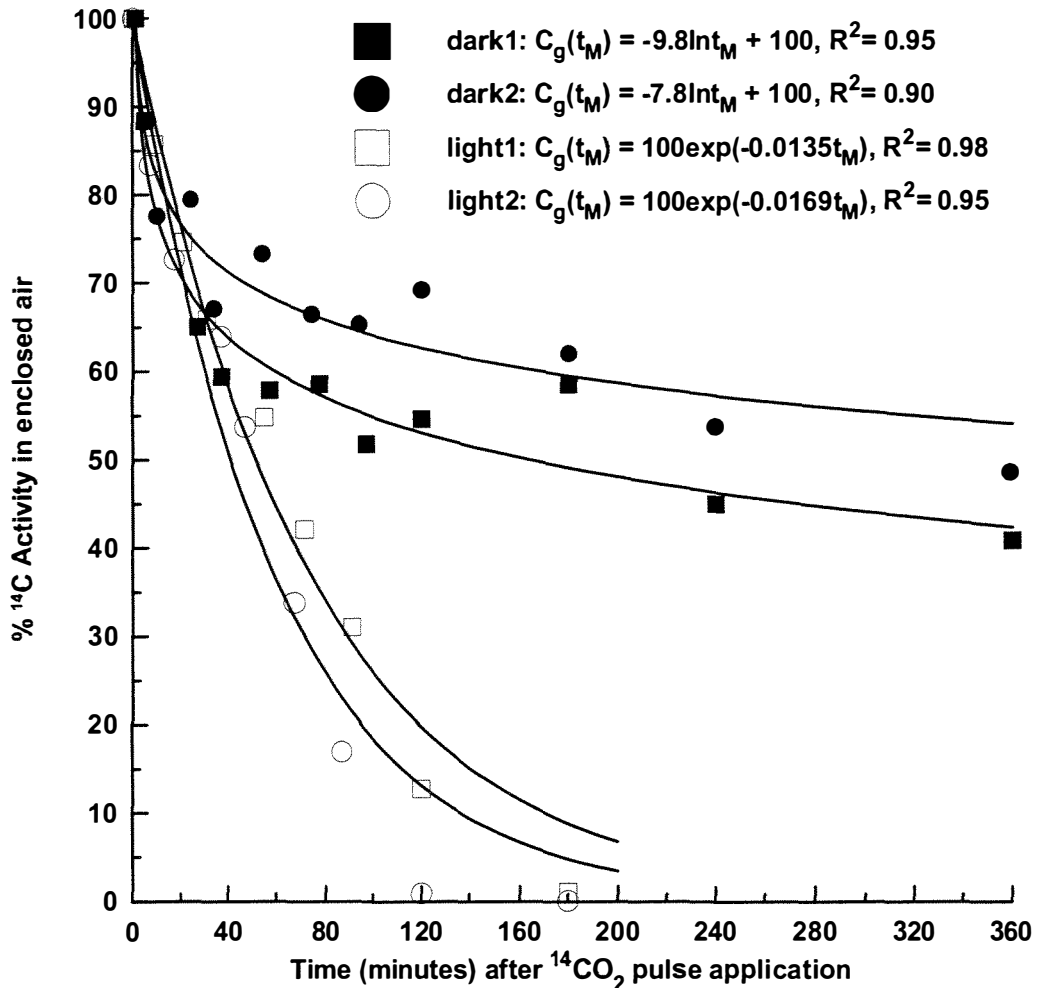
Figure 7.2: Percent ^{14}CO$_2$ activity (of the total initial injected activity) at different times (minutes) after injection of ^{14}CO$_2$ pulse above the pasture swards of both light and dark lysimeters

$\dagger$ The initial time for the logarithmic relationships fitted to the ^{14}CO$_2$ activity decline in the dark lysimeters (Figure 7.1) was set to 1 minute since log $0 = \infty$

= 0) in both the light lysimeters. In the light2 lysimeter, the ^{14}C activity declined very rapidly to <1% of initial ^{14}C activity within 2 h of pulse application, which indicates that either the pasture swards of the light2 lysimeter are more vigorous (see Section 5.3.2) and therefore fixed ^{14}CO$_2$ more rapidly, or that the rate of translocation of ^{14}CO$_2$ by diffusion into the soil was more rapid compared to the light1 lysimeter.

In the first hour after pulse application, the rate of decline in ^{14}C activity was very rapid in the dark lysimeters and was quite similar to the decline in the light lysimeters (Figure 7.2). With time, the rate of decline in ^{14}C activity in the enclosed air above the dark lysimeters slowed down considerably. Calculations estimated that the partial pressure of the total CO$_2$ in the enclosed air after the injection of ^{14}CO$_2$ (for volumes of dark and light enclosures – see Sections 7.2.5.1 and 7.2.5.2) was raised from $10^{-3.5}$ atm of ^{12}CO$_2$ alone to $10^{-1.89}$ atm of ^{12}CO$_2$ + ^{14}CO$_2$ for the dark1, $10^{-2.48}$ atm for the dark2, $10^{-1.97}$ atm for the light1 and $10^{-1.81}$ atm for the light2 lysimeters. The partial pressure of ^{12}CO$_2$ in soil atmosphere in pasture soil is reported to be between $10^{-1.6}$ and $10^{-1.3}$ atm (see Section 5.3.2) which is higher than the total partial pressure of the ^{12}CO$_2$ + ^{14}CO$_2$ in the enclosed air above both the dark and light lysimeters (i.e. between $10^{-2.48}$ and $10^{-1.81}$). This indicates that the initial CO$_2$ diffusion gradient will be out of the soil and ^{14}CO$_2$ diffusion gradient will be into the soil but after a certain period of time, the number of molecules of ^{14}CO$_2$ diffusing into and out of the soil will reach equilibrium (see discussion in Section 5.3.2). In the dark lysimeters, ^{14}CO$_2$ assimilation was very small (see next Section 7.3.2); therefore, diffusion of ^{14}CO$_2$ into the soil was the major sink responsible for the initial (0–1 h) decline in ^{14}C activity in the enclosed air. One hour after the injection of ^{14}CO$_2$, the number of ^{14}CO$_2$ molecules rapidly diffusing into and out of the soil tended to equilibrate (Figure 7.2). Researchers considering photo–assimilation as the only process for ^{14}CO$_2$ decline in the air, for short periods after pulse labelling, may overestimate photo–assimilation rates. Therefore, there is a need to account for the ^{14}CO$_2$ diffusion into the undisturbed soils when short–term photo–assimilation rates are estimated from decreasing atmospheric ^{14}CO$_2$ activity, and when the root respiration rates of ^{14}C–assimilate are being estimated from appearance of "recent" ^{14}C activity in soil water and air (see Chapter 8 for an attempt at this using data obtained in this chapter).

7.3.2   *^{14}C distribution in plant top herbage, roots and soil at harvest*

At 30 h after ^{14}C pulse labelling, the ^{14}C activity and specific activity of the plant shoots in the dark lysimeters was $\leq 0.5\%$ of that observed in the light lysimeters (Figure 7.3a,b). This indicates that the plants in the dark assimilated a very small amount of $^{14}CO_2$.
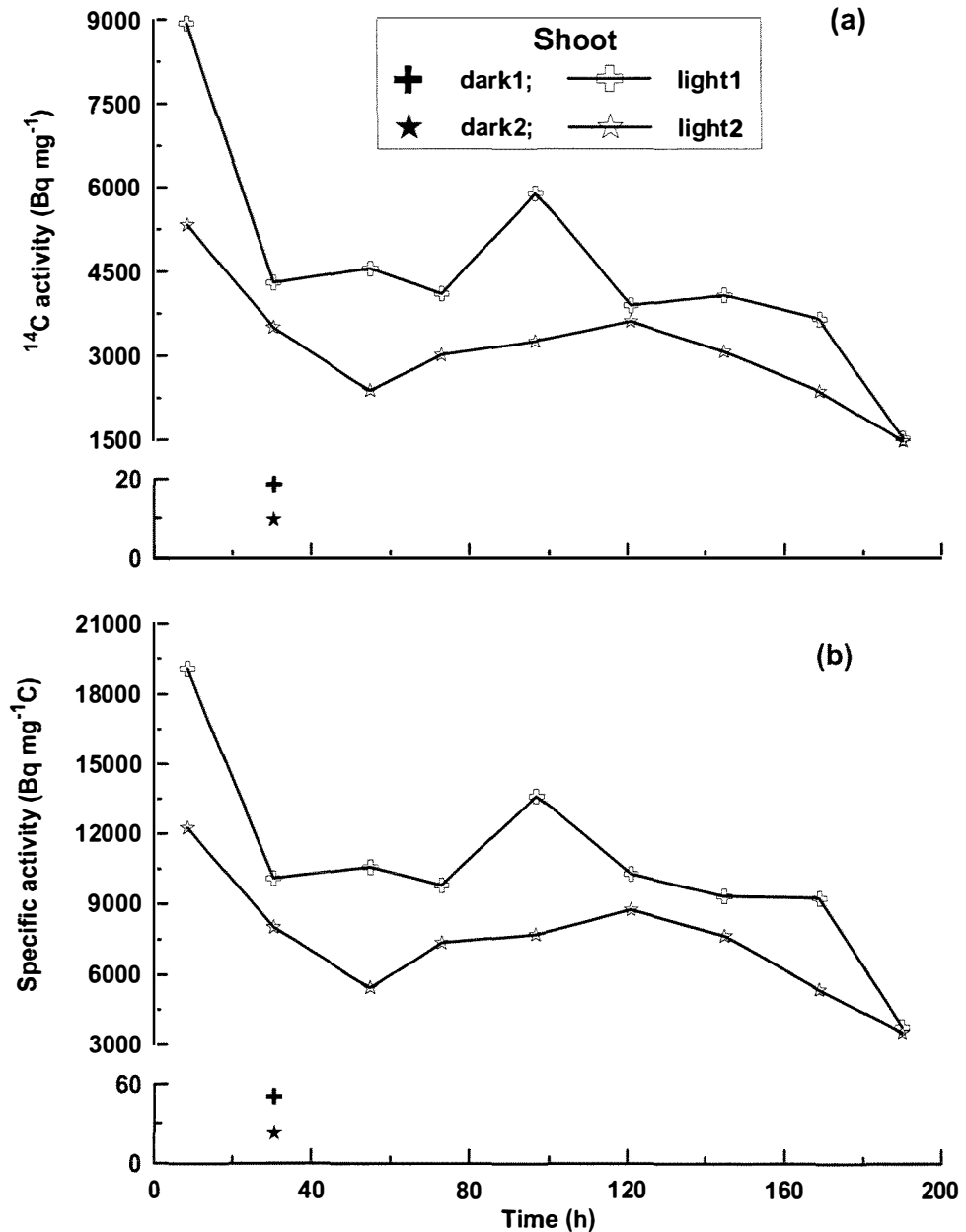
Figure 7.3: Change in (a) ^{14}C activity (Bq mg^{-1}) and (b) specific activity (Bq mg^{-1}C) of pasture shoot at different times after $^{14}CO_2$ pulse labelling under both dark (symbols only) and light conditions (line plus symbols).

This could result from:

a) a short exposure (< 15 seconds) of pasture plants of the dark lysimeters to light during the placement of the "dark canopy" resulting in the initiation of ATP and $NADPH_2$ synthesis in chloroplast required for the 'dark reaction' CO_2 assimilation to occur;

b) some absorption of the $^{14}CO_2$ gas on and into the plant leaves (exchange with HCO_3^- in plant cells) and some uptake of the $^{14}CO_2$ (or $H^{14}CO_3^-$) dissolved in soil water through transpiration flow of water to shoot at ~ 25°C in the dark (see below); and

c) photo–assimilation of $^{14}CO_2$ or $H^{14}CO_3^-$ from the soil water after removal of the "dark canopy" till harvest at 30 h.

In the light lysimeters, the ^{14}C activity (Bq mg^{-1}) and specific activity (Bq $mg^{-1}C$) of the plant shoots sampled at different times was highest at the first sampling time (8.5 h after injection of the $^{14}CO_2$ pulse) and decreased to between one third to one fifth at harvest time (190 h after injection of the $^{14}CO_2$ pulse on day 8). The variability in the decreasing trend of the shoot ^{14}C activity and specific activity (Figure 7.3a,b) was probably the result of uneven herbage sampling with respect to plant parts of active $^{14}CO_2$ assimilation (see discussion in Section 5.3.4.1). The similar trends in changes of ^{14}C activity and specific activity show that the ^{14}C isotope was distributed relatively evenly through the plant shoot tissues (*cf.* Figure 7.3a and 7.3b). The ^{14}C activity and specific activity of the shoots from the light lysimeters was markedly higher than those from the dark lysimeters even though the light lysimeters were harvested 190 h after pulse labelling compared to 30 h after for the dark lysimeters (Figure 7.3a,b). This indicated that dark conditions had indeed minimised $^{14}CO_2$ assimilation.

At harvest, between 30–32% of the total injected ^{14}C was recovered in the plant shoots and between 39–42% was in soil+roots in the light lysimeters compared to a small proportion in the plant shoots (0.3–0.7%) and soil+roots (1.2–2.5%) in the dark lysimeters (Table 7.1). In the light lysimeters, between 26–31% of the total ^{14}C injected above plant has been lost from the system till harvest probably by photorespiration, soil (root and microbial) respiration and back diffusion (after canopy removal) from soil into the atmosphere (Table 7.1). In the dark lysimeters, only 3.2% of the injected ^{14}C activity was

recovered in the shoots, and soil and roots at harvest. This indicated that the plants assimilated and translocated only a very small proportion of injected ^{14}C under dark conditions, which could be due to the various reasons mentioned above. Most of the injected $^{14}CO_2$ activity was lost when the 'dark canopy' was removed after pulse labelling.

At harvest, the majority of the ^{14}C in the soil+roots (i.e. 78–87% in the light and 65–74% in the dark lysimeters) was in the upper 0–3 cm soil layer (Figure 7.4a,b). In the light lysimeters, the roots contributed between 62–72% of the ^{14}C recovered in soil+roots. This root ^{14}C was mainly present in roots in the top 0–3 cm soil layer (54–59%) and decreased considerably (8–13%) in the lower depths (Figure 7.4b). Whereas in the dark lysimeters roots constituted 30% of the small amount (0.3–0.7%) of ^{14}C in the soil+roots at harvest (Table 7.1). Again, this root ^{14}C was mainly present in the roots in the top 0–3 cm soil layer (i.e. between 21–26%) (Figure 7.4a).

Table 7.1: Distribution of total injected ^{14}C (as MBq and %) in different components of pasture shoot–root–soil system under both dark and light conditions at harvest.

Component	MBq of total injected ^{14}C recovered (% of total injected ^{14}C are in brackets)			
	dark1	dark2	light1	light2
Total ^{14}C injected into atmosphere	11.71 (100)	2.71 (100)	11.33 (100)	12.01 (100)
^{14}C remaining in shoots (above–ground)	0.034 (0.29)	0.018 (0.65)	3.39 (29.9)	3.91 (32.5)
^{14}C remaining in soil+roots (sum of all the depths)	0.14 (1.2)	0.07 (2.5)	4.45 (39.3)	5.23 (43.5)
^{14}C lost from the plant–root–soil system[*] [total injected ^{14}C minus ^{14}C in pasture–soil system	11.54 (98.5)	2.62 (96.7)	3.49 (30.8)	2.87 (23.9)
^{14}C remaining in roots (sum of all the depths)	0.043 (0.37)	0.02 (0.75)	3.21 (28.3)	3.24 (26.9)

[*]*Under light conditions, possible pathways for $^{14}CO_2$ loss are shoot, root/soil respiration and back diffusion from soil into the atmosphere after removal of transparent polyethylene enclosure, whereas under dark conditions, most of the $^{14}CO_2$ was lost when dark canopy was taken off at the end of pulse labelling (i.e. after 6 h), as there was negligible assimilation by plants.*

The depth allocation of the ^{14}C assimilates indicates that, irrespective of the amount of ^{14}C in both the dark and light lysimeters (Table 7.1), the depth distribution pattern of ^{14}C in the roots and soil+roots was quite similar i.e. higher proportions of ^{14}C in the top 3 cm depth and small proportions in the lower depths. However, in the dark lysimeters, the relatively high proportion of belowground ^{14}C was present in the soil rather than in the roots in contrast to the light lysimeters (Figure 7.4a,b; Table 7.1). This may suggest that, in addition to absorption of dissolved $H^{14}CO_3^-$ by the soil particles, some chemolithotrophs e.g. nitrifying bacteria may fix $^{14}CO_2$ or $H^{14}CO_3^-$ (Alexander, 1977). Their population can be expected to be higher on the rhizoplane and in rhizosphere soil (Curl and Truelove, 1986).
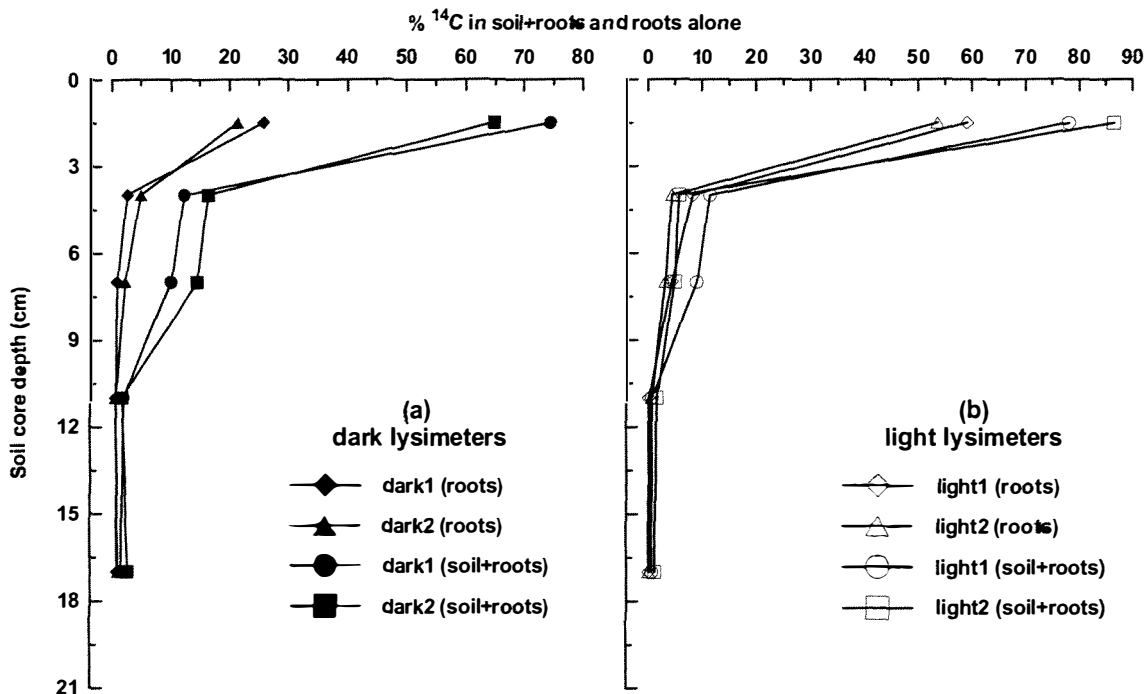
Figure 7.4: Percent distribution by depth of the total ^{14}C recovered below ground at harvest in roots and soil+roots of both (a) dark and (b) light lysimeters. The data points are plotted at mean depth of a soil slice.

The distribution of recently photo–assimilated ^{14}C in the pasture shoot–root–soil system within 8 days of pulse labelling (in this experiment) is comparable to those reported by Rattray *et al.* (1995) and Saggar *et al.*, (1999) for a similar type of plant material within a week of pulse labelling. Swinnen *et al.* (1994) reported that at day 19 after applying the

$^{14}CO_2$ to wheat plants, about half of the initial ^{14}C activity was recovered in shoots and the rest of the labelled ^{14}C was either respired by shoots (36%) or recovered in belowground parts (15%). Swinnen *et al.* (1994) also found that the ^{14}C content of soil organic C and roots decreased exponentially with depth. Moreover, a greater proportion of ^{14}C was concentrated in roots than soil in upper soil layer and vice–versa in deeper soil layers. Zagal (1994) found that between 34–50% of net assimilated ^{14}C was recovered in shoots and 32–42% transferred under the soil surface of *Lolium perenne* grown in a pot and exposed to a ^{14}C–labelled atmosphere. Only 10% was in the soil and the rest in the roots.

In the present study, the roots in the top 3 and 5 cm soil depths constituted up to 75% of the total ^{14}C activity in the soil+roots at these depths in the light lysimeters and 57% in the 9 cm depth and decreased further in the lower soil depths (Figure 7.4b). The lower accumulation of root–released ^{14}C in the upper soil layer (also see Section 5.3.4.2) is ascribed to higher temperatures and enhanced aeration (Swinnen *et al.*, 1994), and high microbial activity (Lavahun *et al.*, 1996) of the soil layers closer to the soil surface compared to the lower soil depth. Thus, in agreement with Swinnen *et al.* (1994), rapid respiration and $^{14}CO_2$ loss is proposed as the mechanism of apparent low recovery of root–released ^{14}C.

In both the dark and light lysimeters, the ^{14}C activity (Bq mg^{-1}, Figure 7.5a,c) and specific activity (Bq mg^{-1}C, Figure 7.5b,d) in samples of soil+roots and roots alone were higher in the top 3 cm soil depth compared to the lower soil depths. However, the ^{14}C activity and specific activity values in the light lysimeters are very large compared to those in the dark lysimeters (*cf*. Figure 7.5a,b and 7.5c,d). The ^{14}C activity and specific activity
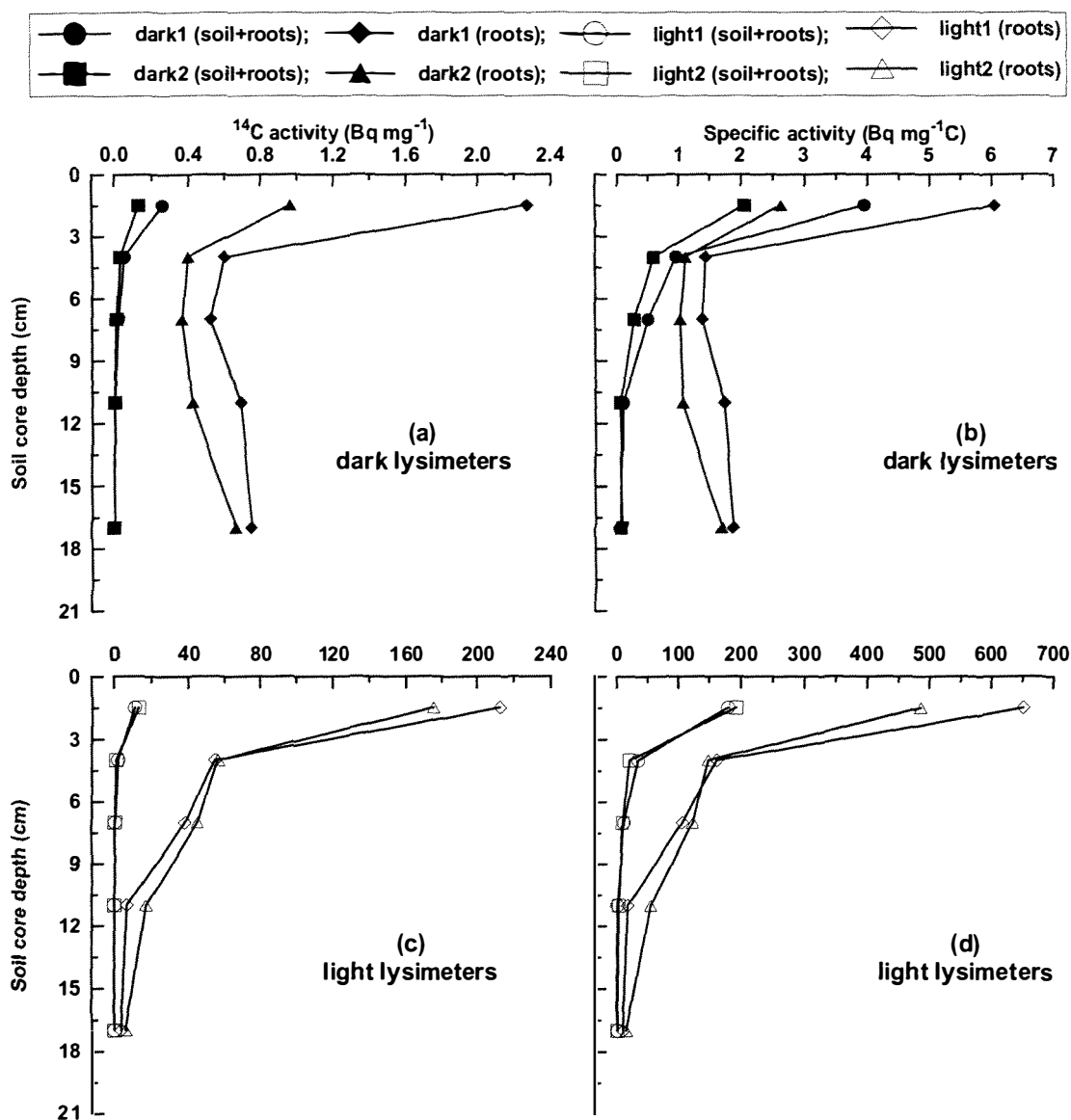
Figure 7.5: ^{14}C activity (Bq mg^{-1}) and specific activity (Bq mg^{-1}C) of soil+roots and only roots at different soil depths in both (a,b) dark and (c,d) light lysimeters at harvest i.e. 30 h *after pulse labelling for dark and* 190 h after for light lysimeters. The data points are plotted at mean depth of a soil slice.

of roots was considerably higher than soil+roots at all the soil depths (Figure 7.5a,b,c,d) except that the specific activity of the roots was lower than the soil+roots at the 3 cm soil depth in the dark1 lysimeter (Figure 7.5b). The higher specific activity of roots indicates that the roots contained a greater proportion of newly allocated ^{14}C counts relative to root ^{12}C. As the pasture sward in the dark lysimeters assimilated a very small amount of injected ^{14}C, the higher specific activity of roots than soil+roots in the dark lysimeters could be attributed to translocation of the small amount of ^{14}CO$_2$ assimilated by the shoots to roots, and uptake of the small amount of ^{14}C labelled bicarbonates in the soil water by roots. In the light lysimeter, the considerably higher specific activity at the 3 cm depth than the dark lysimeters suggests that many new roots per unit of existing root were formed in this depth and these new roots have used a greater proportion of recently photo–assimilated ^{14}C that was translocated below ground compared to the roots at lower soil depths (Figure 7.5b,d). Selected new white roots also show this pattern (Figure 7.6a,b). Considerably lower specific activity at the lower depths indicates a significant decrease in root activity in terms of ^{14}C fixed against a background of unlabelled root ^{12}C.

The ^{14}C specific activity (Bq mg^{-1}C) of the new white roots from light lysimeters was much greater than the dark lysimeters at all the soil depths (Figure 7.6a,b). This indicates a greater assimilation of radioactive ^{14}C in light lysimeters and a greater translocation and storage in the new white roots that are formed after the pulse labelling. Interestingly, in the dark lysimeters, the ^{14}C specific activity (Bq mg^{-1}C) of the white roots at the 3 cm soil depth is about the same as that of the shoots at harvest (*cf.* Figures 7.6a and 7.3b); whereas in the light lysimeters, the ^{14}C specific activity of the white roots at the 3 cm is about two times to that of the shoot and was still higher at 5 cm depth (*cf.* Figures 7.6b and 7.3b). This difference could result for two reasons: either a) turnover of shoot C was probably greater than the root C or b) shoot samples contained a larger mass of unlabelled ^{12}C at labelling and newly synthesised ^{14}C always remained a small proportion of the total shoot C. White roots, however, which were selectively sampled, were relatively new in nature having higher proportion of pulse ^{14}C.
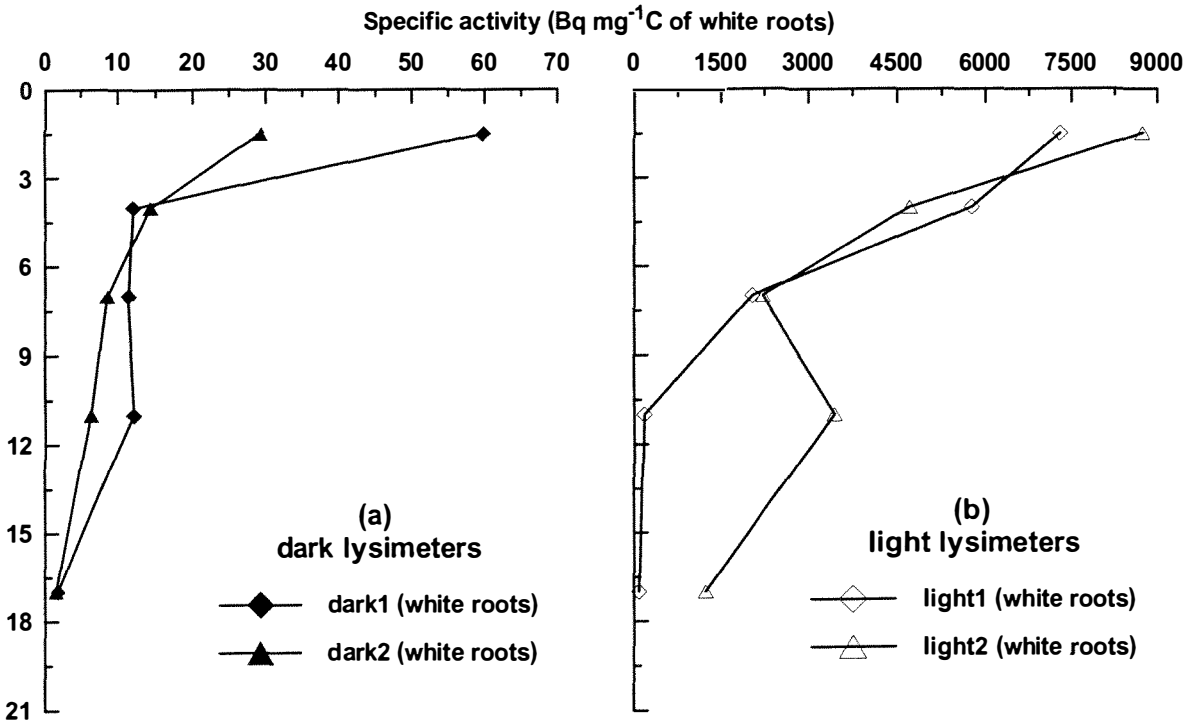
Figure 7.6: Specific activity (Bq mg^{-1}C) of only white roots from both (a) dark and (b) light lysimeters at different soil depths. The data points are plotted at mean depth of a soil slice.

7.3.3 ^{14}C activity in soil solution

Negligible counts in soil solution extracts after acidification confirmed that virtually all ^{14}C activity in soil solution was ^{14}CO$_2$ or H^{14}CO$_3^-$ (see Section 5.3.3.1).

Both in the dark and light lysimeters, the ^{14}C activity (MBq m^{-3}) in soil solution reached peak values at different times after ^{14}CO$_2$ pulse labelling of the plants depending on soil depth and was lower at greater soil depths (Figures 7.7a,b and 7.8a,b). In the 3 and 5 cm depths, respectively, the maximum ^{14}C activity was reached within 2 and 3 h of pulse labelling in the light lysimeters, compared to 6 and 9 h in the dark lysimeters. At the 9 and 13 cm depths, the ^{14}C activity in soil solution was low and peak activity was not discernible within 28 h in the dark1 lysimeter (Figure 7.7a).

The ^{14}C activity in the soil solution of dark2 lysimeter was less than the dark1 because it received approximately 4 times less $^{14}CO_2$ (see Table 7.1). However, the chronological patterns of peak ^{14}C activity in the soil water of both dark lysimeters were similar (cf. Figures 7.7a and 7.7b). Interestingly, the ^{14}C activity per cm^3 of soil water from 3 and 5 cm depths at peak heights is similar for the light (Figure 7.8a,b) and dark lysimeters [compared with the dark1 lysimeter (Figure 7.7a) because it received approximately the same ^{14}C counts].

In the light lysimeters, the appearance of atmospheric $^{14}CO_2$ into the soil water was very rapid – occurring within 10 and 50 minutes after the introduction of the $^{14}CO_2$ pulse in the 3 cm (containing between 47–59% of plant root mass; Figure 7.9) and 5 cm (containing

Figure 7.7: ^{14}C activity (MBq m^{-3}) in soil solution from (a) dark1 and (b) dark2 lysimeters at different soil depths (see legends) and times (h) after $^{14}CO_2$ pulse application to pasture swards.

between 15–25% of plant root mass; Figure 7.9) soil depths, respectively (Figure 7.8a,b). However, in the dark lysimeters, the ^{14}C activity in soil solution was low within the first hour and even at the time of occurrence of peaks (2–3 h) in the light lysimeters at different soil depths (Figure 7.7a,b). The physical structure of all soil cores, root distribution and

soil water content were expected to be more similar than different, between the light and dark cores, therefore, earlier appearance of $^{14}CO_2$ and peaking in the light lysimeters is attributed to rapid photo–assimilation, translocation and root respiration of $^{14}CO_2$.

The ^{14}C allocation in shoots and roots discussed in Section 7.3.2 (Figures 7.3 and 7.5; Table 7.1) confirmed that: a) in the light lysimeters, $^{14}CO_2$ assimilation was appreciable and therefore appears to dominate diffusion as a mechanism for atmospheric $^{14}CO_2$ to be transferred to the soil and roots, whereas in the dark lysimeters diffusion is the major process, and b) much more $^{14}CO_2$ was released/respired by the roots of the light lysimeters compared to the dark lysimeters. Therefore, the difference in the pattern of ^{14}C activity in soil solution of the light lysimeters, compared with the dark lysimeters, was the large contribution of root-derived ^{14}C (since $^{14}CO_2$ diffusion and dissolution in soil water, and $^{14}CO_2$ assimilation by chemolithotrophs occurred in both the dark and light treatments) (*cf.* Figures 7.7a,b and 7.8a,b). In Chapter 8, these patterns in the dark lysimeters are shown, by modelling, to be consistent with diffusion theory (Figure 8.4).

Simple calculations show that at harvest of the dark lysimeters, about 34–63% of the total MBq of ^{14}C recovered in shoot–root–soil system could have been taken up as $H^{14}CO_3^-$ by roots via transpiration flow (assuming 5 mm of daily water uptake by pasture plants – see Section 7.2.8) during the 30 h of growth period after pulse labelling. This suggests that uptake of $H^{14}CO_3^-$ dissolved in soil water by roots was a significant factor responsible for the small amount of activity that was detected in the pasture shoot–root system exposed to the ^{14}C pulse under dark conditions. Whereas, in the light lysimeters, the uptake of $H^{14}CO_3^-$ over the period of 8 days by the pasture roots via transpiration, in proportion to the total ^{14}C activity in the pasture shoot–root–soil system was very small (approximately between 2.6–3.0% of the total ^{14}C activity in shoots and soil+roots). Plus it is likely that the $^{14}CO_2$ gradient is higher at the root surface in the light lysimeters. This indicates that the contribution of $H^{14}CO_3^-$ dissolved in the soil water to the total ^{14}C–assimilates in the pasture plant of the light lysimeters was very small.
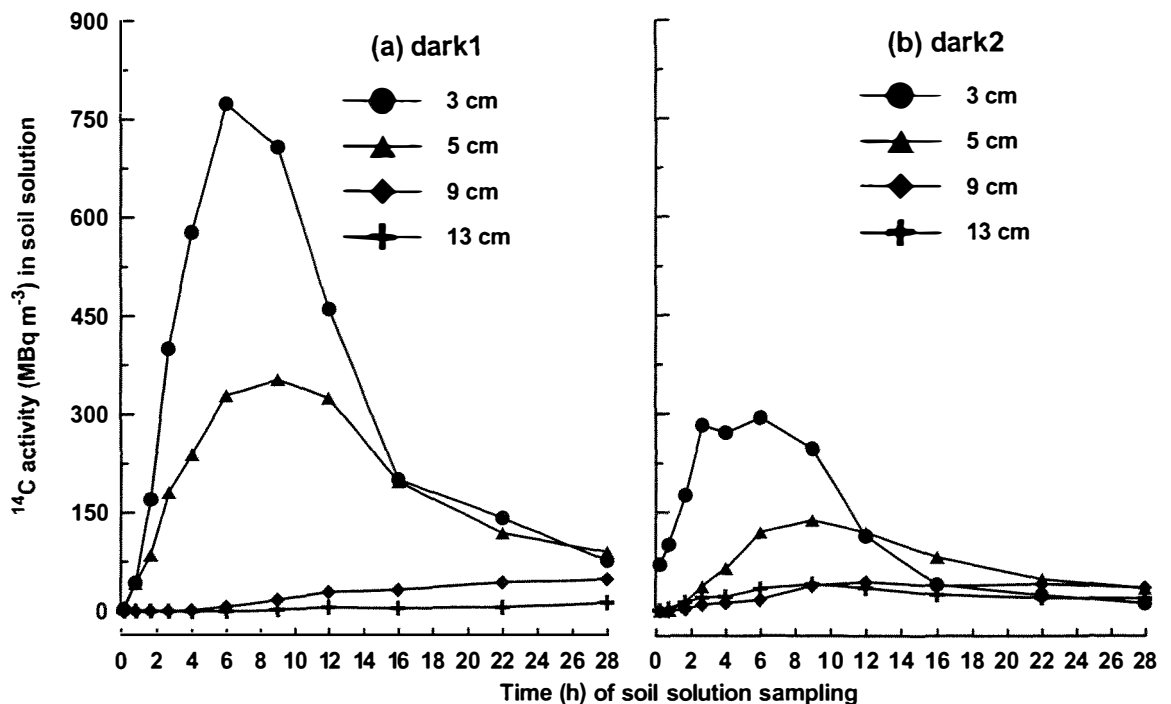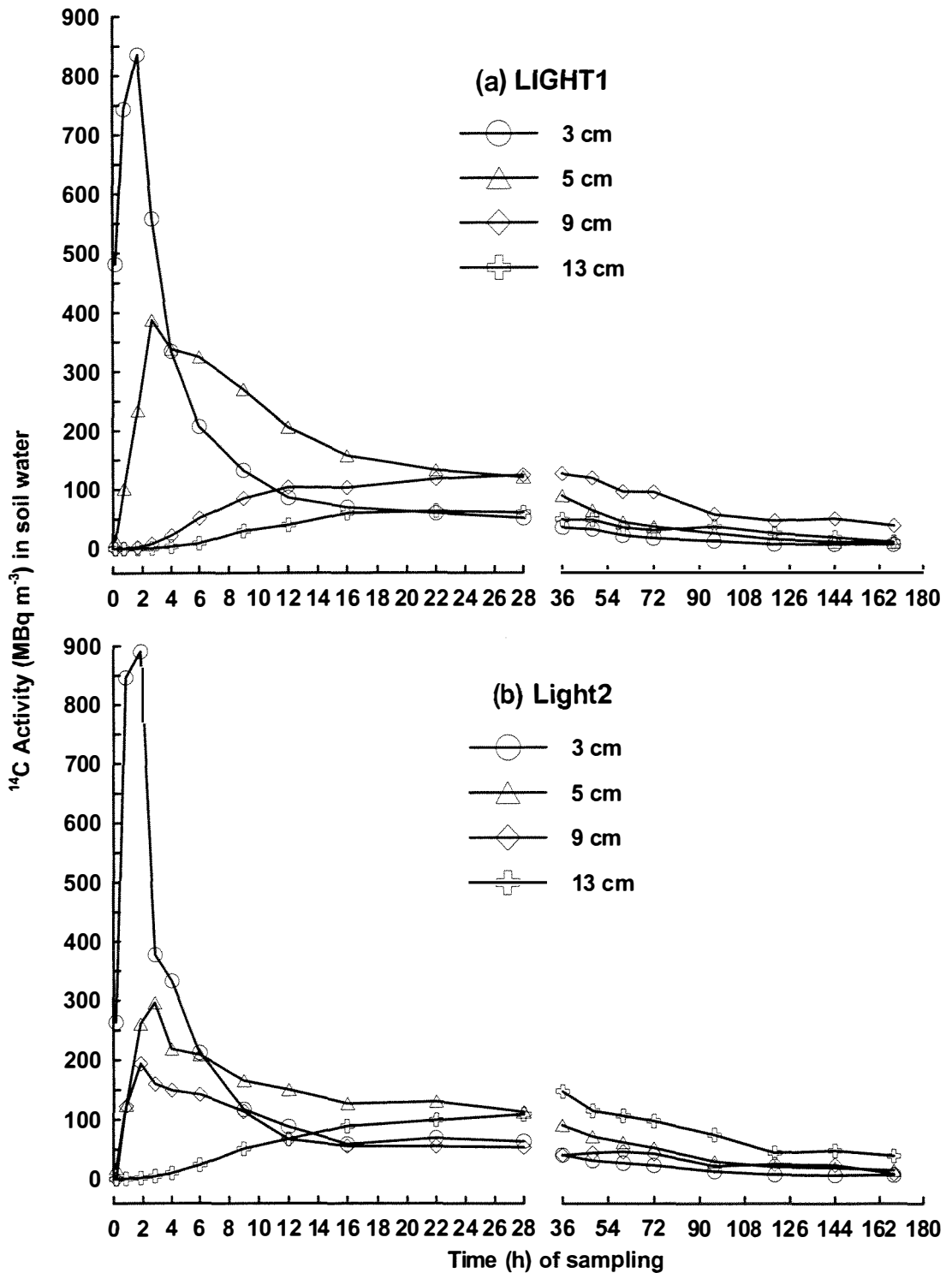
Figure 7.8: ^{14}C activity (MBq m^{-3}) in soil solution from (a) light1 and (b) light2 lysimeters at different soil depths (see legends) and times (h) after $^{14}CO_2$ pulse application to pasture swards.

In the light lysimeters, at the 3 and 5 cm depths, the ^{14}C activity in soil water declined very rapidly after reaching the peak compared to the other soil depths (except 9 cm depth in light2 lysimeter) (Figure 7.8a,b). The ^{14}C activity in soil water at all the depths reached almost to background level by the harvest time (Figure 7.8a,b). This indicates the absence of ^{14}C–labelled root exudates and the low specific activity of root respired CO_2 by the harvest time. It can be concluded that most of the residual ^{14}C found in roots and soil at the harvest time (see Section 7.3.2 above) was mainly located in the non–metabolic pool of the roots and rhizosphere microbes. Similarly, Kuzyakov *et al.* (1999) noticed a negligible $^{14}CO_2$ evolution from the soil 8 days after pulse labelling of *Lolium perenne* and concluded that most residual ^{14}C in the soil was located in the structural pool of roots. The greater proportion of ^{14}C in soil than roots in the dark lysimeters indicates that the non–metabolic pool of chemolithotrophs should also be considered in the description of C pools.

7.3.4   *Attempting a ^{14}C balance in lysimeters*

In the light lysimeters at 3 h after injection, $\leq 1\%$ of injected $^{14}CO_2$ remained in the atmosphere. Therefore, $\geq 99\%$ of the injected $^{14}CO_2$ activity appeared to be present as shoot, root, soil, soil air and soil water ^{14}C (Figure 7.2). This is consistent with 69–74% of the total injected $^{14}CO_2$ activity being in shoot, root and soil at harvest (i.e. 190 h after pulse labelling) (Table 7.1). In the dark lysimeters (i.e. with negligible assimilation), however, it was shown in Section 7.3.1 that the ^{14}C activity in the enclosed air was reduced to only 41–49% of the total injected ^{14}C 6 h after injection (Figure 7.2). Therefore, the difference from that injected [6.9 MBq (i.e. 59% of the total $^{14}CO_2$ activity) in the dark1 lysimeter and 1.4 MBq (51% of the total $^{14}CO_2$ activity) in the dark2 lysimeter] should be recovered into the soil pore space. However, estimates of the total ^{14}C activity in soil water (Section 7.2.8) of the dark lysimeters at 6 h after pulse labelling recovered considerably less ^{14}C activity in the soil water and air (i.e. <0.8 MBq for dark1 and <0.2 MBq for dark2 lysimeters) than the difference between injected (at the start of the pulse labelling) and measured enclosed–air $^{14}CO_2$ activity at 6 h (*cf.* Figures 7.1 and 7.7a;b). Therefore, in the dark lysimeters, we either have a loss of atmospheric $^{14}CO_2$ due to leakage, or loss during sampling the soil solution i.e. by degassing of soil water during sampling, as $^{14}CO_2$ samples may have been withdrawn from dead end pores. Also, incomplete mixing of $^{14}CO_2$ in soil water with $^{14}CO_2$ in soil air pore space could be a factor.

7.3.5　　　　*Factors affecting below ground allocation of* ^{14}C

The greater proportion of root mass (between 47–59%) is recovered in the top 0–3 cm layer of the soil cores and decreased down the soil cores (Figure 7.9), which is reflected in decreasing proportion of ^{14}C recovered in lower soil depths. The initial amount of the recently photo–assimilated $^{14}CO_2$ released by the roots is also likely to depend on the root mass distribution with depth because of greater translocation to the top soil layers initially, and with time, the ^{14}C assimilates are translocated to the lower soil layers (see Section 5.3.3.2). However, in the present study, at harvest, the ^{14}C allocation per unit of root mass and specific activity of the roots in the light lysimeters were considerably less in the lower depths (Figure 7.5c,d), indicating that the roots in the lower depths are less active compared to roots in top 3 cm soil depth or fewer new roots are formed in the lower
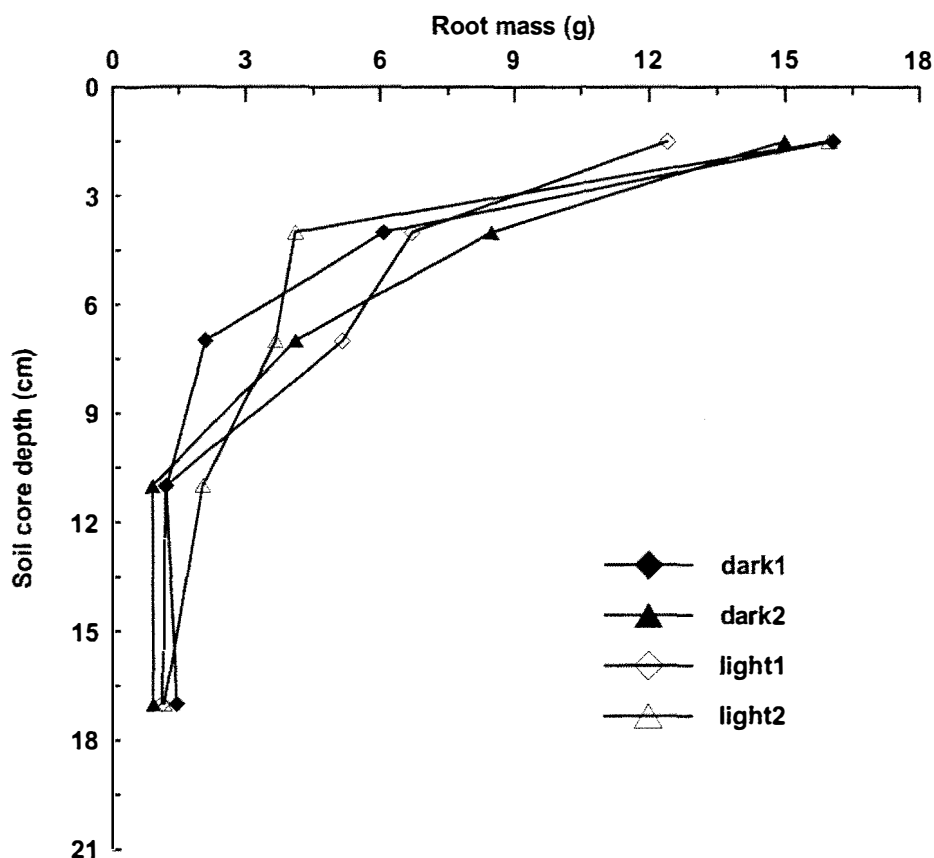
Figure 7.9: Root mass (g) at different soil depths in both dark and light lysimeters.

depths. Therefore, root respiratory activity per unit mass of root may not be constant with soil depth and may not be proportional to root density.

A number of studies have reported that the recently assimilated ^{14}C is translocated and released very quickly from the metabolic pool of the roots (Prosser and Farrar, 1981; Warembourg *et al.*, 1982; Cheng *et al.*, 1993; Rattray *et al.*, 1995; Kuzyakov *et al.*, 1999 Saggar *et al.*, 1999 – see discussion in Section 5.3.3). The present study and the preliminary study reported in Chapter 5 confirmed the findings of other workers and found that the RSMS are very effective way of monitoring the rapid translocation of recently assimilated ^{14}C by plants, which occurred within the 10 minutes of the introduction of pulse. In Chapter 5, the RSMS monitoring showed that, with time, the recent ^{14}C assimilate from the shoot was transported to the roots in lower depths and released in greater amount in soil water (see Section 5.3.3.2), which indicated that the roots were actively respiring in the lower soil layers. But in contrast to the results of Chapter 5 (Figure 5.4), the root activity in the lower soil depths in the present study was found to be low and did not produce a peak of the ^{14}C activity in soil water to the same height as obtained in the 3 cm soil depth (*cf.* Figures 5.4 and 7.8a,b). The peak heights, especially at the 9 and 13 cm depths, remained quite low (Figure 7.8a,b), which again suggest that the roots in these depths are not actively respiring.

The main reason for the difference in the ^{14}C activity in soil water, obtained in the present study and that found in Chapter 5, was probably due to their relatively high soil moisture content. They were taken from the field after ponding the sampling site with water using infiltration rings (see Section 7.2.2). Also, water was applied from the top of the soil cores (see methods Section 7.2.3) one day before pulse labelling. The high water content caused the air–filled porosity to decrease (Table 7.2). This would place constraints on gaseous exchange between the soil air and the atmosphere. This could have reduced the oxygen concentration in the soil to critical concentrations required by the plant roots and soil microbes to function normally.

The oxygen concentration in the soil air space at a given depth in the soil is regulated by the diffusive resistance of the soil and by the rate of oxygen demand of the plant roots and soil biota (Jury *et al.*, 1991). As the oxygen is depleted it must be replaced by oxygen moving from the atmosphere above the surface into the soil. Even relatively high values of

oxygen in the soil air may not be sufficient enough to prevent aeration problems in plant roots surrounded by thick water films as the water film around the root represents an

Table 7.2: A comparison of some key properties of soil cores of two ^{14}C pulse labelling studies (present study and study described in Chapter 5) taken from the same pasture field – one is relatively wet than the other.

Soil core depth	Bulk density (ρ_b as Mg m^{-3} soil)	Volumetric water content (V_w as m^3 m^{-3} soil)	Air–filled Porosity (f_g as m^3 m^{-3} soil)
-----------------	----------------	--Measured values in the present study---	----------------
0–3 cm	0.67 (0.07)*	0.52 (0.08)	0.21 (0.11)
3–5 cm	0.87 (0.15)	0.59 (0.09)	0.06 (0.15)
5–9 cm	0.98 (0.06)	0.57 (0.08)	0.03 (0.11)
9–13 cm	1.12 (0.08)	0.48 (0.03)	0.07 (0.06)
13–21 cm	1.23 (0.07)	0.44 (0.03)	0.07 (0.05)
	------------Measured values of the study described in Chapter 5-----------		
0–2 cm	0.88 (0.17)#	0.36 (0.05)	0.29 (0.12)
2–4 cm	1.07 (0.04)	0.48 (0.01)	0.09 (0.01)
4–8 cm	1.00 (0.01)	0.46 (0.00)	0.13 (0.01)
8–12 cm	1.08 (0.12)	0.43 (0.05)	0.14 (0.1)
12–16 cm	1.28 (0.03)	0.43 (0.01)	0.06 (0.00)
16–20 cm	1.31 (0.16)	0.41 (0.03)	0.06 (0.09)

Values in brackets are S.E. of mean of four soil cores
#*Values in brackets are S.E. of mean of two soil cores*

enormous barrier to diffusion (approximately 10, 000 times less diffusion in water than in air) (Kristensen and Lemon, 1964 – as cited by Jury *et al.*, 1991).

A model was constructed to examine the likely effect of soil water content on root respiration in this experiment. Recently, Sierra and Renault (1998) have shown that the

maximum oxygen consumption rate (averaging 35 L m^{-3} day^{-1} at 20°C) occurred in the top 30 cm layer of a Gleyic Luvisol (FAO classification; Aqualf – USDA classification) soil profile under ryegrass crop (*Lolium multiflorum*). In the present study, the oxygen consumption rate of 35 L m^{-3} day^{-1} was assumed as constant in space and time for the soil cores of 21 cm length from Typic Fragiaqualf (USDA classification).

The rate of change in oxygen concentration at various soil depths can be obtained by solving the equation of continuity of gas flow (Equation 8.3) and then simulating the oxygen transport and consumption process at various air–filled porosity values in a spreadsheet (Figure 7.10). The following assumptions can be made: a) a steady state flow of oxygen from the atmosphere to the soil air (as the oxygen concentration in the atmosphere is constant); b) a constant oxygen consumption rate (i.e. 35 L m^{-3} day^{-1}); and
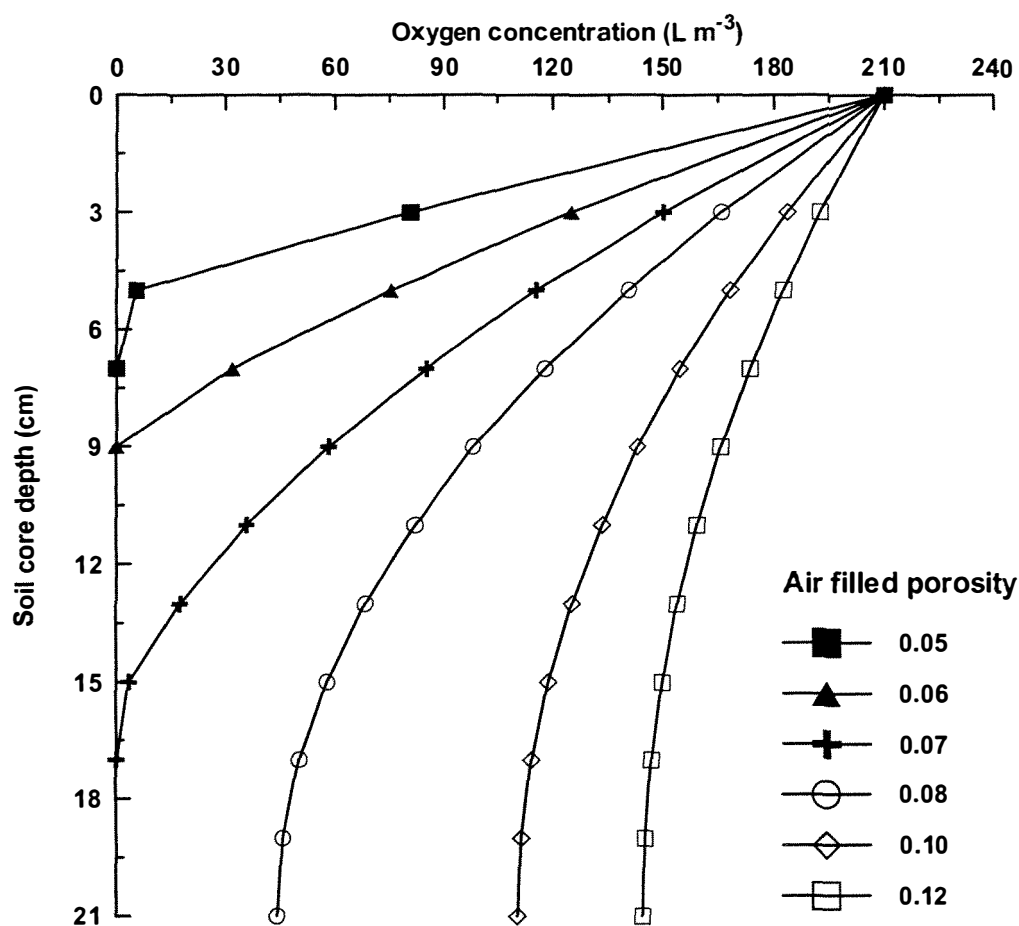
Figure 7.10: The simulated effect of change in air–filled porosity (m^3 m^{-3} of soil) on the oxygen concentration in soil air with depth.

c) the biologically active soil depth was 21 cm, and d) there was no escape below the root zone.

The simulated oxygen concentration in the soil air with depth clearly shows that the soil, with air–filled porosity values (f_g) of 0.06 and 0.05 m^3 m^{-3} of soil, becomes anaerobic at 9 and 7 cm depths, respectively (Figure 7.10). As the measured (Table 7.2) and modelled (see Chapter 8) air–filled porosity values for soil cores in the present study are within this range, so the lower ^{14}C activity recovered in soil water at lower soil depths (Figure 7.8a,b) is almost definitely due to the reduced root and microbial activity caused by oxygen availability constraints.

However, the root activity per unit of root mass in terms of ^{14}C activity retained (Figures 7.5c,d and 7.6b) and released by roots into the soil (Figure 7.8a,b) was high in the top 0–3 cm soil layer. The reason for high root activity in the top 0–3 cm soil layer may well be explained by various reasons: a) the layer is closer to the soil surface that probably caused the better gaseous exchange between the soil air and the atmosphere and ensured continuous supply of oxygen for optimum root respiration, b) water can move down under the influence of gravity, and lost from the surface layer by evapotranspiration, which makes this layer relatively less wet than the other lower soil layers; and c) bulk density is low and the measured porosity value is high (Table 7.2).

7.4   CONCLUSIONS

This study shows that CO_2 is rapidly photo–assimilated, translocated and released (within 10 minutes) by roots. *In situ* sampling of soil solution with RSMS is a novel method to monitor rapid translocation of atmospheric $^{14}CO_2$ (i.e. whether it is by diffusion or by assimilation and release by plants) in soil water and air.

The proportion of total photo–assimilated ^{14}C allocated to roots and soil decreases with depth as root mass decreased down the soil profile. The considerable decrease in specific activity of the roots with depth suggests that root respiratory activity may not be proportional to root density with depth. Lack of oxygen in the soil air, due to high water content and low air–filled porosity, can significantly affect the root activity at depth. In order to enhance the soundness of SOM dynamics models for plant–root–soil systems,

further studies that look at the extent to which depth allocation of C, and C turnover as influenced by different soil moisture levels, would be very useful.

The following comments are relevant to isotopic $^*CO_2$ pulse labelling of plant–soil systems. While a pasture sward is actively photo–assimilating isotopically labelled $^*CO_2$, diffusion of $^*CO_2$ into soils and uptake of dissolved $^*CO_2$ ($H^*CO_3^-$) by roots does not appear to be a significant pathway for $^*CO_2$ translocation. In non–photosynthetic periods, however, diffusion, plant uptake of isotopically labelled $^*CO_2$ (H^*CO3^-) and assimilation by chemolithotrophs should be considered in accounting for the fate of isotopic $^*C$.

Finally, the PVC pipe, adhesive tape and polyethylene enclosures used in this study appear to allow significant $^{14}CO_2$ leakage if photo–assimilation is not rapidly occurring. In studies where ^{14}C balance calculations are necessary to calculate rates of C transfer; this system should not be used.

CHAPTER 8   MODELLING $^{14}CO_2$ DIFFUSION AND ACTIVITY IN THE SOIL SOLUTION OF UNDISTURBED SOIL CORES

8.1   INTRODUCTION

To study the allocation and transformations of recently assimilated ^{14}C in the pastoral soils, it was considered that intact soil cores taken directly from the field, rather than repacked soil, should be used. However, sealing of pasture soil surface is not feasible (see Section 7.1) and examining soil respiration (root and microbial) under these conditions could lead to incorrect estimates because of interference from diffusion of $^{14}CO_2$. There is, therefore, a need to account for the $^{14}CO_2$ diffusion. Exposing these intact soil cores to a $^{14}CO_2$ pulse under dark conditions resulted in negligible photosynthetic assimilation of CO_2 by the pasture (Section 7.3.2). Therefore, $^{14}CO_2$ moves into the soil solution of the 'dark' lysimeters solely by molecular diffusion and dissolution in the soil water. In the 'light' lysimeters, $^{14}CO_2$ gets into the soil solution both by gaseous diffusion of CO_2 into the soil and by respiration of photosynthetically fixed ^{14}C by plant roots (Section 7.3.3). The results (Figures 7.1 and 7.8a,b) show that $^{14}CO_2$ is rapidly assimilated by the plants, translocated and released by the roots. In the present chapter, $^{14}CO_2$ diffusion model was developed for the pasture–soil system exposed to dark and light conditions in an attempt to predict the different contributions made to the ^{14}C concentration in the soil water resulting from $^{14}CO_2$ diffusion and photo–assimilation, translocation followed by root or rhizosphere microorganism respiration.

The concentration of CO_2 and O_2 in soil depends upon the rate of respiration of microorganisms and plant roots, the rate of gaseous exchange with the atmosphere, the solubility of gases in water, water content, and soil depth (Jury *et al.*, 1991; Simunek and Suarez, 1993; Suarez and Simunek, 1993). Soil respiration is a driving force for two opposite gas fluxes (O_2 and CO_2) between the soil and the soil surface (Freijer and Leffelaar, 1996). Therefore, in the $^{14}CO_2$ diffusion model in the present study, besides diffusion of $^{14}CO_2$ into the soil (which is a primary mechanism for $^{14}CO_2$ transport under dark conditions), another transport mechanism, $^{14}CO_2$ production in the soil (root and microbial respiration) under light conditions, was considered.

The model is based on explicit finite difference solutions to the appropriate equations (i.e. gas transport equations based on Fick's law – Simunek and Suarez, 1993; Jury *et al.*, 1991). It was developed using Visual Basic for Applications in Microsoft Excel 97, to separate $^{14}CO_2$ transport into the soil air and water by diffusion and dissolution, and by photo–assimilation/translocation/respiration. The surface boundary condition was the measured $^{14}CO_2$ concentration in the air above the soil surface.

To date, there has been limited modelling of CO_2 diffusion in soil (e.g. Suarez and Simunek, 1993) that has been tested by measuring CO_2 concentrations in soil air or water. Similarly, CO_2 diffusion models have not been applied or tested on undisturbed pasture soils. In association with the $^{14}CO_2$ pulse labelling experiment designed to label root C contribution to SOM (Chapter 7), there was an opportunity to develop a $^{14}CO_2$ diffusion model by using the data from RSMS's placed at four soil depths to measure soil water $^{14}CO_2$ concentrations. In the following sections, development and output of the $^{14}CO_2$ diffusion model are discussed. The limitations of the model are also discussed.

The objectives of this study were to look at: a) if the rate of appearance of atmospheric $^{14}CO_2$ in the soil solution, at various soil depths, in the dark soil could be explained by modelling gas diffusion and dissolution, and b) if the contribution to $^{14}CO_2$ made by diffusion and rhizosphere respiration could be separated and identified in the cores exposed to sunlight.

8.2   METHODS AND MATERIALS

8.2.1   *Experimental procedures and analysis*

The site of intact soil core collection, preparation and placement of RSMS's in the soil cores, pulse–labelling techniques for soil cores under dark and light conditions, sampling of the enclosed surface air and the soil solution after pulse–labelling, and their analysis are described in methods Section 7.2.

8.2.2   *List of symbols, their definitions and units*

The symbols and parameters used for simulation of $^{14}CO_2$ transport and production in the pasture–soil system, and their definitions/units are listed in Table 8.1.

Table 8.1: List of symbols and parameters used in modelling $^{14}CO_2$ transport and production in the pasture–soil system

Notation	Units	Definition
a		Fitted parameter of lognormal function – Equation (8.17)
b		Fitted parameter of lognormal function – Equation (8.17)
d		Fitted parameter of lognormal function – Equation (8.17)
f_g	$m^3\,m^{-3}$ of soil air	Gas filled porosity
k	Values in Figure 7.1	Fitted parameter for the surface concentration for dark and light lysimeters
q	$MBq\,m^{-2}\,s^{-1}$	Flux density of $^{14}CO_2$ caused by diffusion in the gas phase
t	s	Time
z	m	Soil depth
p_{CO_2}	atm	Partial pressure of CO_2
A	Values in Figure 7.1	Fitted parameter for the surface concentration for dark and light lysimeters
C_g	$MBq\,m^{-3}$ of soil air	Volumetric concentration of $^{14}CO_2$ in the gas phase
$C_g(0)$	$MBq\,m^{-3}$ of air	Volumetric concentration of $^{14}CO_2$ in the air at the surface ($z = 0$) i.e. above the pasture sward
C_l	$MBq\,m^{-3}$ of soil water	Volumetric concentration of $^{14}CO_2$ in the water phase
D_g	$m^2\,s^{-1}$	Diffusion coefficient of CO_2 in air
D_S	$m^2\,s^{-1}$	Effective soil matrix diffusion coefficient of $^{14}CO_2$ in the gas phase
K	Dimensionless	Solubility coefficient of $^{14}CO_2$ gas in the soil water phase
K_a	Dimensionless	Defined in Equation (8.24)
K_b	Dimensionless	Defined in Equation (8.27)
K_D	Dimensionless	Defined in Equation (8.18)
K_H	$mole\,L^{-1}\,atm^{-1}$	Henry's law constant
M	$MBq\,m^{-3}$ of soil	Total volumetric concentration of $^{14}CO_2$ in the soil gas and liquid phase
R	$L–atm\,mole^{-1}\,K^{-1}$	Gas constant
R_c	Dimensionless	Defined in program modules (Section 8.6.1)
S	$MBq\,s^{-1}\,m^{-3}$ of soil	$^{14}CO_2$ production/sink term i.e., the net resultant of root and microbial respiration, or root uptake of dissolved $^{14}CO_2$ in the soil water)
T	K	Absolute temperature
V_W	$m^3\,m^{-3}$ of soil air	Volumetric water content
γ_{gas}	Dimensionless	Tortuosity factor in the gas phase

8.3 MODEL DEVELOPMENT

8.3.1 Model assumptions

The model assumes (a) a homogeneous soil; (b) that any convective transport of $^{14}CO_2$ in the soil air or water is small relative to molecular diffusion and can be neglected; (c) that the $^{14}CO_2$ transport in the unsaturated zone of soil occurs solely in the gas phase and molecular diffusion is the transport mechanism (de Jong and Schappert, 1972; Solomon and Cerling, 1987); (e) that dissolved $^{14}CO_2$ removed from the soil by root water uptake was negligible and ignored (see Sections 7.2.8 and 7.3.3); (f) that the diffusion of dissolved $^{14}CO_2$ in the liquid phase is neglected because the diffusion coefficient of a gas in the gas phase is about 10 000 times higher than the diffusion coefficient of dissolved gas in the liquid phase (Jury et al., 1991); and (g) in the absence of detailed information on the geometry of the soil gas and liquid phase that controls the rate of gas/liquid exchange of CO_2, following Simunek and Suarez (1993) and Suarez and Simunek (1993) instantaneous equilibrium between the gas and liquid phase is assumed. For large negative soil water potentials i.e. a relatively dry soil profile (e.g. −100 kPa, around 20% volumetric water content), only pores of neck size <3 μm would hold water (McLaren and Cameron, 1996) and water films on air–filled pores would be <0.01 μm thick. With more than 60% air–filled porosity, many air–filled pores will be interconnected and allow for rapid diffusion and redistribution of $^{14}CO_2$. With thin water films under this condition, instantaneous equilibrium of soil air $^{14}CO_2$ with water is a fair assumption and is unlikely to alter the accuracy of the model. At low negative soil water potentials, i.e. a wet soil profile (e.g. −15 kPa), larger pores of unknown geometry become partly or wholly water filled and gas diffusion distances may be large due to the lack of free space and the increased tortuosity. Therefore, $^{14}CO_2$ exchange with the whole water volume may not be instantaneous relative to the gas diffusion rate of CO_2. A conceptual diagram of the gas and liquid geometry of soil adjacent to a RSMS is shown in Figure 8.1.
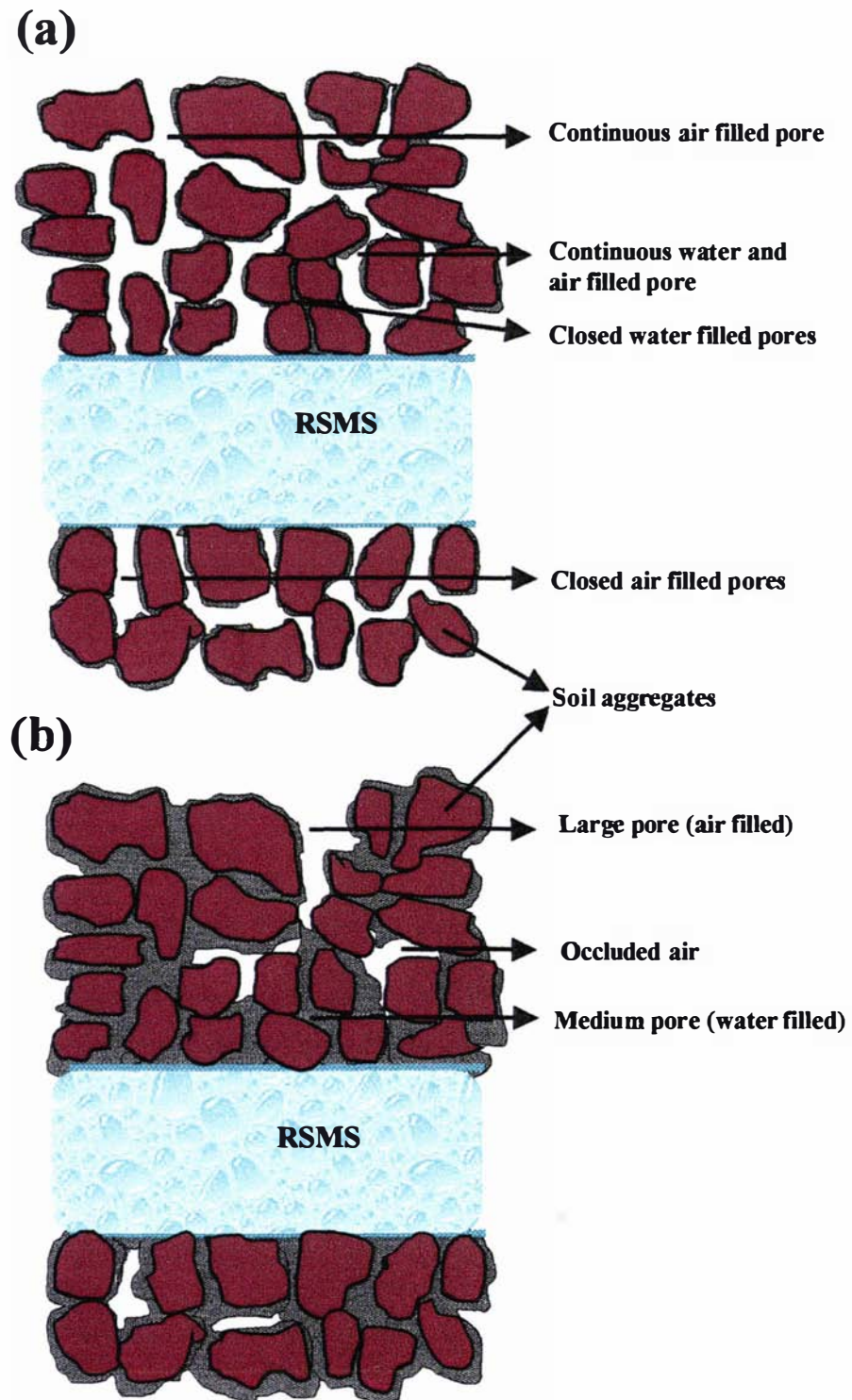
Figure 8.1: The enlarged conceptual view of the local water and gas geometry adjacent to a RSMS – (a) relatively dry soil with thin water films rapidly reaching equilibrium with the gas phase, and (b) relatively wet soil with large diffusion distances meaning that disequilibrim between the gaseous and dissolved CO_2 is likely.

8.3.2   Basic transport equations

Molecular diffusion of gases in soil is described by Fick's first law (Jury *et al.*, 1991; Simunek and Suarez, 1993)

$$q = -f_g D_S \frac{dC_g}{dz} \tag{8.1}$$

For transient diffusion, the equation of continuity for gas flow from rhizosphere sources or sinks (S) and diffusion from the surface is also relevant. It is

$$\frac{\partial M}{\partial t} = S - \frac{\partial q}{\partial z} \tag{8.2}$$

Combining Equations (8.1) and (8.2) gives

$$\frac{\partial M}{\partial t} = S + f_g D_S \frac{\partial^2 C_g}{\partial z^2} \tag{8.3}$$

The gaseous diffusion coefficient in the soil (D_S) can be formally defined as

$$D_S = \gamma_{gas} D_g \tag{8.4}$$

One empirical study (Campbell, 1985) suggests

$$\gamma_{gas} = 0.9 f_g^{1.3} \tag{8.5}$$

for low gas filled porosity conditions. This relationship was assumed to be valid for soil conditions in the present study. The Equations (8.1), (8.4) and (8.5) imply that the pore size does not explicitly affect the rate of diffusion, in contrast to the dominant effect of pore size on viscous laminar flow. Unless the pore size approaches the mean free path length of a molecule between collisions, molecules bounce around just as fast in small pores as they do in large pores, so given the same connectedness and shape, molecular diffusion is just as fast in small and large pores.

The total volumetric concentration (M) is the sum of the $^{14}CO_2$ in the gas and water phases, i.e.

$$M = f_g C_g + V_w C_l \tag{8.6}$$

The volumetric concentration of $^{14}CO_2$ dissolved in the water phase (C_l) is related to the volumetric concentration of $^{14}CO_2$ in the gas phase (C_g) by a solubility coefficient K. This relationship is described below:

$$C_l = K C_g \tag{8.7}$$

or

$$C_g = \frac{C_l}{K} \tag{8.8}$$

Assuming instantaneous equilibrium between the gas and liquid phases, and substituting Equation (8.8) in Equation (8.6) gives

$$M = \frac{f_g}{K} C_l + V_w C_l = \left(\frac{f_g}{K} + V_w\right) C_l \tag{8.9}$$

Substituting Equations (8.8) and (8.9) into Equation (8.3) gives

$$\left(\frac{f_g}{K} + V_w\right)\frac{\partial C_l}{\partial t} = S + \frac{f_g}{K} D_s \frac{\partial^2 C_l}{\partial z^2} \tag{8.10}$$

8.3.2.1   Transport equations in the absence of photo–assimilation

For the dark lysimeters, there was negligible photo–assimilation by the plants and therefore $^{14}CO_2$ respiration by roots (Section 7.3.2). Therefore $S = 0$ in the dark conditions and Equation (8.10) equals

$$\left(\frac{f_g}{K}+V_W\right)\frac{\partial C_1}{\partial t}=\frac{f_g}{K}D_S\frac{\partial^2 C_1}{\partial z^2} \tag{8.11}$$

Rearranging Equation (8.11) and dividing both sides by $\dfrac{K}{f_g}$ gives

$$\frac{\partial C_1}{\partial t}=\frac{D_S}{\left(1+\dfrac{K}{f_g}V_W\right)}\frac{\partial^2 C_1}{\partial z^2} \tag{8.12}$$

8.3.2.2   Transport equation in the presence of photo–assimilation

For the light lysimeters, $S\neq0$ as some of the recently photo–assimilated ^{14}C translocated to the roots is respired and released. Therefore soil becomes a source of [14]C. The value of S can be obtained by rearranging Equation (8.10) as

$$S=\left(\frac{f_g}{K}+V_W\right)\frac{\partial C_1}{\partial t}-\frac{f_g}{K}D_S\frac{\partial^2 C_1}{\partial z^2} \tag{8.13}$$

8.3.3   Surface boundary conditions for dark and light lysimeters

To solve Equations (8.12) and (8.13) initial and surface boundary conditions for both dark and lysimeters need to be defined. The initial condition was

$$C_1(z,0)=KC_g(z,0)=0 \text{ for } z\geq0 \text{ and } t=0 \tag{8.14}$$

After injection of the [14]CO_2 pulse above the leaves of the pasture plants in the enclosed environment, the surface boundary condition (for $z=0$ and $t\geq0$) was approximated as

$$C_1(0,t)=KC_g(0,t)=K\left(A\ln(t)+k\right) \tag{8.15}$$

for the dark lysimeters, and

$$C_1(0,t) = KC_g(0,t) = K(A\exp(-kt))$$ (8.16)

for light lysimeters. $C_g(0,t)$ is the MBq of $^{14}CO_2$ per m^3 of air above the pasture sward (z = 0) at time t. These equations (i.e. the best fit of the measured $^{14}CO_2$ activity) were used in the model to represent the surface boundary conditions (i.e. z = 0) with the parameter values given in Figure 7.1.

8.3.4   Optimisation of diffusion parameters

As accurate measures of f_g were not known (high standard errors – see Table 7.2), optimised values for f_g were found by using Equation (8.12) to obtain estimated C_1 values close to the measured C_1 values in the dark lysimeters. The optimised f_g values were then used for modelling the diffusion component in the light lysimeters in Equation (8.13), on the assumption that soil structure was more similar (root distribution, porosity, soil water content etc.) than different in each core. Equation (8.12) was first solved for the dark lysimeters where S = 0. Best–fit values for f_g (0.06 m^3 m^{-3} soil) were then used to solve Equation (8.13) for the light lysimeters to provide values of S at different soil depths (see Section 8.6). This average porosity factor is a poor representative of the complicated geometry of continuous and discontinuous pore space (Figure 8.1) in the undisturbed soil column. The model output is very sensitive to the air–filled porosity value. A small change, such as 0.01–0.02 m^3 m^{-3}, can change the output considerably. Furthermore, the assumption that the equilibrium between the gas and liquid phase is instantaneous is unlikely to hold in these relatively wet soil cores. Slow diffusion of dissolved $^{14}CO_2$ through a proportion of narrow water filled pores will lead to the model compensating by optimising values for air–filled porosity. Definition of pore space and gas/liquid geometry in soils is an area that requires further research and is beyond the scope of this thesis.

8.3.5   Simulation of ^{14}C activity in soil water as a function of depth in light lysimeters

The measured values of ^{14}C activity (MBq m^{-3}) in the soil water in the light lysimeters were only known at four soil depths and at certain times (Figure 7.8a,b). As the measured ^{14}C activity (MBq m^{-3}) in soil water was similar shape to a lognormal distribution function, values of C_1 at all depths and times for use in Equation (8.13) were obtained by fitting a

lognormal function to the experimental–data ($^{14}CO_2$ in soil water) using Solver in Microsoft Excel 97 (Wraith and Or, 1998). The lognormal distribution function fitted to the experimental data for the four soil depths is

$$C_l = \frac{a}{dt} \exp\left\{\frac{-[\ln t - b]^2}{2d^2}\right\}$$

(8.17)

Solver was used to obtain the predicted C_l by minimising error in the C_l. A deviate (or error) between the measured C_l (Figure 7.8a,b) and predicted C_l values was squared for each data point i.e., $(C_{l\ measured} - C_{l\ modelled})^2$ and these squared deviations were summed (sum of squared errors, SSE) and minimised by varying a, b and d using Solver in Microsoft Excel. These steps (minimising SSE and optimising the fitting parameters) were

(e) **light1 lysimeter**
$a = -13796999.8z^3 + 2158426.8z^2 - 54339.2z + 2130.6$
$R^2 = 1$

(f) **light2 lysimeter**
$a = 36272850.7z^3 - 8265592.3z^2 + 562735.3z - 8831.3$
$R^2 = 1$

(g)
$b = -2684.4z^3 + 81.8z^2 + 65.9z - 0.5$
$R^2 = 1$
$d = -1777.6z^3 + 418.1z^2 - 34.8z + 2.1$
$R^2 = 1$

(h)
$b = 27210.5z^3 - 6655.5z^2 + 505.7z - 8.8$
$R^2 = 1$
$d = 2617.2z^3 - 788.7z^2 + 67.9z - 0.3$
$R^2 = 1$
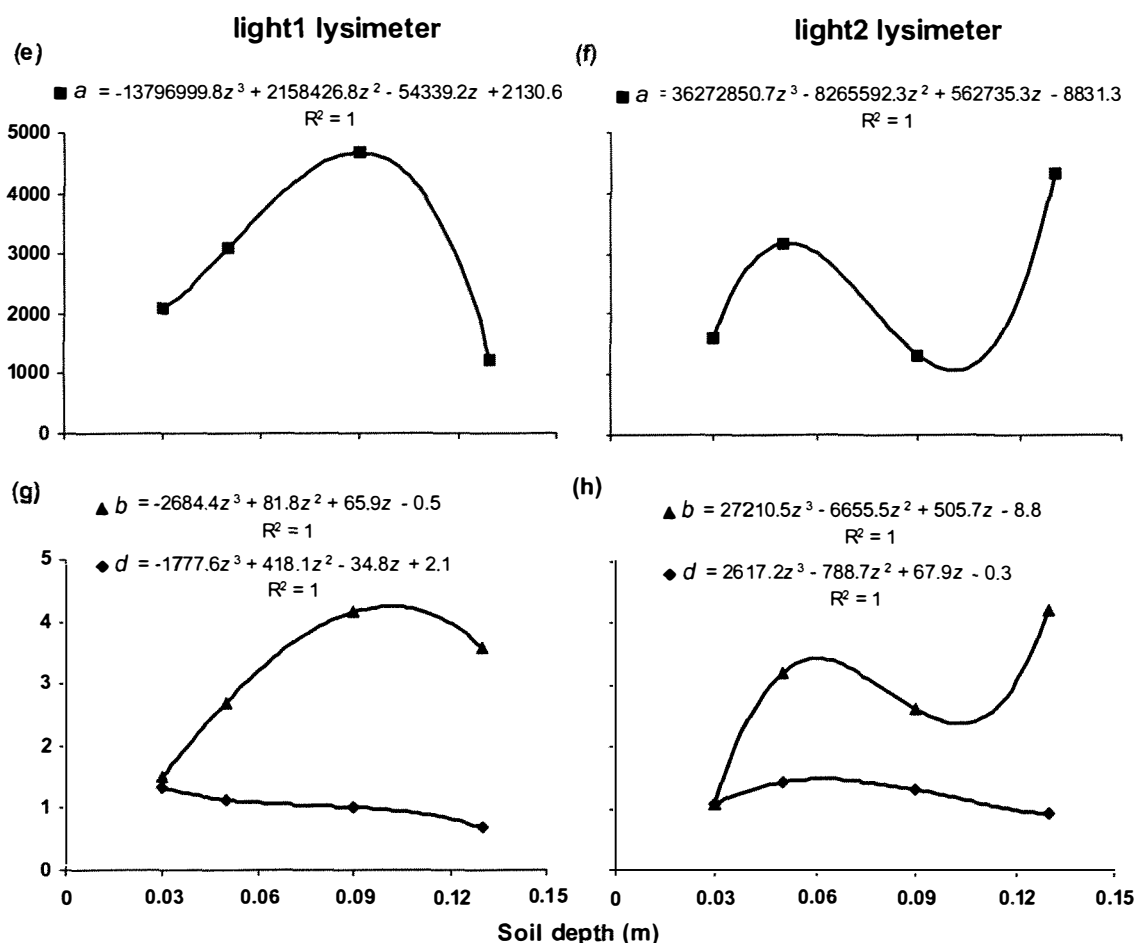
Soil depth (m)

Figure 8.2: Log normal function (Equation 8.17) parameter values fitted for different soil depths of both light1 (e,g) and light2 (f,h) lysimeters.

repeated for the four experimental soil depths. Polynomial equations were then fitted (Figure 8.2e,f,g,h) to describe how the parameters of the lognormal equations varied as a function of soil depth. These equations were coded into the model for light lysimeters to allow the model to account for the change in these parameter values with soil depth. Once the predicted values of C_l at all the depths and times were obtained, the model could solve Equation (8.13) to obtain S values.

8.3.6   *Chemical consideration (dissolution of $^{14}CO_2$) in the diffusion model*

In order to explain solubility of $^{14}CO_2$ in the water phase, various expressions for CO_2 solubility equilibria (Stumm and Morgan, 1981) are given as

$$\text{Distribution (mass law) constant, } K_D = \frac{[CO_{2(l)}]}{[CO_{2(g)}]} \tag{8.18}$$

In Equation (8.18), $[CO_{2(g)}]$ can be expressed by Dalton's law of partial pressure

$$[CO_{2(g)}] = \frac{p_{CO_2}}{RT} \tag{8.19}$$

Combination of Equations (8.7), (8.18) and (8.19) gives

$$[CO_{2(l)}] = \left(\frac{K_D}{RT}\right) p_{CO_2} = K_H p_{CO_2} \tag{8.20}$$

where $K_H = K_D / (RT)$

The value of K_H as a function of temperature is taken from Ellis (1959) – as cited by Drever (1982). Henry's law states that the concentration of CO_2 in the water phase is proportional to partial pressure of CO_2 in the gas phase or in other words, if the aqueous system is in equilibrium with the gas phase, "the partial pressure of the CO_2 in solution" is equal to the partial pressure of the CO_2 in the gas phase.

The distribution coefficient (K_D) as given by Equation (8.18) is always a constant value at a particular temperature (e.g., 0.935 at 293K and 1.7 at 273K) at any partial

pressure of CO_2. This is because the volume fractions or the mole fractions of the gas that dissolve in a certain volume of water are proportional to the partial pressure.

The normal convention is to refer to all the dissolved carbon dioxide as carbonic acid (Drever, 1982) and thus dissolution of CO_2 in water can be represented by the equation

$$CO_{2(g)} + H_2O \Leftrightarrow H_2CO_3 \qquad (K_H = 0.039 \text{ mole L}^{-1} \text{ atm}^{-1} \text{ at 293K}) \qquad (8.21)$$
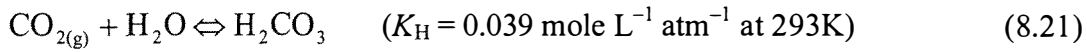
The equilibrium constant consistent with this convention can be written as

$$[H_2CO_3] \cong [CO_{2(l)}] = K_H p_{CO_2} \qquad (8.22)$$

The total water phase CO_2 concentration is defined as the sum of CO_2 dissolved $[CO_{2(l)}]$ and various carbonate species in the solution phase (Lindsay, 1979). In solution carbonic acid dissociates to give

$$H_2CO_3 \Leftrightarrow H^+ + HCO_3^- \qquad (8.23)$$

The equilibrium constant for this reaction can be written as

$$K_a = \frac{[H^+][HCO_3^-]}{[H_2CO_3]} \text{ or } [HCO_3^-] = \frac{K_a[H_2CO_3]}{[H^+]} \qquad (8.24)$$

Rearranging $[HCO_3^-]$ in terms of p_{CO_2} and $[H^+]$, by incorporating Equation (8.22) in the above Equation (8.24) gives

$$[HCO_3^-] = \frac{p_{CO_2} K_H K_a}{[H^+]} \qquad (8.25)$$

The bicarbonate ion also dissociates:

$$HCO_3^- \Leftrightarrow H^+ + CO_3^{2-} \qquad (8.26)$$

The equilibrium constant for this reaction can be written as

$$K_b = \frac{[H^+][CO_3^{2-}]}{[HCO_3^{2-}]}$$ (8.27)

Total carbonates in solution consist of $[H_2CO_3 + HCO_3^- + CO_3^{2-}]$, therefore, the mole fraction of various carbonate species (Lindsay, 1979) can be written as

$$^{MF}H_2CO_3 = \frac{[H_2CO_3]}{[H_2CO_3 + HCO_3^{2-} + CO_3^{2-}]};$$

$$^{MF}HCO_3^- = \frac{[HCO_3^-]}{[H_2CO_3 + HCO_3^- + CO_3^{2-}]} \text{ and}$$ (8.28)

$$^{MF}CO_3^{2-} = \frac{[CO_3^{2-}]}{[H_2CO_3 + HCO_3^- + CO_3^{2-}]}$$

At pH 6.38 and 10.38, and temperature 293K, the molar ratio of bicarbonates to carbonic acid and carbonates to bicarbonates, respectively, is equal to unity. This ratio increases by 10–fold for each unit increase in pH and decreases 10–fold for each unit decrease in pH (Lindsay, 1979). Below pH 6, essentially all the dissolved carbonate species are in the form of H_2CO_3, and above pH 7 essentially all are in the form of HCO_3^-. Above pH 10.38 the activity of HCO_3^- rapidly becomes small as bicarbonates dissociate in alkaline pH conditions of solution. So, the pH plays an important role in the distribution of carbonate species in solution.

Since soils are open to the atmosphere, there is continual opportunity for loss or gain of CO_2. In most agricultural soils, the partial pressure of CO_2 in the soil pore space is higher than in air, because CO_2 is continually being released by the respiration of roots and other organisms in soil. Soil CO_2 concentration for pasture soils in NZ is about 70 to 140 times (2.1% to 4.2%) the atmosphere level (0.03%) (Parfitt et al., 1997). For calculations, a partial pressure of 0.021 atm of CO_2 was used as a reference level for pasture soils pore space.

Figure 8.3 shows the actual log activities of various carbonate species and the solubility coefficient in soil solution (pH $\leq$ 8) as a function of pH in equilibrium with two different partial pressure of CO_2 (0.021 and 0.0003 atm) in the gas phase at 293K (Lindsay 1979). In this figure, H_2CO_3 is present at $10^{-3.088}$ M and is independent of pH at constant
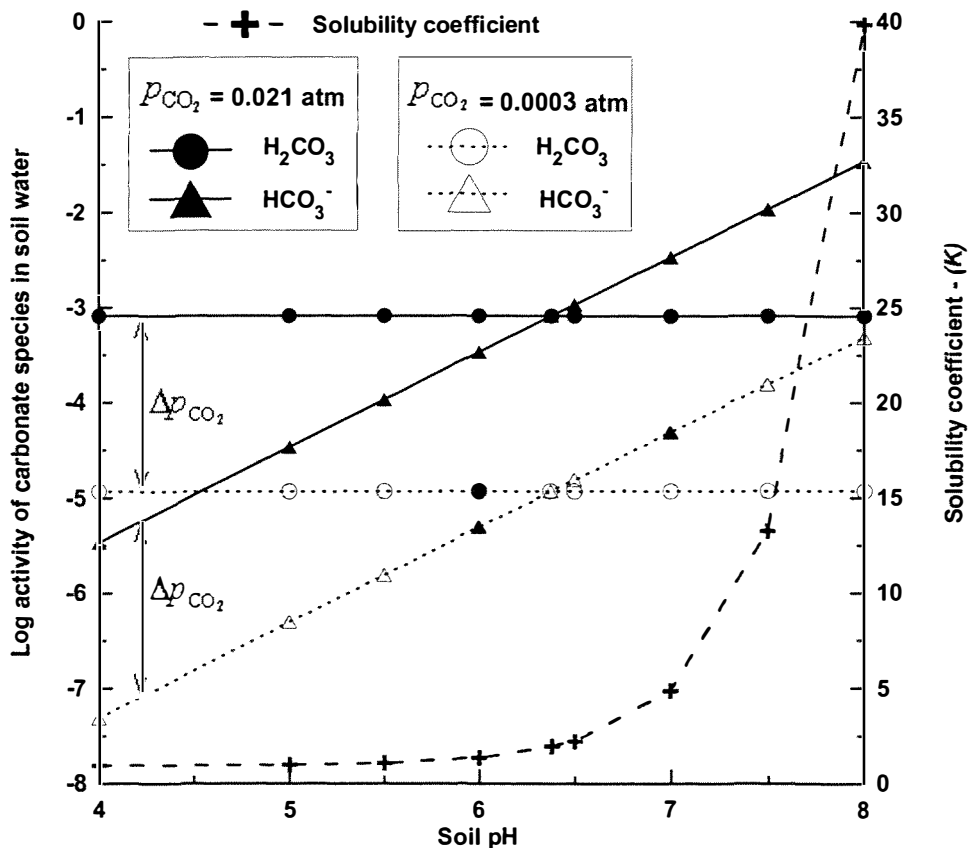
Figure 8.3: The effect of soil pH ($\leq$ 8) on the activities of carbonate species (adapted from Lindsay, 1979) and solubility coefficient of CO_2 in soil solution (K) in equilibrium with two different partial pressure of CO_2 (i.e. 0.021 and 0.0003 atm) in the gas phase. The Δp_{CO_2} indicates the change in log activities of carbonate species (i.e. log of moles per litre) with change in partial pressure of CO_2 from 0.021 to 0.0003 atm and vice versa at different pH values.

partial pressure of CO_2. The log activity of HCO_3^- increases 10–fold for each unit increase in pH at any partial pressure of CO_2. Increasing the partial pressure of CO_2 by 10–fold shifts all the lines, in the Figure 8.3, upward by one log unit and vice–versa. This shows the dependence of the log activities of dissolved CO_2 on the pH of the soil solution and the

concentration of the gas in the soil air. This graph can be used to obtain concentration (mole L^{-1}) of various carbonate species in soil solutions (pH ≤ 8), and the solubility coefficient (i.e. the ratio of the amounts of various carbonate species present in soil solution to the amount of CO_2 in the soil air) at different pH (pH ≤ 8) and partial pressure of CO_2 in the soil gas phase. Symbol K represents this solubility coefficient (see Equation 8.7). The solubility coefficient does not change with the change in partial pressure of CO_2 in the soil air but varies with the change in soil pH at any particular partial pressure of CO_2 (Figure 8.3).

8.4   MODEL OUTPUT AND EVALUATION

8.4.1   *$^{14}CO_2$ diffusion in the absence of photo–assimilation*

In the dark lysimeters, where negligible photo–assimilation occurred (i.e. absence of a source term), the modelled $^{14}CO_2$ concentration in soil water as a function of time (h) agreed reasonably well with the measured $^{14}CO_2$ concentration at the 3 and 5 cm depths for

Figure 8.4: The measured versus modelled ^{14}C activity (MBq m^{-3}) in soil solution from (a) dark1 and (b) dark2 lysimeters at different soil depths (see against legends and dotted lines) and times (h) after $^{14}CO_2$ pulse labelling of pasture swards.

the dark1 lysimeter, and at the 3, 5 and 9 cm depths for the dark2 lysimeter (Figure 8.4a,b). Although, at the 3 and 5 cm soil depths, the $^{14}CO_2$ activity of the simulated data at the maximum value appeared somewhat different (erred by up to 43%) from the measured data (especially in the dark1 lysimeter), the peaks of the simulated curves occurred at the same times as the measured $^{14}CO_2$ activity (Figure 8.4a,b). The large differences in the measured and modelled ^{14}C concentration in soil water at lower depths (9 and 13 cm) can perhaps be attributed to soil solution sampling problems experienced at the lower soil depths (i.e. soil solution suction was not effective due to poor contact between the porous tube of RSMS and the soil). When the cores were dissected it was found that there were some earthworms around the sampling points at the lower depths, which could have weakened the good contact between the soil and the porous tube of the RSMS required for proper sampling of the soil solution.

The overall reasonable agreement between the modelled and measured $^{14}CO_2$ concentration in the dark lysimeters indicates that the amount and rate of appearance of atmospheric $^{14}CO_2$ in soil solution can be explained by gas diffusion in soil. In the absence of photo–assimilation, the activity of $^{14}CO_2$ in the soil solution is controlled by the $^{14}CO_2$ diffusion coefficient in soil (which also affects the $^{14}CO_2$ fluxes into and out of the soil) and the solubility coefficient (K) in soil water. The diffusion coefficient is strongly dependent on the gas filled porosity (f_g) (see Equations 8.4 and 8.5), which was optimised by the model (Section 8.3.4). The best–fit f_g assumed in the model (see Section 8.6.1 below) was within the range of the measured values, except at the 3 cm soil depth (see Table 7.2). Incorrect measured f_g values for the surface layer of the intact soil cores are highly likely, because accurate soil volumes were difficult to obtain as the surface layer (i.e. 0–3 cm in the present study) was uneven and the soil volume of each slice was measured only approximately.

8.4.2 $^{14}CO_2$ *diffusion and production in the presence of photo–assimilation*

In the light lysimeters, the simulated $^{14}CO_2$ activity in soil water as a function of time, obtained by fitting experimental data for four soil depths to the lognormal distribution function (see Section 8.3.5), matched closely the measured $^{14}CO_2$ activity (Figure 8.5a,b – data plotted for only the first 30 h of soil solution sampling after pulse labelling).
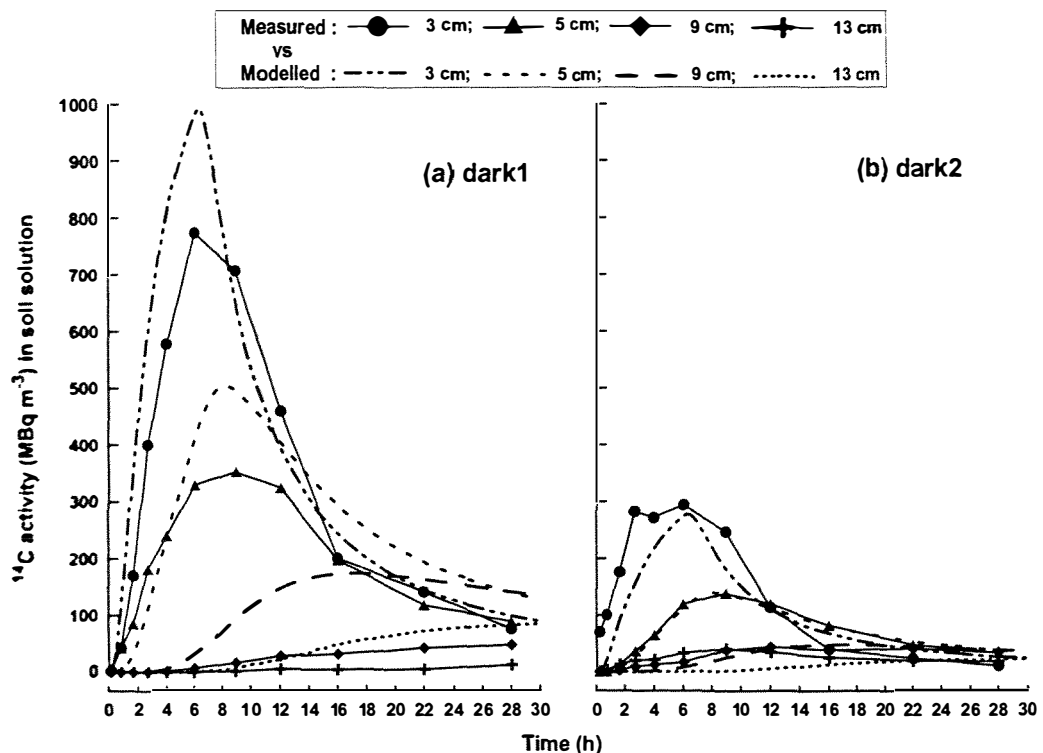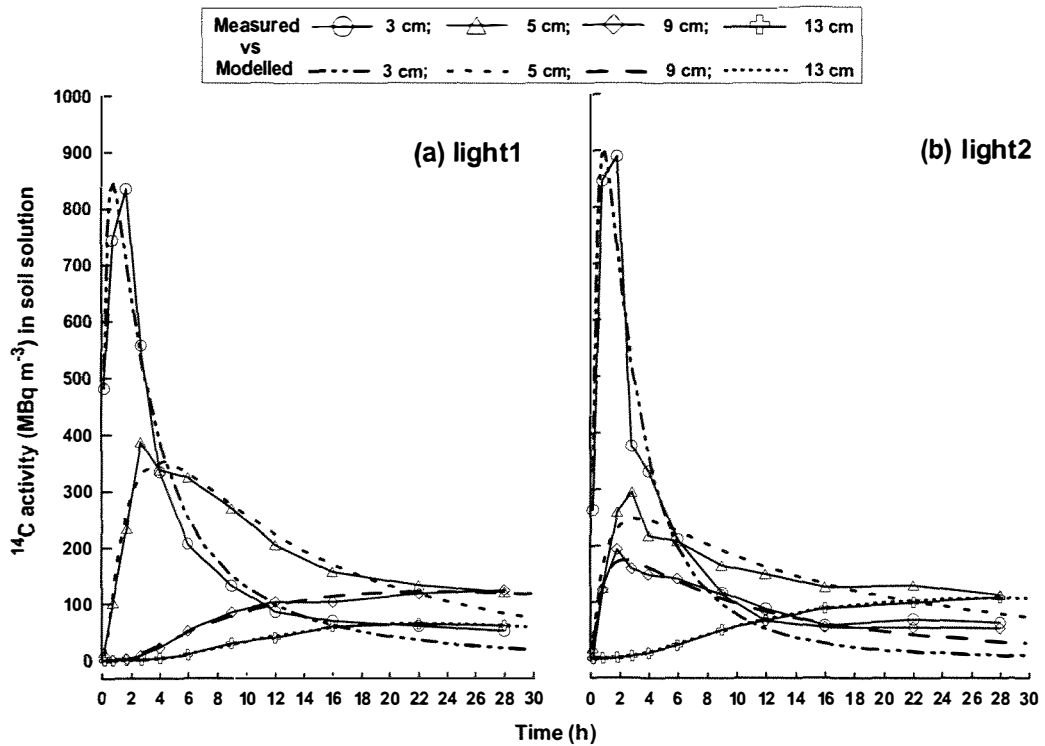
Figure 8.5: The measured versus modelled ^{14}C activity (MBq m^{-3}) in soil solution from (a) light1 and (b) light2 lysimeters at different soil depths (see legends) and and times (up to 30 h) after $^{14}CO_2$ pulse labelling of pasture swards.

The predicted value of S (source/sink – MBq s^{-1} m^{-3} soil – Figure 8.6a,b) was obtained from the model (Equation 8.13) at the experimental depths as a function of time and depth (see Section 8.3.5). The predicted S shows that the 3 cm soil depth (containing 47–59% of the total root mass – Figure 7.9) was the major source for the release of recently assimilated ^{14}C. The release of recently assimilated ^{14}C is estimated to occur very quickly (i.e. within 10 minutes of the introduction of a $^{14}CO_2$ pulse in the enclosed air above the pasture swards). In the dark lysimeters, where negligible photo–assimilation occurred (see Section 7.3.2), a small amount of $^{14}CO_2$ activity appeared within the first hour at the 3 and 5 cm depths (Figure 8.4). Therefore, any $^{14}CO_2$ produced at the 3 and 5 cm soil depths would also be expected to diffuse out of the soil surface within the first hour. Moreover, since the 0–3 cm soil layer is at the soil surface, there can be a rapid exchange of $^{14}CO_2$ produced by the roots with the atmosphere. The rapid decline in $^{14}CO_2$ respired (MBq s^{-1} m^{-3} soil) (i.e. within the first hour of introduction of the $^{14}CO_2$ pulse) at the 3 cm depth also suggests the possible rapid efflux of $^{14}CO_2$ from the soil surface (Figure 8.6a,b). Cheng et $al.$ (1993) recorded the beginning of $^{14}CO_2$ evolution by roots to occur within 30 minutes of pulse labelling the leaves of $Triticum$ $aestivum$, where soil

surfaces were sealed to exclude diffusion of $^{14}CO_2$. Evolution of $^{14}CO_2$ peaked within 2 h after labelling the shoots. Similarly, Kuzyakov *et al.* (1999) reported that $^{14}CO_2$ efflux from a sealed root–soil column began within one hour after pulse labelling shoots of *Lolium perenne* and reached its maximum within the first day of pulse labelling. However, in my modelling of the light lysimeters, the *S* value ceased to be a source of $^{14}CO_2$ at the 3 cm soil depth after 1 h of pulse labelling in the light1 lysimeter (Figure 8.6a) and after 2 h in the light2 lysimeter (Figure 8.6b). Estimated errors in the model are too large to suggest that the 3 cm layer became a sink (i.e. root uptake of dissolved $^{14}CO_2$ in soil water) for $^{14}CO_2$.

The *S* (MBq s^{-1} m^{-3} soil) values obtained at the other soil depths (5, 9 and 13 cm) were very small compared to the 3 cm soil depth. It has been discussed in Section 7.3.5 that the possible reason for the low ^{14}C activity at the lower soil depths in the light lysimeters was the lack of oxygen, which hampered the root activity and therefore ^{14}C production by the roots. Furthermore, in the light2 lysimeter, the model suggests some small sink values at the 5 and 13 cm soil depths within the first 2 h of pulse labelling
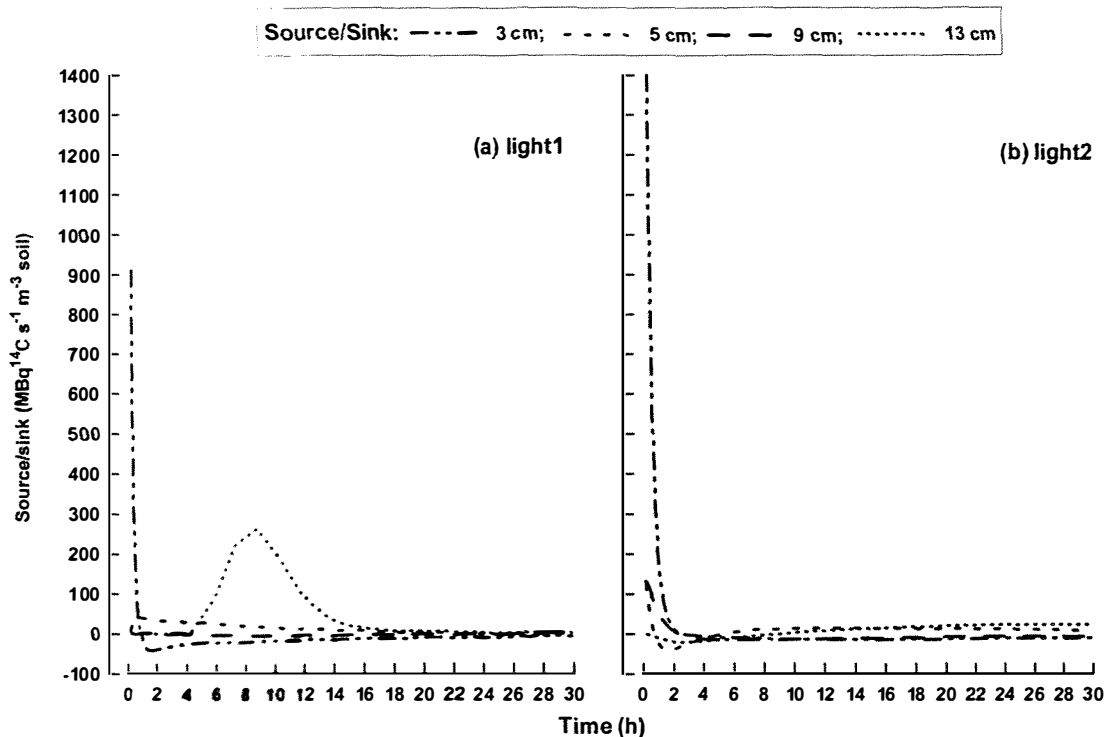
Figure 8.6: The predicted ^{14}C activity (MBq s^{-1} m^{-3} soil) of the source/sink term in soil from (a) light1 and (b) light2 lysimeters at different soil depths (see legends) and times (h) after $^{14}CO_2$ pulse application to pasture swards.

(Figure 8.6b). These are most probably caused by errors in predicting actual $^{14}CO_2$ diffusion rather than actual net $^{14}CO_2$ uptake by ^{14}C labelled roots. As no one else has measured $^{14}CO_2$ production from roots at different soil depths, no comparison with other data can be made.

In the light lysimeters, the ^{14}C activity in soil water was a function of both $^{14}CO_2$ diffusion and the release of assimilated $^{14}CO_2$ by roots and soil microbes, therefore the diffusion model was developed to separate $^{14}CO_2$ diffusion from rhizosphere respiration as a function of soil depth and time. The ^{14}C activity in soil water was only known at four soil depths and nineteen times (*cf.* Figure 7.8a,b). In the model development, functions were used to firstly interpolate the ^{14}C activity in the soil solution at all the depths and times, and then the model used these functions to calculate the source/sink as a function of depth and time. The problem is that even small errors in the estimated diffusion of $^{14}CO_2$ can lead to large errors in the calculated S values, as the model involves numerical differentiation. Also, the model assumed an effectively instantaneous equilibrium between the gas phase and the soil solution (as sampled by RSMS), which is probably unrealistic [The K value (i.e. air to soil solution ^{14}C ratio) assumed in the model is 1:1.18 at pH 5.8]. Therefore, the values of S obtained in using the model may not be reliable. In particular, $^{14}CO_2$ sink values for ^{14}C labelled roots are unlikely to be real nor is the large source peak at 13 cm soil depth in the light1 lysimeter at 9 h (Figure 8.6a,b). If it is real, rather than being caused by root respiration, it is more likely the result of an open macro pore network operating to the mini RSMS at this (13 cm) depth.

8.5   CONCLUSIONS

The $^{14}CO_2$ diffusion model developed in the present study appeared to be reasonably successful in modelling the rate and activity of $^{14}CO_2$ diffusion into the soil in the absence of photo–assimilation (dark lysimeters). However, an optimised gas filled porosity (f_g) was used in the model. This could have masked the effect of disequilibrium between the liquid and gas phases.

In the dark lysimeters, errors in estimating the peak $^{14}CO_2$ activities at soil depths of 3 and 5 cm were up to 43% of the observed activities. In the light lysimeters, errors in matching modelled $^{14}CO_2$ diffusion and $^{14}CO_2$ input from rhizosphere respiration sources

(S) to actual ^{14}C measurements in soil water are therefore probably similarly large, so that using the data to calculate accurately respiration sources (S) in the soil below 3 cm depth was impracticable. This may be a hurdle that prevents precise small scale modelling of gaseous diffusion in field soils. The possible reasons, why the model did not accurately calculate S in the light cores, are listed below.

a) The limited amount of dissolved $^{14}CO_2$ data available from the soil solution samples. A much smaller depth increments and more frequent soil solution samplings than used in this study would be needed.

b) The probable unreliability of the assumption of local equilibrium between the CO_2 concentration in the gas and liquid phases. Simultaneous soil air and soil solution samples would also be required to assess the establishment of an instantaneous equilibrium between the gas and liquid phase.

c) Uncertainty to D_S and f_g. The diffusion coefficient of a gas in soil can be measured using relatively insoluble and inert gases (e.g. N_2 or Ar) (Rolston, 1986). But unless the soil water content is constant with depth and time (which is not true for a dynamic pasture–soil system), extrapolating the data from such measurements for use in Equation (8.13) to determine S will not be valid.

The present study showed that the ways to accurately model gas diffusion in pastoral soils are extremely tedious. Quantitative information on the effect of pore space and gas/liquid phase geometry on relative rate of gas diffusion and gas/liquid exchange in undisturbed soils merits further attention.   ,

8.6 PROGRAM MODULES FOR $^{14}CO_2$ DIFFUSION IN DARK AND LIGHT LYSIMETERS

8.6.1 Dark lysimeters

```
Sub darkgas()
'Numerical gas diffusion program in the absence of photo–assimilation; units m, s
'Initial Section
Dim Cg(100), q(100), M(100), Cl(100)
nl = 21: totz = 0.21: dz = totz / nl
ttot = 30: ttot = ttot * 60 * 60
dt = 0.0025 * 60 * 60
Dg = 0.000014: fg = 0.06: Ds = 0.9 * fg ^ 1.3 * Dg: Vw = 0.56:
K = 1.18: Rc = 1 + Vw / fg * K
'(K = 14CO2 solubility coefficient at pH = 5.8)
counter = 100: Maxcounter = 100
q(nl) = 0: Row = 2: t = 60
'Dynamic Section
Do Until t > ttot
'Surface ( i.e. z = 0) boundary curve for dark lysimeters)
'Cg(0) = –425.7 * Log(t / 60) + 4337.15  '(dark1 lysimeter)
Cg(0) = –78.52 * Log(t / 60) + 1005.22  '(dark2 lysimeter)
'Interchange remark on the surface boundary curves depending on the lysimeter to
be modelled (present model is for the dark2 lysimeter)
If t > 6 * 60 * 60 Then Cg(0) = 0:
If counter >= Maxcounter Then
Worksheets("Sheet2").Cells(Row, 2).Value = t / 60 / 60
For n = 0 To nl
Worksheets("Sheet2").Cells(Row, n + 3).Value = Cg(n)
Worksheets("Sheet2").Cells(Row, n + 3 + nl + 2).Value = Cl(n)
Next n
counter = 0: Row = Row + 1
End If
q(0) = (Cg(0) – Cg(1)) / (dz / 2) * Ds * fg
For n = 1 To nl – 1
q(n) = (Cg(n) – Cg(n + 1)) / dz * Ds * fg
Next n
For n = 1 To nl
dM = (q(n – 1) – q(n)) * dt / dz
M(n) = M(n) + dM
Cg(n) = M(n) / fg / Rc
Cl(n) = Cg(n) * K
Next n
counter = counter + 1
t = t + dt
Loop
End Sub
```

8.6.2   Light lysimeters

```
Sub lightgas()
'Numerical gas diffusion program in the presence of photo–assimilation; units m, s
'Initial Section
```
Dim $C_g(100)$, $q(100)$, $M(100)$, $C_l(100)$, $S(100)$
Dim $a(100)$, $b(100)$, $d(100)$, $C_g$old(100)
nl = 10: totz = 0.21: dz = totz / nl
ttot = 168: ttot = ttot * 60 * 60
dt = 0.005 * 60 * 60: D_g = 0.000014: f_g = 0.06: V_W = 0.56
K = 1.18: D_s = 0.9 * f_g ^ 1.3 * D_g: R_c = 1 + V_W / f_g * K
For n = 1 To nl
z = 0.02 * n – 0.01
'Variation in fitted parameters of lognormal equation as a function of depth
'(Light1 lysimeter)
'a(n) = –13796999.8 * z ^ 3 + 2158426.8 * z ^ 2 – 54339.2 * z + 2130.6
'b(n) = –2684.4 * z ^ 3 + 81.8 * z ^ 2 + 65.9 * z – 0.48
'd(n) = –1777.6 * z ^ 3 + 418.1 * z ^ 2 – 34.8 * z + 2.05

'(Light2 lysimeter)
a(n) = 36272850.7 * z ^ 3 – 8265592.3 * z ^ 2 + 562735.3 * z – 8831.3
b(n) = 27210.5 * z ^ 3 – 6655.5 * z ^ 2 + 505.7 * z – 8.84
d(n) = 2617.2 * z ^ 3 – 788.7 * z ^ 2 + 67.9 * z – 0.32
'Interchange remark on the lognormal equation parameters depending on the lysimeter to be modelled
Next n
counter = 40: Maxcounter = 40
q(nl) = 0: Row = 2: t = dt
'Dynamic Section
Do Until t > ttot
'Surface (i.e. z = 0) boundary curve for light lysimeters)
'C_g(0) = 3580.2 * Exp((–1) * (0.0135)) * (t / 60) *'(Light1 lysimeter)*
C_g(0) = 5222.5 * Exp((–1) * (0.0169)) * (t / 60) *'(Light2 lysimeter)*
'Interchange remark on the surface boundary curves depending on the lysimeter to be modelled (present model is for light2 lysimeter)
If t > 3 * 60 * 60 Then C_g(0) = 0: Maxcounter = 300
If counter >= Maxcounter Then
Worksheets("Sheet2").Cells(Row, 2).Value = t / 60 / 60
For n = 1 To nl
Worksheets("Sheet2").Cells(Row, n + 3).Value = S(n) * 60 * 60
Worksheets("Sheet2").Cells(Row, n + 3 + nl + 2).Value = C_l(n)
Next n
counter = 0: Row = Row + 1
End If
q(0) = (C_g(0) – C_g(1)) / (dz / 2) * D_s * f_g
For n = 1 To nl – 1
q(n) = (C_g(n) – C_g(n + 1)) / dz * D_s * f_g
Next n
For n = 1 To nl

$C_g(n) = a(n) / K / (d(n) * t / 60 / 60) * \text{Exp}((-1) * (\text{Log}(t / 60 / 60) - b(n)) \wedge 2 / 2 / d(n) \wedge 2)$
'log = natural log i.e. ln
Next n
For n = 1 To nl
$dM = (C_g(n) - C_g\text{old}(n)) * f_g * R_c$
$S(n) = dM / dt - ((q(n-1) - q(n)) / dz)$
$C_l(n) = C_g(n) * K$
$C_g\text{old}(n) = C_g(n)$
Next n
counter = counter + 1
$t = t + dt$
Loop
End Sub

CHAPTER 9   SUMMARY AND CONCLUSIONS, AND IMPLICATIONS FOR FUTURE RESEARCH

9.1   SUMMARY AND CONCLUSIONS

The work presented in this thesis can be summarized as follows:

9.1.1   Literature review

Changes in land management practices can alter the quantity and quality of SOM. Marked decreases may indicate unsustainable practices. Therefore, it is important that appropriate measurements of SOM are developed to identify change. A number of procedures have been applied to the measurement and characterization of SOM relative to its turnover and nutrient availability but only limited success has been achieved in separating SOM into dynamic pools that show change with landuse. Chemical fractionation procedures have been useful to monitor the effect of treatments on both quantity and quality of SOM, even though it is achieved at the expense of some changes in chemical structure of organic matter. Results from physical fractionation studies indicate that, other than the chemical structure, the stability and turnover of SOM also depends on its location within the soil matrix. Isotopes of C (e.g. ^{13}C or ^{14}C) in SOM studies can be used to follow the fate of specific C sources against the background of large amounts of SOM. Using isotopically labelled SOM in fractionation studies, specific fractions that are biologically active can be identified. Thus, a combination of chemical and physical approaches along with an isotope tracer, can be helpful to elucidate the complex nature of SOM.

Existing techniques used to characterise SOM into pools/fractions have been unable to classify plant available S in a single defined pool. Information on the effect of changes in land management practices on labile soil organic S fractions is scarce. The literature review emphasized a need to look for new techniques that provide a few biologically meaningful labile organic S fractions, which are responsive for short– and long–term changes in management practices.

9.1.2   *Standardising analytical procedures for S and C fractions*

A modified soil P fractionation procedure was used to fractionate soil S and C. Ionic forms of S and C were released to ion exchange resins, and organic S and C extracted in alkali of increasing concentration, to obtain fractions of decreasing lability. Various digestion procedures were investigated for measuring HI reducible and total S, and total C, in the alkaline extracts.

Digestion and analysis of methionine and sulphanilamide organic S standards in alkaline solutions indicated that a modified method of NaOBr oxidation gave the best recovery of S from alkaline soil extracts.

A modified dichromate digestion procedure, for the simultaneous determination of total C and ^{14}C in soil and plant materials, gave the most consistent and complete recovery of C and ^{14}C from soil extracts and plant materials. A procedure was developed for correcting chloride interference with this digestion method.

9.1.3   *Characterisation of changes in S and C fractions*

In Chapter 4, the ability of the sequential chemical fractionation procedure to characterise organic S and C fractions into pools important in the cycling and release of plant available nutrients was evaluated. Soil samples, representing a range of S fertiliser histories (pasture soils, Mt.Thomas), period of cultivation (permanent pasture, and short– and long–term cultivated maize/barley sites, Kairanga) and restoration crops (annual and perennial ryegrass, Wakanui), were fractionated. Increasing rates of fertiliser S application increased both total soil S and C. Continuous maize cultivation decreased total soil S and C, whereas restoration to pasture built up soil S and C. The chemical fractionation comprised three sequential extractants: ion exchange resin, 0.1 M NaOH, 1 M NaOH, and a residual digested fractions. The major fractions of soil S and C were either extracted in 0.1 M NaOH (49–69% S and 38–48% C) or remained unextracted in the residual fraction (17–38% S and 46–53% C). Only small proportions of soil S and C were extracted by resin (1–13% S and 5–6% C) or 1 M NaOH (5–16% S and 6–8% C). Resin–S was higher in fertilised pasture soils than unfertilised pasture or cropping soils. Under conditions of organic S and C build up or depletion (caused by changes in management practices) both

the 0.1 M NaOH and residual fractions showed a greater change than the 1 M NaOH or resin fractions. Change in the large alkali–insoluble residual fraction with landuse indicated that increasing strength of alkali could not readily fractionate labile soil organic S and C into fractions that are becoming increasingly less bioavailable. Further studies were conducted, using recent root and root–released ^{14}C, to evaluate the nature of SOM extracted in the 0.1 M NaOH and residual fractions (see Chapter 6).

9.1.4 *Distribution of recently fixed ^{14}C by depth in intact pasture–soil system*

Permanent pasture renovation phases were successful in increasing SOM and its S content (Chapter 4). Pulse labelling with [14]CO_2 of undisturbed cores of ryegrass/clover pasture swards was undertaken: a) to study the allocation of ^{14}C to different soil depths, b) to evaluate mini RSMS for monitoring the turnover of root and root–derived ^{14}C at the plant/soil–water interface, and c) to produce recently ^{14}C–labelled rhizodeposited SOM (including roots) for subsequent fractionation studies.

The depth distribution of recently photo–assimilated ^{14}C to pasture roots showed that at harvest (a week after pulse labelling), the allocation of ^{14}C was greatest in the topsoil (0–2 cm) and decreased with depth. This allocation pattern was mostly similar to the root mass distribution with depth. In the topsoil (0–2 cm), the ^{14}C was mainly present in the roots (>90%) rather than in the surrounding soil. At depths below 2 cm, the ^{14}C was more or less evenly distributed between roots and soil. These results suggest that although rhizodeposition could have occurred in greater amounts in the top 2 cm depth, the expected accumulation of root–released ^{14}C could not be observed, probably because of faster decomposition of root-derived C, due to enhanced aeration and higher microbial activity closer to the soil surface.

In situ soil solution sampling with RSMS, at different depths below the sward surface, was a very effective 'real time' method of monitoring [14]CO_2 appearance in the undisturbed soil profile. Peaks of [14]CO_2 activity that were displaced with time for different soil depths gave a good indication of the rate of ^{14}C allocation by pasture sward to different soil depths. The soil water sampled by the RSMS contained only [14]CO_2 and not ^{14}C as soluble exudates, which suggests that the exudates were probably rapidly metabolised and respired by rhizosphere microbes. However, as the pasture soil surface was not covered, the

contribution from diffusion of $^{14}CO_2$ from the atmosphere to the soil water was uncertain. Studies were undertaken to model diffusion of $^{14}CO_2$ into undisturbed pasture soils (Chapter 8).

9.1.5   *Characterisation of recent root and root–released ^{14}C*

In Chapter 4, the sequential chemical fractionation of total soil S and C indicated that both the 0.1 M NaOH soluble and alkali–insoluble residual fractions changed with the change in management practices. As the recently rhizodeposited ^{14}C produced from a $^{14}CO_2$ pulse labelling study was predominantly present in roots (Chapter 6), soil and root samples from the pulse labelling experiment were used to elucidate the 'functional nature' of C separated into the chemical fractions. As the study progressed, density fractionation of soil containing ^{14}C labelled material was also conducted.

The chemical fractionation distributed recent root and root–released ^{14}C among all the fractions with approximately 50% remaining in the alkali–insoluble residual fraction. The pattern of ^{14}C distribution amongst resin, 0.1 M NaOH, 1 M NaOH and residual fractions resulted from the form of root C, and was not influenced significantly by the presence of soil. More recently synthesised root C (high specific activity) was preferentially extracted by resin and 1 M NaOH extracts, whilst older root C (lower specific activity) was extracted in the 0.1 M NaOH and residual fractions.

A density fractionation procedure was conducted with the aim of separating recent root and root–released ^{14}C into a single fraction. However, the procedure distributed the ^{14}C between light (~ 42%) and heavy (~ 45%) fractions. The 5% NaCl soluble fraction recovered <13% of the root–derived ^{14}C. The light fraction contained a major contribution from fresh roots (high specific activity), but the heavy fraction also included a considerable proportion of the recent root and root–released ^{14}C. The density fractionation of roots alone, and roots remixed with the non–radioactive soil showed that adhesion of soil particles to the fine ^{14}C labelled roots and/or adsorption on soil of soluble organic anions released from roots caused the ^{14}C activity to appear in greater amounts in the heavy fraction.

It can be concluded that neither the chemical nor the density fractionations were capable of characterising recently rhizodeposited "labile" [14]C into one main fraction. Similarly, density separation followed by chemical fractionation provided no improved categorisation of dynamic SOM into pools of distinct lability. Rather, this combined separation procedure redistributed the ^{14}C among all the fractions.

An anaerobic incubation and various acids and oxidising agents were also tried to recover a greater proportion of root and root–released ^{14}C that otherwise appeared in the alkali–insoluble residual fraction. The most promising avenue appeared to be the 30% H_2O_2 pre- and the hot 1 M HNO_3 post-alkali extraction treatments, which were more successful in separating high specific activity C from the residual fraction than other treatments. Future investigations of soil S and C fractionation could include these pre- and post-alkali extraction treatments, however, differences in the alkaline or acid solubility of root–derived SOM suggest that recovery of dynamic soil C pools in fractions of a more contiguous nature is unlikely.

9.1.6   *[14]CO₂ diffusion and photo–assimilation in pasture–soil system*

In Chapter 5, the use of mini RSMS allowed the measurement of $^{14}CO_2$ activity in soil water resulting from root and soil (microbial) respiration. As the pasture soil surface was not covered, the exact contribution from rhizosphere respiration activities was uncertain because of diffusion of $^{14}CO_2$ into the soil. Similarly, photo–assimilation rates could be overestimated. Thus, an attempt to account for $^{14}CO_2$ diffusion into soil was made by $^{14}CO_2$ pulse labelling of undisturbed cores of pasture swards under dark and light conditions. Soil solution was then sampled with RSMS to monitor the appearance of recently photo–assimilated ^{14}C and diffused $^{14}CO_2$ at different depths and times after pulse labelling.

In the light lysimeters, photo–assimilation of $^{14}CO_2$ and translocation of ^{14}C metabolites to the roots, and subsequent $^{14}CO_2$ release into the soil solution occurred within 10 minutes of pulse labelling, at the 3 cm depth. However, in the dark lysimeters, $^{14}CO_2$ activity (MBq m^{-3}) in the soil solution was low for the first hour. These results indicated that the *in situ* soil solution sampling technique with mini RSMS was a very

effective 'real time' method of monitoring the rapid appearance of a $^{14}CO_2$ pulse through photo–assimilation/translocation/root respiration.

In the light lysimeters, the allocation of ^{14}C to roots was mostly proportional to the root mass distribution with depth. Roots at lower depths, however, had lower ^{14}C specific activity, which suggested that the root respiratory activity decreased with depth. This may reflect the effect of low O_2 availability due to high water content of soil cores.

A comparison of the extent of ^{14}C assimilation under dark and light conditions indicated that photo–assimilation/translocation/root respiration was the main pathway for $^{14}CO_2$ movement into soils in light conditions. In dark conditions, diffusion was the main mechanism for $^{14}CO_2$ movement into soils. A small amount of ^{14}C assimilation under dark conditions probably resulted from a very short (< 15 seconds) photo–assimilation period, uptake of $H^{14}CO_3^-$ by roots, or assimilation by chemolithotrophs in rhizosphere soil.

It was possible to conclude that pulse labelling of photosynthetically active pasture swards with unsealed soil surfaces can be carried out without significantly overestimating photo–assimilation rates by isotopically labelled $^*CO_2$ diffusion and uptake of dissolved $^*CO_2$ (H^*CO_3).

9.1.7   Modelling $^{14}CO_2$ diffusion and activity in intact pasture soils

In Chapter 8, a $^{14}CO_2$ diffusion model was developed for intact pasture soil cores exposed to dark and light conditions. The objective was to see if the activity and rate of appearance of atmospheric $^{14}CO_2$ in soil solution of the dark lysimeters could be explained by modelling gas diffusion and dissolution, and whether the effect of $^{14}CO_2$ assimilation/translocation/rhizosphere respiration could be separated from $^{14}CO_2$ diffusion into the soil cores exposed to sunlight.

The model assumed that $^{14}CO_2$ transport in the unsaturated zone of soil occurred solely in the gas phase and that molecular diffusion was the transport mechanism. Also, instantaneous equilibrium between the gas and liquid phase was assumed, which may not be true for the discontinuous nature of air–filled pore space in the soil cores used in this study.

The simulation model appeared to be successful in describing the $^{14}CO_2$ diffusion into soil under dark conditions; however, the simple model was fitted to the experimental data by optimising the gas filled porosity value. Thus the optimised gas filled porosity also accommodated for the variability due to soil pore space discontinuity and any gas/liquid exchange disequilibria that existed. In the dark lysimeters, errors in estimating the peak $^{14}CO_2$ activities at soil depths of 3 and 5 cm were in the order of 1–43% of the observed activities. In the light lysimeters, errors in matching estimated $^{14}CO_2$ diffusion and rhizosphere respiration to measured ^{14}C activity in soil water were therefore too large to accurately estimate the $^{14}CO_2$ input from rhizosphere respiration sources. The possible reasons for inaccurate estimates of $^{14}CO_2$ production values in the light soil cores are: a) limited amount of dissolved $^{14}CO_2$ data (i.e. only four soil depths and nineteen times) were available from the soil solution samples, b) probable unreliability of the assumption of local equilibrium between the CO_2 concentration in the gas and liquid phases, and c) uncertainty of the pore space geometry.

It was concluded that small–scale gaseous diffusion modelling in field soils is impracticable unless the soil gas/liquid phase geometry could be determined in order to calculate accurate gaseous diffusion coefficients for the undisturbed soil. In this regard, simultaneous air and soil solution samples, taken using RSMS, at much smaller depth increments and more frequently would help to indicate any disequilibrium between the gas and liquid phases.

9.2 IMPLICATIONS FOR FUTURE RESEARCH

The 30% H_2O_2 pre- and hot 1 M HNO_3 post-alkali extraction treatments of the residual fraction have shown some ability to recover a 'labile' root C that appeared in the alkali–insoluble residual fraction. These two treatments should be investigated further on the C depletion series of cropped soils to better characterise soil organic S and C into pools of distinct lability. The use of recently rhizodeposited 'labile' ^{14}C in fractionation schemes holds promise to simplify the search process for 'potential' labile fractions. Researchers, wishing to validate C cycling models for pasture and cropped soils, may have to settle on measures of key SOM components rather than distinct chemical fractions that purport to be representative of dynamic C pools.

As mentioned above, anaerobic soil conditions caused by a high water content will affect root activity and plant growth. To enhance the soundness of SOM dynamics models for plant–root–soil systems, further studies that look at the extent of rhizodeposition at different soil depths, and C turnover as influenced by different soil moisture levels, would be very useful.

The $^{14}CO_2$ diffusion modelling study showed that ways to accurately model gas diffusion in pastoral soils are extremely tedious. There is no information on the rate of gas/liquid exchange in undisturbed soils of different moisture contents. It would be worthwhile conducting some simple isotope dilution experiments with inert tracer gases in order to quantify the information on the geometry of the gas and liquid phase in undisturbed soils.

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