A COMPARISON OF THE FATE OF ELEMENTAL SULPHUR AND SULPHATE SULPHUR BASED FERTILIZERS IN PASTURE SOILS

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ABSTRACT

Nitrogen fixation by legumes has a particular requirement for adequate soil sulphur status. Sulphur (S) is a mobile nutrient and is easily leached from aquic soil environments, therefore regular topdressing with S fertilizer is required to maintain legume vigor and pasture production in most New Zealand pasture soils. Escalating fertilizer costs have focused attention on the efficiency of use of S fertilizers, particularly superphosphate (SSP) and alternative elemental S (S^0) based fertilizers less liable to leaching loss in this aquic environment.

Field and glasshouse trials, using the resident clover/ryegrass sward on undisturbed soil cores (150 mm diameter, 100 mm depth), were undertaken to determine the comparative short-term fate of SSP and different particle sizes of S^0. Methods for manufacturing radioactively labelled (^{35}S) fertilizers were developed. In addition, the effect of sheep dung on the short-term immobilization of soil and fertilizer S was also investigated. A simple computer simulation model explaining the observed transformation of soil sulphur and ^{35}S labelled fertilizer was developed.

Initially, the effect of sheep dung on the short-term immobilization of soil and fertilizer S was investigated. Very small amounts (about 2-5%) of plant (clover/ryegrass pasture) S and P, within 100 mm of the area surrounding the dung pellet, were derived from the dung. Under the experimental conditions that prevailed, dung S behaved as a slow release S form causing neither greater immobilization of soil or fertilizer S nor mineralization of soil organic S. It was concluded that the impact of dung return on short-term (< one year) S fertilizer fate need not be considered.

An initial field trial comparing the fate of microfine S^0 (< 0.010 mm) relative to sulphate-based SSP was undertaken on Tokomaru silt loam, a New Zealand yellow-grey earth (Fragiaqualf). The microfine S^0 oxidized within 30 days of application but initially (up to 60 days) was slightly less effective than SSP in terms of plant uptake. Over longer periods of time (150 days) their performances were comparable. Final cumulative plant uptake at 150 days accounted for 13.6% of microfine S^0 and 16.3% of the SSP-sulphate.

The major transformation of ^{35}S from microfine S^0 and ^{35}S labelled gypsum In SSP to soil organic ^{35}S forms occurred in the first 30 days after application. The organic ^{35}S activity formed from $^{35}S^0$ was twice that formed from sulphate-based fertilizer and was mainly carbon-

bonded ^{35}S in the top 33 mm of the pasture soil profile. The amount of organic ^{35}S remaining as carbon-bonded ^{35}S decreased with soil depth and the reverse occurred for the ester-sulphate ^{35}S. By 150 days, greater activity from the microfine $^{35}S^{o}$ remained in the soil organic S fraction than from the sulphate-^{35}S fertilizer, indicating that more soil organic S reserves may be formed through the use of fine S^{o} fertilizer than from the sulphate-based fertilizer. This also indicated the advantage of using S^{o} in minimizing the S leaching losses in this aquic environment.

An inverse dilution technique using an isotope injector developed at Massey University to uniformly label undisturbed soil cores with carrier-free $^{35}SO_4^{=}$ solution was used to measure the impact of S^{o} and sulphate-based fertilizers on the fate of soil S. Results were consistent with the labelled fertilizer technique and both techniques indicated rapid incorporation of ^{35}S into soil organic S and that the carbon-bonded S formed was likely to be a subsequent source of mineralized S available to plants.

Soil samples from the preliminary field study were used to evaluate soil preparation and extraction techniques. Soil sampling and preparation techniques were evaluated on the basis that an extract sampling the plant available S pool in soil should have the same ^{35}S specific activity as plant growing on that soil. The average ^{35}S specific activity in a calcium dihydrogen phosphate (CaP-S) (0.04 M) extract from a freeze-dried sample of the top 60 mm of a pasture soil was most closely related to the ^{35}S specific activity of plants growing on that soil. CaP-S extracts from field-moist soil or 0.01 M $CaCl_2$ extracts from field-moist or freeze-dried soils had higher specific activities than plants. It was concluded that plants were able to extract soil S from soils which was not exchangeable with added $^{35}SO_4^{=}$ fertilizers during either the field experiment or extraction with 0.01 M $CaCl_2$.

The second series of field and glasshouse trials were conducted to investigate the fate of ^{35}S labelled SSP, gypsum and S^{o} of varying particle sizes (<0.150 mm, 0.150-0.250 mm and 0.250-0.500 mm, in granulated and non-granulated forms) in two pasture soils contrasting in mineralogy and fertility status. Under glasshouse conditions, 50 mm of simulated rainfall was applied to each of the undisturbed soil cores during the first 56 days after fertilizer application. For the remainder of the period, cores were watered from below using a saucer. Field cores remained subject to the local climate. Both the rate of oxidation in soil and the efficiency of plant use of S^{o} was improved by decreasing its particle size. Relative to soluble $SO_4^{=}$-S applied as gypsum or SSP, the plant utilization of ***oxidized*** S^{o} was similar.

Granulation of finer S^{o} with or without finely ground phosphate rock had little effect on the

long-term (180 days) oxidation rate or the efficiency with which, after oxidation, finely ground S^o was taken up plants.

Apart from S^o of large particle size (>0.150 mm) which had not oxidized, the major fate of fertilizer ^{35}S, either under glasshouse or field conditions, was again in soil organic matter mostly formed in the top 33 mm of the soil. Applications of gypsum and SSP caused ^{35}S to move to the 33-100 mm soil depths but there was no additional influence of P on the depth to which $SO_4^=$ was leached.

A preliminary computer simulation model describing the fate of $^{35}SO_4^=$-S fertilizer was developed. The model provided a very accurate method of predicting plant uptake of S from both SSP fertilized and unfertilized soil cores. The model also indicated that, at any particular soil depth, on average, actual rates of mineralization and immobilization may exceed root uptake of S by 1.5 to 2 fold (mg S turned over per unit of S taken up by plants). The accuracy of the estimated turnover rate could not be validated because the model gave relatively inaccurate predictions of the measured movement and transformations of ^{35}S tracer added to the soil as SSP. There was, however, relative similarity between the observed and predicted proportional distribution of ^{35}S between soil and plant S forms. Such a distribution supported the concept of using root activity as a modifier of mineralization and immobilization rates in order to describe the extent of these processes at different soil depths.

The study has emphasized the greater importance of the surface few millimeters of pasture soil in S transformations, important in the fate of fertilizer and pasture plant nutrition. There appears to be scope in manipulating S^o particle size to improve the efficiency of the S fertilizer used.

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CHAPTER 1

INTRODUCTION

Sulphur is an essential element for plant growth and is required by plants mainly for protein and enzyme synthesis. It is an important component of the nitrogenase enzymes which are involved in nitrogen fixation (Lehninger, 1982). Pasture production in New Zealand depends on atmospheric nitrogen fixation by legumes.

Traditionally, fertilizer use in New Zealand pasture soils was determined mainly by phosphorus (P) requirements. Single superphosphate (SSP), 9% P and 11% S, has been the major fertilizer used to correct P deficiency and it is fortuitous that in the process sufficient sulphur (S) was applied (Boswell, 1985, Boswell and Swanney, 1986; Till *et al.,* 1987). Currently, New Zealand imports S worth approximately NZ$40 million every year mainly for the SSP fertilizer industry.

Recent studies on the mass balance of the S cycle in grazed hill country pastures, in the North Island of New Zealand, have shown that in areas where S is applied annually as SSP, large S leaching losses can occur (up to 70% of applied S per annum) which are mostly dependent upon the rate of application (Saggar *et al.,* 1990a, 1990b). In other areas of New Zealand, particularly in high rainfall areas, elemental S (S^0) fertilizers which slowly oxidize to release sulphate-S have been recommended to reduce S leaching loss (Sinclair and Saunders, 1984). It has long been established that elemental S (S^0) is a suitable S fertilizer form for New Zealand pastoral soils (Ludecke, 1965; Sinclair *et al.,* 1985; Williams and Morton, 1985; Swanney *et al.*, 1988; Boswell and Swanney, 1991). Elemental S is an important S source because of its high analysis and slow rate of release of plant-available sulphate-S, which can be controlled by its particle size distribution. Under severe leaching conditions the greater efficiency of S^0 over $SO_4^=$ is clearly obvious and general recommendations (Sinclair and Saunders, 1984) for S^0 use are available based on slow release (S^0 oxidation) concepts (Swanney *et al.,* 1988). Apart from studies measuring the amounts of S^0 residues in soil (Lee *et al.,* 1987), no studies have been conducted to examine the fate of S^0 in soils and whether this fate is the same as S from $SO_4^=$ based fertilizers.

In contrast to excessive use of S fertilizer, in the economic climate of the past six years many farms in New Zealand have now not received fertilizer or have received S-free phosphatic fertilizers, such as diammonium phosphate, reactive phosphate rocks and phosphoric acid

acidulated reactive phosphate rocks. This reduced S input, combined with the potential of New Zealand soils to become S-deficient by leaching, has created some striking responses to reapplied gypsum (M.J. Hedley, personal communication) and a general need for re-assessing agronomic requirements for sulphur (Sinclair *et al.,* 1985). Very little information on the S status of New Zealand soil is available, however, with current knowledge it is difficult to predict accurately the S fertilizer requirements of pastoral systems varying widely in soil fertility status.

Many questions remain to be answered prior to determining the efficiency with which soil and fertilizer S is utilized in pastoral systems; e.g. what are the rates of mineralization of soil organic S and immobilization of fertilizer S into organic S reserves in soils under different environmental conditions and farming systems, and in the long term can the use of the insoluble slow release S^0 lead to more efficient S fertilizer use ? In general, efficient use of fertilizer S occurs when the rate at which S is cycling in the soil-plant-animal system is maximized and when the number of cycles completed are large compared to the rate of non-product losses from the cycle. The fate of S applied to pastoral soils in sulphate-S fertilizer form (gypsum or superphosphate) was studied in drier areas of New Zealand (Gregg, 1976) and Australia (Till and May, 1970a, 1970b) but there remains a lack of understanding of the fate (i.e. movement in soil profile, leaching losses, incorporation into soil organic S and the forms of available soil S utilized by pasture plants) of applied S^0 fertilizers in pastoral soils in moisture regimes like those of the central North Island of New Zealand. Such research is important as S becomes an increasingly costly input into New Zealand pastoral farming.

The objective of this thesis is to develop radioisotope techniques to study the fate of sulphate-based and S^0 fertilizers in field soils and having developed these techniques to investigate the factors which influence the fate and plant availability of these fertilizers, in order to provide information that can be used to formulate and improve fertilizer recommendations for pastures.

This thesis comprises nine chapters. Following this introduction are a review of literature on aspects of sulphur cycling in grazed pasture systems and a chapter on the materials and methods used. Methods for radioactively labelling fertilizer sulphur were developed and were employed to produce fertilizers for studies of the short-term fate of fertilizer S which are discussed in Chapter 5 and 7. The main emphasis of this thesis is focused on the short-term fate of different particle sizes of S^0 applied onto undisturbed soil cores cut from permanent ryegrass/clover pasture of contrasting fertility status (Chapter 5-7). These particle size diameters range from less than 0.010 mm to 0.500 mm. Sulphate-based S, applied as gypsum and superphosphate, was also used as a reference in the studies. The effect of sheep dung on the short-term immobilization of soil and fertilizer S is discussed In Chapter 4.

A preliminary computer simulation model describing S cycling was developed (Chapter 8) in an effort to explain the observed transformation of S and ^{35}S in order to calculate actual mineralization and immobilization rates in an environment where sulphate leaching occurs. General conclusions and implications are given in Chapter 9.

CHAPTER 2

A REVIEW OF LITERATURE ON ASPECTS OF SULPHUR CYCLING IN GRAZED PASTURE SYSTEMS

2.1 INTRODUCTION

A pasture system revolves around the functioning of several components: soils, plants, grazing animals and their residues and soil micro- and macro- flora and fauna. These components are responsible for recycling nutrients from senescing materials to plant available forms again. An understanding of the cycling of the specific plant nutrient, sulphur, requires an understanding of the functioning of the individual components. Tracing sulphur's movement through soils, plants and animals involves a multiplicity of complex reactions, transfers and transformations (Whitaker, 1970). Naturally, there is no one single pathway for the cycling of an element in a grazed pasture system. Several pathways exist. The objectives of this chapter are to review and develop an understanding of the nature of S and factors affecting its circulation through and losses from soil-plant-animal systems with particular emphasis on grazed pasture soils.

2.2 NATURE, FORMS AND DISTRIBUTION OF SOIL SULPHUR

In the late 1970s, a greater awareness of the importance of S in crop production, particularly protein synthesis, and of soil as a source of S for plants resulted in several studies of the nature and forms of S in soils (Metson, 1969, 1979a, 1979b, 1979c; Williams, 1975; Halstead and Rennie, 1977; Biederbeck, 1978).

A knowledge of the forms and amounts of soil S is essential to our understanding and interpretation of S cycling. It should be stated at the outset that our understanding of the forms and amounts of soil S is limited by current analytical and separation techniques. Most specific S compounds associated with both organic and inorganic soil components cannot be identified without chemical treatment and separation or extraction from soil. The soil **S** cycle can only be represented by the dominant fractions that can be measured and will change as new techniques provide more information on the natural chemical structures of soil organic S.

2.2.1 Total sulphur

The total S content of soils has been recently reviewed by Metson (1969, 1979a, 1979b, 1979c), Blair (1979) and Freney and Williams (1983). S, the thirteenth most abundant

element, comprises about 0.052% of the Earths crust (Day, 1963). In nature, this element with six valance electrons can exist in a variety of organic and inorganic combinations, in solid, solution or gaseous forms and in various states of oxidation ranging from -2 (sulphide) to +6 (sulphate). The total S content of soils ranges from approximately 0.002 to 5% (w/w). High levels are found in tidal marsh soils, where sulphide accumulates, in the soils in arid areas containing inorganic sulphates such as gypsum, and soils subjected to severe industrial pollution (Syers and Curtin, 1987). In organic soils (e.g. peats) total S may exceed 0.5% dry weight (Halstead and Rennie, 1977). Most agricultural soils or mineral soils have S contents ranging from 50 to 1000 mg kg^{-1} in the surface 15 cm (Freney and Williams, 1983; Syers and Curtin, 1987).

The proportions of S in organic or inorganic form vary according to soil type, depth in profile (Williams, 1974), climate and cultural conditions (Bettany *et al.*, 1979, 1980). Sulphate-S ($SO_4^=$) is the form most available to plants.

2.2.2 Inorganic sulphur

In the surface horizons of most agricultural soils, inorganic S occurs almost entirely as sulphate but represents a relatively small proportion of total S, less than 25% of total S in most agricultural soils (Halstead and Rennie, 1977) and more commonly less than 5%. It is derived from wet and dry deposition of mainly sulphates and sulphur dioxide, weathering of soil parent rocks (oxidation of reduced inorganic forms of S, e.g. sulphide) and oxidation (termed mineralization) of organic S (Roy and Trudinger, 1970). Weathering reactions are thought to be a minor input of S in current topsoils (Metson, 1979a). This is mainly because mineral sulphides are quickly weathered in aerobic, topsoil environments.

In general, inorganic soil S includes easily soluble sulphate in soil solution, sulphate adsorbed on positively charged surfaces of soil particles, insoluble sulphate e.g. insoluble sulphate co-precipitated with $CaCO_3$ and reduced inorganic compounds e.g. sulphides (Brown, 1982).

2.2.2.1 Readily soluble sulphate-S

Generally, the surface of most agricultural soils contain only small amounts of this fraction although in semi-arid areas where evapotranspiration exceeds drainage, or in poorly drained conditions, high levels of sulphate may accumulate (Freney and Williams, 1983; Blakemore *et al.*, 1968).

The occurrence of only small amounts of soluble sulphate in soils has been explained by Metson (1979a) to result from:

1. Retention on soil colloids as adsorbed sulphate in anion retentive soils;
2. Leaching downward or laterally out of the soil profile;
3. Utilization for nutrition of plants and microorganisms;
4. Precipitation as insoluble sulphate.

Soil sulphate levels are often subject to seasonal fluctuation depending upon the net balance between addition from rainfall, irrigation water, mineralization of organic matter, applied fertilizers, and losses from leaching and plant and micro-organisms uptake (Williams, 1968; Staunes, 1985). Data from Nguyen (1982), Cornforth *et al.,* (1983), Nguyen *et al.,* (1989a, 1989b) and Ghani *et al.* (1990) confirm the importance of these processes. Nguyen (1982), Nguyen *et al.,* (1989a, 1989b) and Comforth *et al.* (1983) found that in the North Island (New Zealand) the amounts of extractable soil sulphate present in spring are lower than in autumn, possibly due to the increase in leaching loss of sulphate and the slow rate of mineralization during the winter time. Ghani *et al.* (1990) found that amounts of soil sulphate can decrease in short spaces of time, particularly, after rainfall events causing drainage.

2.2.2.2 Adsorbed sulphate-S

As mentioned above, the concentration of sulphate in the soil solution, an important factor in influencing plant uptake and leaching losses, is affected by several factors including sulphate adsorption-desorption and S mineralization-immobilization reactions resulting from microbial activity and plant uptake. Sulphate adsorption-desorption is therefore an important process affecting the rate of S cycling (Johnson and Todd, 1983; Fuller *et al.*, 1985).

Soils vary widely in their capacity to adsorb sulphate. Sulphate adsorption is primarily related to soil aluminium and iron sesquioxide content (Harward and Reisenauer, 1966; Tabatabai, 1987). Heavily weathered iron- and aluminium- rich soils usually have greater adsorption capacities than less weathered soils (Blakemore *et al.*, 1968). Tabatabai (1987) outlined the following factors that influence sulphate adsorption:

1. Clay content and type of clay mineral. Adsorption of soil sulphate increases with the clay content of the soils. Capacities of hydrogen saturated clays for sulphate adsorption are kaolinite > illite > bentonite.
2. Hydrous oxides. Hydrous oxides of Al and to a lesser extent of Fe, show marked

tendencies to retain sulphate. These compounds are probably responsible for most of the sulphate adsorption in many New Zealand soils.

3. Soil depth. Sulphate adsorption is often greater in subsoils due to the presence of more clay, Al and Fe. Subsoils commonly have lower concentrations of other anions (e.g. $H_2PO_4^-$ competing for sorption sites (see point 7, below)).
4. Effect of pH. Sulphate adsorption is favoured by strongly acidic conditions which protonate hydrous oxides into positively charged $(M\text{-}OH_2)^+$ groups. It becomes almost negligible at pH >6 when hydrous oxides lose their net positive charge.
5. Sulphate concentration and temperature. The amount of sulphate adsorbed is concentration and temperature dependent. Adsorbed sulphate is in kinetic equilibrium with sulphate in solution. Temperature has a relatively small effect on sulphate adsorption by soil.
6. Effect of time. Sulphate retention increases with the length of time it is in contact with adsorbing substances.
7. Presence of other anions. Sulphate is generally considered to be weakly held with the strength of retention decreasing in the order hydroxyl > phosphate > sulphate=acetate > nitrate=chloride. Phosphate will displace or reduce the adsorption of sulphate but sulphate has little effect on phosphate (Bolan *et al.*, 1986).
8. Effect of cations. The amount of sulphate retained is affected by the affinity of the associated cation of the salt or by the exchangeable cations on the soil surface. Increased surface positive charge results in a greater amount of sulphate in the diffuse double layer of cations and anions at clay and organic matter surfaces (Curtin and Syers, 1990). This effect follows the lyotropic series: $H^+ > Sr^{2+} > Ba^{2+} > Ca^{2+} > Mg^{2+} > Rb^+ > K^+ > NH_4^+ > Na^+ > Li^+$, which is essentially ranking cations in order of charge per unit surface area of their hydrated ion.

Our current understanding of adsorption mechanisms have been reviewed by Barrow (1985) and Bohn *et al.* (1986). In general, the mechanisms of adsorption in soils are usually divided into two reactions: nonspecific, where the adsorbate is held as a counter-ion in the diffuse double layer next to a positively charged colloid surface, and specific or chemi-sorption or ligand (all define the same reaction), where the ions enter into coordination with the oxides of metals and become bonded to the metal in the structure as well as displacing other ligand ions (Rajan, 1978). Both mechanisms occur simultaneously in a given soil horizon. All major ions are considered to be involved in nonspecific adsorption, but only a few are subject to specific adsorption or ligand exchange (Hingston *et al.*, 1967; Bohn *et al.*, 1979, 1986). Both mechanisms are mainly associated with surface oxides or hydrous oxides of Al^{+3} and Fe^{+3} as well as some clay minerals (Parfitt and Smart, 1978).

Nonspecific adsorption is pH dependent. Oxide surfaces may become protonated and require anions to balance the charge (Stumm and Morgan, 1970). At high pH, these same surfaces may lose protons and have net negative charge (Harward and Reisenauer, 1966; Hingston *et al.*, 1967). The pH at which the surface has no charge is the zero point of charge (ZPC). The ZPC of Al^{+3} and Fe^{+3} oxides is near a pH of 9, so that these sesquioxides would have a net positive charge in most acid soils and can retain large amounts of soil sulphate or phosphate.

Specific adsorption, which is also pH dependent, occurs when anions exchange with hydroxyl ions of hydrous oxides at weathered mineral edges and enter into coordination with Al^{+3} and Fe^{+3} ions (Bohn *et al.*, 1979). This reaction will make the surface of the oxide more negative and in some cases cause a release of OH^- ions (Stumm and Morgan, 1970).

Sulphate adsorption capacity frequently increases with soil depth and this plays an important role in retaining sulphate against leaching (Williams, 1975; Gregg, 1976; Gregg and Goh, 1978, 1979, 1982; Goh and Gregg, 1982a, 1982b) and minimizes the luxury uptake of sulphate (Barrow, 1975). It is commonly observed that subsoils show an accumulation of adsorbed sulphate, and higher affinities for sulphate than topsoils (Blakemore *et al.*, 1968; Gregg and Goh, 1978; Metson, 1979b, 1979c). This is not only because of changes in mineralogy but because of decreases in pH (Ensminger, 1954; Kamprath *et al.*, 1956 and Harward and Riesenauer, 1966) and lack of competition from the more strongly adsorbed phosphate ion (Ensminger, 1954; Barrow, 1967a).

Some important features of sulphate accretion by soil were identified by Fox *et al.* (1983). These are:

1. The capacity of sulphate sorption increases with soil weathering. In the case of the Andepts, the order of increasing sorption is Typic Dystrandept < Hydric Dystrandept < Typic Hydrandept; and in the case of soils developed from crystalline minerals the order is Alfisol < Ultisol < Oxisol.
2. Well-drained soils usually contain little sorbed sulphate, even if such soils have developed considerable sorption capacity, unless sulphate accrues in the soil from an outside source, such as fertilizer.
3. Amounts of soluble sulphate and its availability do not follow the quantity of total S in the soil. S deficiency is associated with low sulphate saturation.

According to Fox *et al.* (1983), considerable uncertainty exists about the nature and plant availability of adsorbed sulphate. For example, although plants do utilize adsorbed sulphate, it

has been observed that S deficiency may develop in crops on soils of the tropics which contain several thousand kg ha^{-1} of $SO_4^=$-S within the root zone. The capacity of a soil layer to absorb sulphate (Barrow, 1969a, 1970) influences the rate at which it is taken up by plants. Results conducted by Barrow (1969a) indicated that soil with a low ability to adsorb sulphate released sulphate into soil solution more rapidly than do high sulphate sorbing soils.

In addition to the adsorbed sulphate in the surface horizons, the agronomic value of the subsoil sulphate to pasture and deep-rooting crops such as lucerne has been recognized by various workers, (Ensminger, 1958; Blakemore *et al.*, 1969; Gregg *et al.*, 1977). For example, in a New Zealand yellow-brown pumice soil, Toxopeus (1970) found no relationship between the available sulphate in topsoil (0-7.5 cm) alone and yield response to S fertilizer. Including the amount of available sulphate in the subsoils, to a depth of 60 cm, improved the prediction of the pasture response.

The availability of subsoil S was shown in a greenhouse study; corn plants became S deficient when grown on Ap horizons alone, but had adequate S in their tissues when placed over B horizons with large amounts of adsorbed sulphate (Camberato and Kamprath, 1986).

Fox and colleagues (Hasan *et al.*, 1970; Fox, 1976; Fox *et al.*, 1977), Barber (1984), Hue *et al.* (1984) and Camberato and Kamprath (1986), have determined approximate soil solution concentrations of sulphate S required for 95% maximum plant growth. These results indicated a soil solution sulphate concentration of 0.156 mM L^{-1} $SO_4^=$-S for fine textured soils and 0.025 mM L^{-1} $SO_4^=$-S for coarse textured soils were optimum for growth. Fox (1980) showed that a number of subsoils maintained insufficient soil solution sulphate concentration despite large amounts of adsorbed sulphate.

In general, it would appear that although the subsoil sulphate can provide S to plants, the size of its contribution depends upon soil chemical and physical factors (Gregg *et al.*, 1977; Metson, 1979a) as well as plant rooting depths. In strongly weathered soils of low pH, where adsorbed sulphate is not easily desorbed by soil colloids, this sulphate pool may not be available to plants (Metson, 1979a; Fox, 1980) because the sulphate solution concentration is too low or plant root development may be limited by aluminium toxicity (Ensminger, 1954). In contrast, as mentioned by Rennenberg (1984), it was considered that the acidification of soils enhances the availability of Al^{+3} to roots (Rorison, 1973); polyvalent cations such as Al^{+3} and Ca^{+2} may under some circumstances stimulate the uptake of sulphate by root cells (Franklin, 1971; Skjelbreid and Nissen, 1980; Jones and Smith, 1981).

Isotopic studies on the uptake of S by pasture plants (Gregg *et al.*, 1977) have indicated that soil physical factors such as the availability of moisture within the soil profile and soil porosity, can affect the penetration of plant roots and consequently influence the magnitude of S uptake.

Although it is difficult to judge the benefit of subsoil S for plant nutrition (Bohn *et al.*, 1986), an increased ability of soils to retain sulphate is considered to reduce fertilizer S requirements (Sinclair, 1983). To calculate the fertilizer requirements of pasture soils, the anion retention characteristics of the soil are required (Sinclair and Saunders, 1984). The phosphate retention capacity (Saunders and Hogg, 1971) is normally used as the anion retention index in New Zealand soils but specific sulphate retention soil tests have been developed elsewhere; e.g. Barrow, 1967a; During and Martin, 1968; Saunders and Hogg, 1971. The New Zealand Ministry of Agriculture and Fisheries use the P retention value in their calculations as an index of S leaching potential. Typically, soils derived from siliceous parent materials have low S retention values, while soils derived from volcanic parent materials tend to have a higher ability to retain sulphate (Saunders and Hogg, 1971).

2.2.2.3 Other inorganic S forms

In some calcareous soils a significant fraction of total S is present as insoluble calcium sulphate co-precipitated with $CaCO_3$. These compounds are generally unavailable to plants particularly if associated with coarse particles of carbonate mineral. Williams and Steinbergs (1962) measured the amounts in calcareous soils ranging from 25 to 3000 ppm S.

High concentrations of sulphate transported to root surfaces by mass flow, in excess of plant demand, may accumulate at root surfaces (Mengel and Kirkby, 1978). In this manner gypsum may sometimes precipitate in rhizopheres of actively growing plants particularly in glasshouse soils heavily fertilized with superphosphate (Barber *et al.,* 1963).

Sulphides and some reduced S forms and even elemental S (S^0) can occur under waterlogged conditions or poorly drained subsoils (Brown, 1985, Zucker and Zech, 1985). In well-drained, well-aerated soils the amount of mineral S occurring as compounds of lower oxidation states are negligible (Metson, 1979a).

2.2.3 Organic sulphur

Both plants and microbes incorporate sulphate into various organic compounds. These organic S forms enter soils as plant litter, animal excreta and corpses or are formed ***in situ*** by the soil biota. In most well drained and non-calcareous agricultural soils, the major proportion of the total S in surface soils is in organic form (Fitzgerald, 1976, 1978; Biederbeck, 1978; Freney and Williams, 1983; Stevenson, 1986). This is mainly because the majority of soil organic S is insoluble in water and not susceptible to leaching losses. The forms of organic S, mainly deposited in soils, include the S containing amino acids and the sulfonates in which ***S is directly bonded to carbon***, and the true organic esters of sulphuric acid, in which ***S is bonded to oxygen*** in the form of ***C-O-SO$_3^-$*** linkages. In sulphamates, S occurs in the form of ***N-O-SO$_3^-$*** and ***N-SO$_3^-$***.

Little is known of the macro-molecular nature of organic S in soils (Freney and Stevenson, 1966; Freney, 1967; Freney and Williams, 1983). Freney (1967) and Lowe (1969a, 1969b) have shown a wide variety of S compounds were produced by organisms either in or on soils. Most of these were susceptible to decomposition, did not accumulate in their mono-molecular form, and were not readily identified in the soils.

The amount of soil organic S is closely associated with the amounts of carbon and nitrogen. Over a large sample of soils, the mean C:N:S ratio was 130:10:1.3 (Freney and Williams, 1983), however, the amount of S in relation to carbon and nitrogen varies between soils. The difference may be attributed to differences in parent material, soil forming factors (climate, vegetation, leaching intensity, drainage, and temperature), cultivation and management practices (Freney and Williams, 1983).

The work of Freney and co-workers (Freney, 1961, 1967; Freney *et al.*, 1969, 1970, 1971, 1975) suggested that most of the organic S in soils can be separated into the following forms by the use of chemical analytical techniques:

1. Organic S which is reduced to H_2S by a mixture of hydriodic acid, formic acid and hypophosphorous acid is known as hydriodic acid reducible S (HI-reducible S) and S in this fraction is not bonded directly to carbon. This fraction is believed to be mainly ester-$SO_4^=$, C-O-SO_3^- (arylsulfatase, choline sulphate, sulphate polysaccharides, etc.) and N-O-SO_3^-, (Freney, 1961).
2. Organic S which is not reduced by the above mixture is believed to comprise all of the S which is bonded directly to carbon, C-S, (Freney, 1961).

3. Organic S which is reduced to inorganic sulphide by Raney-Nickle/NaOH consists almost entirely of S in the form of amino acids (Lowe and De Long, 1963; Freney *et al.*, 1975) and is known as Raney-Nickel reducible S.

Since the HI-reduction method will also convert S^0 and sulphate-S to H_2S (Freney, 1958), the true amount of ester-$SO_4^=$ in soil is usually obtained by subtracting the amount of S^0 and sulphate-S from the amount of HI-reducible S (unless sulphate-S was extracted before reduction). Details on ester-$SO_4^=$ formation, transformation, and its significance in the S cycle were reviewed by Fitzgerald (1976, 1978). It has been considered that carbon-bonded S may be derived from both leaf litter and root inputs, as well as microbial synthesis. Ester-$SO_4^=$ are generated predominantly by soil microbial populations (David *et al.*, 1984 and Fitzgerald, 1978).

Laboratory studies have shown that the relative proportions of carbon bonded and ester-$SO_4^=$ formed depend on the availability of carbon or sulphate or the C:S ratio of the organic substrate for microbial growth (Saggar, 1980; Saggar *et al.*, 1981b; Ghani *et al.*, 1988, 1991).

In general HI-reducible S accounts for 30-70% of the total soil organic S (Williams and Steinbergs, 1959; Freney, 1961; Lowe and De Long, 1963; Tabatabai and Bremner, 1972b; Bettany *et al.*, 1973; Neptune *et al.*, 1975). In some soils the percentage of soil S present as HI-reducible S remains constant with depth (Williams, 1975), but in others the percentage increases with depth (Tabatabai and Bremner, 1972b; Williams, 1975). This probably reflects differences in the carbon and S availability in different soils and soil depths. Upper horizons, ramified more intensely by roots, would have higher carbon input rates.

The carbon-bonded S fraction includes amino acids and protein S such as methionine and cysteine. De Long and Lowe (1962) proposed a procedure for the determination of carbon-bonded S in soils which claimed to recover all forms of organic S other than covalent sulphate and alkyl sulphones. This proposed Raney-Nickel reduction method, however, does not reduce the carbon-bonded S in aliphatic sulphones or sulphonic acids and is subject to serious interference from iron and manganese (Freney *et al.*, 1970). In addition, Freney *et al.* (1970, 1972) found that, even under optimal conditions, the amount of Raney-Nickel reducible S was 44% less than the value of carbon-bonded S, calculated by subtracting the HI-reducible S from the total S content of the soil. Thus most workers prefer to estimate the amount of carbon-bonded S by subtracting the HI-reducible S from the total S content of a soil (Freney *et al.*, 1970, 1975; Bettany *et al.*, 1973; Tabatabai, 1982; Landers *et al.*, 1983).

There is conflicting evidence with regard to the agronomic significance of differentiating between carbon-bonded S and HI-reducible S. In mineralization studies using Canadian soils, Lowe (1964, 1965) considered carbon-bonded S to be of little value as a source of mineralizable S, in contrast, Ghani *et al.* (1988, 1991), using New Zealand soils found that most of the sulphate generated from soil incubation studies originated from carbon-bonded S. Freney *et al.* (1975) also showed that over nine months most of the available S (60%), removed by plants in a pot experiment, came from carbon-bonded S although there were changes in all organic fractions.

Sulphur in the HI-reducible S fraction can be easily hydrolyzed into inorganic sulphate by acid or alkali (Freney, 1961). It was considered to be the most labile fraction of soil organic S and/or serve as an immediate reservoir of S that can be mineralized in response to biological demand (McLaren *et al.*, 1985; Strickland *et al.*, 1987). Widdowson (1970), however, found that neither fraction was related to plant S uptake on a range of Iowa soils. Due to this conflict in evidence it was concluded that, separately, these two fractions are unlikely to be of any value for predicting the S requirement of plants.

Cycling between the HI-reducible S and carbon-bonded S has been observed by many researchers (e.g., Freney *et al.*, 1971; McLachlan and De Marco, 1975; Saggar *et al.*, 1981b; Ghani *et al.*, 1988, 1991). McLachlan and De Marco (1975) considered that rather than employ a soil test, determining the rate of change between these two fractions may provide a more appropriate method for determining the soil S status than measuring the actual quantities found in either fraction at any one time. So far, no further work has been done on this aspect.

Although most soil S may be present in the organic form, only a small percentage of this fraction (as little as 10%) may enter the active S cycling pool on an annual basis (Till and May, 1971; Goh and Gregg, 1982a, 1982b; Chapman, 1987a, 1987b).

2.2.4 Other methods for characterizing soil organic sulphur.

As mentioned in Section 2.2.3, it has been observed that characterization of organic S in soil, based on bonding relationships with carbon, does not always provide biologically meaningful fractions (Bettany *et al.*, 1974, 1979; Goh, 1988; Swift *et al.*, 1988). This may be because soil organic matter comprises a complex heterogeneous mixture of living biomass (micro-organisms plus fine root material), partially decomposed and completely transformed plant and animal residues and some exists in intimate association with the mineral components of soil. Some workers have developed extraction techniques with the aim of separating the more labile S from the more inert components irrespective of its bonding relationships to carbon.

Many approaches in the study of organic matter fractionation are as follows: (a) isolation, identification and measurement of individual compounds (Schreiner and Shorey, and co-workers (1908-1938), according to Kononova, 1966); (b) chemical extraction and physico-chemical separation and characterization of various fractions (Hayes and Swift, 1978; Swift *et al.*, 1988); (c) physical separation into size fractions and/or densimetric fractions without significant alteration of organo-mineral complexes (Saggar, 1980); and (d) biological approach aided by radio and stable tracer techniques (Jenkinson, 1976; Jenkinson *et al.*, 1976; Bettany *et al.,* 1979; Saggar, 1980; Ghani *et al.*, 1988).

Chemical extraction and physico-chemical separation is most commonly and widely used, wherein much effort has been concentrated on finding solvents that will extract high proportions of organic materials without significantly altering their composition.

So far, few have attempted the fractionation of S in organic matter. Bettany *et al.*, (1979) and Saggar (1980) used an alkali-pyrophosphate extraction-separation technique on some Canadian soils. Their results showed that the percent of total S as HI-reducible S was found to be greatest in the fulvic acid (FA-A) fraction (alkali soluble and acid soluble) and least in the humic acid fraction (HA-A) (alkali soluble and acid insoluble). Most of these S fractions were associated with the clay size fraction of soils. These results were unable to be interpreted with respect to plant availability of different soil S forms.

Swift *et al.* (1988) proposed a mild fractionation method for organic S using 2 M acetylacetone (pH 8) in combination with ultrasonic dispersion, but the significance of this procedure is unknown.

More research on these various approaches is still needed before it can be stated that they are useful in characterizing the availability of soil organic S to plants.

2.2.5 Microbial sulphur

As mentioned earlier, microbial processes, associated with organic matter decomposition, create plant available S in the soil system. The plant availability of S in soils is largely dependent on the nature and amounts of S present as well as the dynamics of the soil microbial population. The microbial turnover of S may have a great effect on the short term supply of S in soils where most of the S is in organic forms. Thus a knowledge of the amount and nutrient composition of the soil microbial population (microbial biomass), and further, the

response of the microbial biomass to the addition of decomposable carbon energy sources and fertilizer S is necessary to understand the nature of S transformations in soil.

The S content of most microorganisms ranges from less than 0.1 to 1% of dry weight (Alexander, 1977). The most conspicuous cellular constituents in bacteria containing S are amino acids, cysteine and methionine, whereas several fungi can store intra-cellular S as choline sulphate (Harada and Spencer, 1960; Takebe, 1960).

Coughenour (1978), calculated on the basis of the quantity of S amino acids isolated from various bacteria (Laskin and Lechvalier, 1973), that the S content of bacteria ranged from 0.52 to 1.02% and that of bacterial protein between 0.9 to 0.97%. The majority of S amino acids are associated with intercellular or metabolic components of bacteria. The contribution of ester-$SO_4^=$ from within bacterial cells is limited but the ability of bacteria to synthesize these compounds and subsequently release them extracellularly could be of considerable importance in soils (Fitzgerald, 1976).

The S content of fungi, based on the S amino acid composition of various fungal proteins (Coughenour, 1978), ranges between 0.18 and 0.33%. Apart from S amino acids the ability of fungi to synthesize and accumulate choline sulphates and other ester-$SO_4^=$ is well documented (Spencer and Harada, 1960; Takebe, 1960).

Based on the microbial biomass data reported by Clark and Paul (1970), Kowalenko (1978) estimated that bacteria and fungi in the surface of a grassland soil account for between 0.3 and 1% of total soil organic S respectively. Although the microbial population contains a very small proportion of soil S, this fraction is extremely mobile and is considered to be the key driving force for S turnover in soil (Biederbeck, 1978). The latter statement is in agreement with tracer studies which showed that not more than 2-3% of the total organic S in Brown forest soils was in the active phase (van Praag, 1973).

Saggar (1980) and Saggar *et al.* (1981a) developed a method for measuring microbial biomass S in soil. It was found that microbial biomass S in Canadian prairie soils represented about 2.8% of the total S in soil. This was in agreement with the values, measured by other authors, of 2-3% (van Praag, 1973), 1.3% (Kowalenko, 1978), 1.2 and 2.2% (Strick and Nakas, 1984), 0.9 and 2.6% (Chapman, 1987a) and 0.4-1.8% (Ghani *et al.*, 1990).

McLaren *et al.* (1985) estimated the amount of soil microbial biomass S, measured by Saggars technique (Saggar, 1980 and Saggar *et al.*, 1981a), was the same 'active' organic cycling pool as that derived by calculation from their incubation study. Goh and Gregg (1982a, 1982b)

found the active cycling pool of S ranged between 4 to 8 percent of the total organic S in a range of New Zealand soils. Furthermore, Chapman (1987a,1987b) suggested that microbial biomass S can form a significant proportion of the organic S pool which is involved in S cycling and is potentially available to plants. Chapman (1987a) also found that in soils of very low S status and known to respond to S fertilizers, biomass S exceeded 'plant-available' S extracted with phosphate and may thus be an important pool of S in these soils. Recently, Ghani *et al.* (1990) showed that the amounts of microbial biomass S in New Zealand soils were fluctuating within seasons according to soil moisture and temperature changes. Pasture soils had larger amounts of soil microbial biomass S than cultivated and fallow soils. In general, it was also found that soil sulphate levels in soils were inter-related with microbial biomass S concentration.

Useful relationships have been found between soil organic matter, various S constituents (total S, carbon-bonded S, ester-$SO_4^=$, organic S, enzymes S), and microbial activity (Lee and Speir, 1979; Lee *et al.*, 1985). David *et al.* (1982) considered that these relationships indicate the potential for microbial S transformations and the importance of microbial activity in dynamics of S in soil systems.

2.3 BIOLOGICAL TRANSFORMATIONS OF SULPHUR IN SOIL

Processes involved in the decomposition of plant litter, roots and organic matter in excreta, by soil microflora and fauna remain the key processes in maintaining nutrient cycling and, consequently, animal production in grazed pastures. It is generally believed that most of the decomposition reactions releasing S in soils are carried out by micro-organisms and some perhaps by plant roots, although some abiotic chemical reactions are also possible.

Immobilization and mineralization, which are essentially enzymatically catalysed S oxidation and reduction reactions, are the main transformations occurring in soils and are concerned with the internal cycling of S from one soil S form (pool) to another (Freney and Williams, 1983). A diagram representing the S cycle is shown in Figure 2.1. A more complete discussion of the mechanisms and metabolic pathways of various S transformations is available elsewhere (Freney, 1967; Postgate, 1968; Roy and Trudinger, 1970; Fitzgerald, 1976, 1978; Scott, 1985). A summary of these processes is given below. In this review mineralization and immobilization process are discussed.

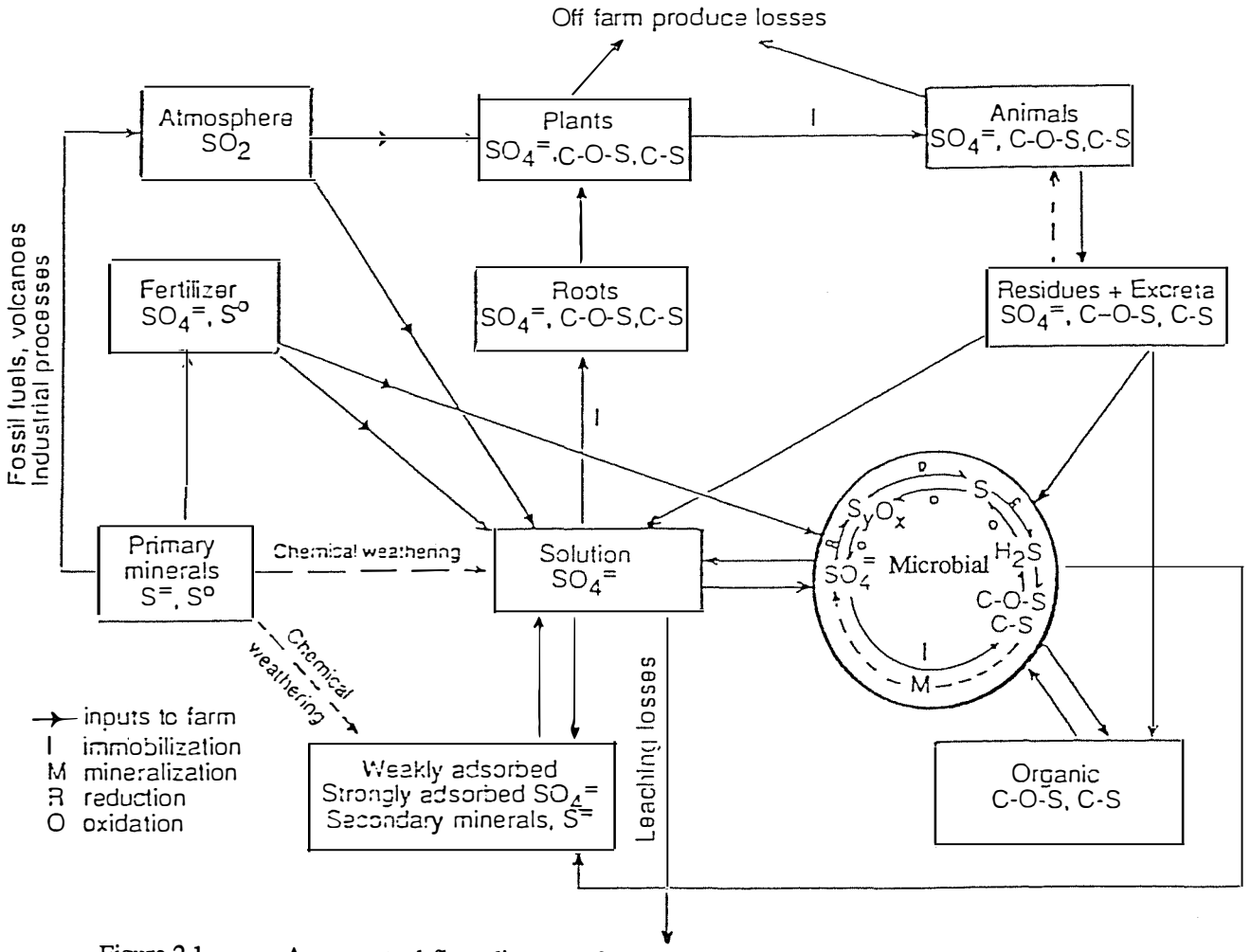


Figure 2.1 A conceptual flow diagram of the main forms and transformations of sulphur in the soil-plant-animal system (M.J. Hedley, 1990. Lecture notes from Soil Fertility and the Environment, Soil Science Department, Massey University, New Zealand).

2.3.1 Mineralization

Organic S mineralization, the hydrolysis and oxidation of organic soil S, is an important process for generating plant available sulphate within all soils, particularly permanent pasture soils, especially when compared quantitatively with the atmospheric and weathering inputs of S. Sorn-srivichai (1980) showed that if S transformations in a pasture soil (Typic Fragiaqualfs) were in steady state the rate of mineralization would be about 19 kg S ha^{-1} $year^{-1}$. This was equivalent to about 2-5% of the soil organic S being mineralized per year and was the single largest input of plant available S.

Although many aspects of the mineralization of S are still poorly understood (Scott, 1985), it is well established that the rate of S mineralization is controlled by factors, influencing the growth of micro-organisms and their output of extracellular hydrolytic sulphatase enzymes, such as temperature, moisture, pH (Chaudhry and Cornfield, 1967a, 1967b; Williams, 1967; Swift 1983), food supply (Barrow, 1960a, 1960b, 1960c) as influenced by soil depth (David *et al.*, 1983), the presence and absence of plants (Freney and Spencer 1960; Maynard *et al.*, 1985), organic matter addition (Saggar *et al.*, 1981b) and the amount of sulphate present (Maynard *et al.*, 1985). Similar factors have been shown to influence the level of hydrolytic sulphatase enzymes in soil (Speir, 1977; Lee and Speir 1979; Speir and McGill, 1979; Tabatabai and Bremner, 1970b).

Williams (1967) and Chaudhry and Cornfield (1967a, 1967b) showed that the optimum temperature for mineralization of S in soil was 40 ^{o}C; mineralization was markedly suppressed at temperatures less than 10 ^{o}C and declined at 50 ^{o}C (Williams, 1967; Stevenson, 1986). Temperature is likely to be an important rate-limiting factor (Syers and Curtin, 1987) and an explanation of the lower levels of sulphate in spring and higher levels in autumn after cooler winter conditions in New Zealand grazed pasture soils as reported by Cornforth *et al.* (1983) and Nguyen *et al.* (1989a).

Australian work has shown that the amount of mineralized S is proportional to increasing pH up to a value of approximately 7.5 (Williams, 1967). Moisture content close to field capacity is optimal for S mineralization and the rate of sulphate production declines sharply at both high and low moisture contents (Williams, 1967).

McGill and Cole (1981) and David *et al.* (1983) considered that mineralization can be divided into two processes, biological and biochemical mineralization, definitions which are directed to the mineralization of carbon-bonded and ester-$SO_4^{=}$ respectively. The concept has it that

carbon-bonded S mineralization is a result of energy demand by microorganisms which subsequently releases inorganic S during oxidation of organic carbon, whereas ester-$SO_4^=$ mineralization is regulated by both energy demand of microbes (to a lesser degree than in C-bonded S mineralization) and microbial nutritional need for sulphate (McGill and Cole, 1981; Lee *et al.*, 1985) (i.e. simple hydrolysis of sulphate ester may satisfy the microbial demand for S). Whereas the concept of biological and biochemical mineralization may be useful in constructing computer simulations of S mineralization it is likely that a 'soup' of microbially produced extracellular enzymes at the 'coal face' of the organic matter mineralization act together to hydrolyze complex soil organics into simple amino acids, sugars and nutrient ions that can be adsorbed by the organisms. The act of cleaving of a sulphate ester may be as much a part of producing a less ionic more transportable energy source as it is releasing sulphate. In addition, it is doubtful whether extracellular enzyme activity in soil is the factor limiting mineralization reactions because measured enzyme activity levels are mostly in excess of available substrate concentrations (Burns, 1978).

The hydrolysis of HI-reducible S by sulphatase enzymes, a likely mechanism of S mineralization in soils (Fitzgerald, 1978), occurs as follows:

$$\text{R-C-O-SO}_3^- + \text{H}_2\text{O} \xrightarrow{\text{sulphatase}} \text{R-C-OH} + \text{H}^+ + \text{SO}_4^=$$

A large number of sulphatases exist in nature (Fiztgerald, 1978). The activity of sulphatase is thought to be controlled by end-product-inhibition by $SO_4^=$ (Speir and McGill, 1979; Freney, 1986) and inhibition by native PO_4^{-3} (Al-Khafaji and Tabatabai, 1979). The mineralization rate of organic S may be slower in the presence of large concentrations of sulphate (Ghani *et al.*, 1991).

The addition of up to 36 ppm sulphate-S did not suppress the mineralization process in studies conducted by Freney and Spencer (1960) and Bettany *et al.* (1974), however, high sulphate concentration (e.g. 108 ppm S; Freney and Spencer 1960) in the presence or absence of plants resulted in immobilization (Freney and Spencer, 1960; Saggar *et al.*,1981b; Maynard *et al.*, 1985).

In laboratory incubations, where nitrogen and S were not growth limiting nutrients, additions of nitrate and sulphate had little effect on the rate of S mineralization (Maynard *et al.*, 1983a; McLaren *et al.*, 1988). The addition of organic material and plant residues, however, does greatly affect the process. Adding readily available carbon sources has been shown to increase the amount of sulphate immobilized (Freney *et al.*, 1971; Saggar et al., 1981b). The

combined C:S ratio of added organic material will determine whether mineralization or immobilization occurs in the short term (Barrow, 1960c; Stewart *et al.*, 1966a, 1966b). Barrow (1960c) has shown that if the C:S ratio of the added material was below 200, mineralization occurred; when the ratio was above 400, sulphate was immobilized into organic matter; and when the ratio was between 200 and 400, sulphate could either be released from or incorporated into the organic matter. In the long term, however, the C:S ratio of residues decreases and the net mineralization of S is expected to occur.

No consistent relationships have been established between S mineralization and total soil organic S or with the C:S, N:S or C:N ratio of soil organic matter. It is suggested that it may be the S content of the recently-added organic matter, rather than total S, that regulates S mineralization (Freney and Williams, 1983; Freney, 1986). As mineralization and immobilization occur concurrently, differing patterns of sulphate release might be expected, depending on the availability of readily utilizable material for the microorganisms.

As a consequence of an inability to find a consistent relationship between the gross elemental ratios in the organic matter and the extent of mineralization, attempts have been made to relate S mineralization to a particular S fraction. McLaren and Swift (1977), McLaren *et al.* (1988) and Ghani *et al.* (1991) using the findings of Freney *et al.*, (1971), have suggested that carbon-bonded S is readily transformed to HI-reducible S which is then mineralized. Bettany *et al.* (1974), however, were unable to demonstrate mineralized S was derived from the HI-reducible S fraction.

Most of the information available on S mineralization has been obtained from short-medium term incubations designed primarily as rapid methods to predict the amounts of S available to plants. How relevant these results are to field S mineralization rates is unclear. Maynard *et al.* (1983a), Syers and Curtin (1987) and Pirela and Tabatabai (1988) found that the apparent rates of S mineralization are very much influenced by the incubation technique used. Maynard *et al.* (1983a) found that the amount of S mineralized in three prairie soils, over a 17 week period of a 'closed' incubation system, averaged 0.7 mg kg^{-1} compared with 11.5 mg kg^{-1} in an 'open' system in which sulphate was periodically flushed out by leaching with dilute $CaCl_2$. Furthermore, Maynard *et al.* (1985) considered that more extensive mineralization in cropped relative to uncropped soils was attributed to the fact that, by maintaining soluble sulphate at low concentrations, this stimulated the production of sulphatase by plant roots and rhizophere microorganisms. In addition, the results obtained by incubation procedures may be biased by soil pretreatments; e.g. Ghani *et al.* (1991) used a phosphate pretreatment to remove adsorbed $SO_4^=$ to encourage S mineralization, the high phosphate concentration remaining in

the soil may inhibit phosphatase and sulphatase enzymes which may be an integral part of mineralization processes.

Under field conditions very little is known about S mineralization rates except for net rates that are represented in the sum of plant uptake and sulphate leaching losses and change in soil sulphate.

Some evidence of long term S mineralization rates in field soil came from a comparison made by fractionating organic matter in some Canadian soils (Saggar, 1980). The results showed that a long term cultivation (65 years) led to depletion of the C, N and S contents of soil organic matter compared to that of an adjacent permanent pasture soil. The decreases were assumed to be the net results of mineralization processes (some may also have resulted from erosion). Relatively lower net losses of S (38%) compared to carbon (44%) and nitrogen (49%) and narrowing C/N/S ratios indicated that S was more resistant to mineralization during the cultivation processes than carbon and nitrogen (Saggar, 1980). It is expected that during the mineralization processes, CO_2 and NO_3^- are lost more easily from the soil than $SO_4^=$ even if the mineralization rates are the same. In a similar study by McLaren and Swift (1977) with a pasture and an adjacent continuously cultivated soil in Scotland, larger amounts of carbon-bonded S (75%) were lost compared to HI-reducible S (25%). Both authors considered that the HI-reducible S has a more transitory nature than carbon-bonded S and it is possibly of great importance in the short-term mineralization of S, whereas in the mineralization of carbon-bonded S it probably passes through an HI-reducible S form prior to release as inorganic sulphate. Castellano and Dick (1988) considered that the ultimate source of plant available S, under long term S depletion, is from the recalcitrant residual S pool. Similar forms of stable organic P were depleted in a Canadian prairie soil subjected to 65 years of wheat-fallow rotation (Hedley and Stewart, 1982).

It appears that no reliable method has yet been developed for assessing the 'potential' mineralization of S on field soils (Swift *et al.,* 1988). This is a difficult task because the rate of this microbially mediated process will fluctuate with soil physical conditions and will be spatially dependent upon opportunistic microbial populations being present with recently added or disturbed organic substrates.

2.3.2 Immobilization

The biochemical pathways of immobilization involve, among other processes, amino acid and sulpholipid synthesis (Leninger, 1982). For amino acid synthesis, sulphate ions are activated in a two-step process, leading to the production of energy-rich sulphate nucleotides APS

(adenosine 5'-phosphosulphate) and PAPA (3'-phosphoadenosine-5'-phosphosulphate). It is thought that PAPS is then reduced (via sulphite) which combines with the amino acid, serine, to form cysteine as follows:

$$SO_4^{=} \rightarrow \text{APS} \rightarrow \text{PAPS} \rightarrow \text{SULPHIDE}$$

$$+$$

$$\text{SERINE} \rightarrow \text{CYSTEINE}$$

Microbial involvement in nutrient immobilization has been the subject of increasing interest as a mechanism for increasing the nutrient retention capacity of soils. In the short term (one year) the amount and rate of fertilizer S incorporation into the soil organic pool appears to be of practical importance in fertilizer use and environmental quality (Gregg, 1976; Freney and Williams 1983). The microbially mediated incorporation of sulphate-S into organic matter has been well documented (Freney 1967; Scott, 1985; Strickland and Fitzgerald, 1985).

It was also considered important that the incorporation of S (or N and P) into an insoluble organic form increases the negative charge associated with the organic colloid fractions and organic cation exchange capacity (CEC). High soil CEC is one of the primary criteria determining soil productivity. In addition to serving as a possible S storage mechanism (Fitzgerald, 1978), the immobilization of sulphate-S may thus play an indirect but important role in soil nutrient status by increasing the retention of cations.

Changes in soil sulphate concentration in incubation studies have given information only on the ***net*** mineralization which had occurred over the period of the experiments (e.g. Freney and Spencer, 1960). Such information is of limited use in a proper understanding of mineralization and immobilization processes (Swift, 1983). Immobilization processes have been investigated by soil incubation under laboratory and glasshouse conditions in the presence and absence of plants and in field conditions through the use of ^{35}S labelled fertilizer sulphate (Gregg, 1976; Saggar, 1980; Ghani *et al.*, 1988). This has enabled investigators to simultaneously monitor the opposing processes (mineralization and immobilization) and provide a more meaningful interpretation of the fate of applied S in its cycling process.

There are two major pathways for the transformation of S from available to organic forms in a soil. Incorporation of available S is either by direct immobilization into microbial organic residues or via plant uptake, where the sulphate is reduced to organic S compounds and plant residues (or subsequent animal residues) and eventually incorporated into soil organic matter. Freney and Stevenson (1966), supported by the data from Scharpenseel and Krausse (1963),

considered that the major pathway was through plants, as ^{35}S tracer evidence showed only limited direct incorporation of S into humic acid occurred but considerable incorporation occurred after the plant residues were allowed to decompose in the soil.

The soluble S in soil if not taken up by plants or lost by leaching, can be immobilized into the soil organic fraction by soil microbial processes. These processes occur very rapidly (Freney *et al.*, 1971; Fitzgerald *et al.*, 1983). All other conditions being equal, the rate at which this occurs depends on the availability of carbon substrates.

Many investigators have used ^{35}S labelled sulphate in short term (ranging from one week to ten weeks) incubation studies in the presence of carbon rich residues. (Freney *et al.*,1971 and 1975; McLaren and Swift, 1977; Goh and Tsuji 1979; Saggar *et al.*, 1981b; Fitzgerald *et al.*, 1982; Schindler *et al.*, 1986; Ghani *et al.*, 1988). In general these studies showed that sulphate-S was rapidly incorporated into organic S. The rapid incorporation of ^{35}S into organic S was also observed in forest soils (Strickland *et al.*, 1987; David and Mitchell, 1987) and showed that incorporation rates of ^{35}S into organic S were high with soils having higher contents of carbon-bonded S. Larger amounts were incorporated into carbon-bonded S in the organic horizon whereas in mineral horizons, most was found as adsorbed sulphate (David and Mitchell, 1987; Schindler and Mitchell, 1987). Ghani *et al.* (1988) showed that, initially, the sulphate-S was rapidly incorporated into both carbon bonded and HI-reducible S within 7-15 days and thereafter the amount incorporated increased slowly and levelled off reaching an equilibrium state or even decreasing depending upon the particular S fraction concerned.

The amount of sulphate-S immobilized therefore varies considerably and can range from 0 to 100% depending on soil and litter characteristics and soil depths, the concentration of $SO_4^=$ added, the presence and absence of plants or a readily utilizable carbon source and environmental conditions.

For example, Goh and Tsuji (1979) and Tsuji and Goh (1979) added ^{35}S labelled gypsum at the rate of 45 kg S ha^{-1} to three soils of different S status and the soils were incubated for 70 days. It was found that about 50% of the added sulphate was incorporated during 70 days. Most of the incorporation occurred during 0-28 days in all soils. The amount Incorporated varied amongst the different soils from 20% to 50%.

Immobilization may be affected by the presence of growing plants (Freney and Spencer, 1960; Maynard *et al.*, 1985). Freney and Spencer (1960), using a simple balance sheet method In studying sulphate changes in the presence and absence of plants, showed that Immobilization

did not occur when low levels (4-36 ppm) of sulphate-S were added to S deficient soils even in the presence of plants, but at a higher level, 108 ppm, even in the absence of plants, sulphate was immobilized to soil organic S. This illustrated the significance of plants in the cycle of S in soils. Similar results were also obtained by Goh and Tsuji (1979) in their incubation of soils with ^{35}S labelled gypsum.

The presence of readily utilizable carbon sources causes rapid increases in the amount of sulphate-S immobilized. This has been also shown by Freney *et al.*, 1971; Saggar *et al.*, 1981b; Ghani *et al.*, 1988, 1991; Swift, 1983. This was firstly demonstrated by Freney *et al.* (1971) in their study of the fate of ^{35}S sulphate added to soil and who found that during 168 days of incubation about 50% was incorporated into soil organic matter. The addition of glucose increased the incorporation to 82%. The majority of the incorporated ^{35}S sulphate appeared in the HI-reducible S fraction. Similar results were also obtained by McLaren *et al.* (1985), Maynard *et al.* (1985) and Schindler *et al.* (1986); Strickland and Fitzgerald (1987). The concentration of S in the added material also affects the immobilization process. Barrow (1960c) showed that immobilization of soil sulphate will occur after adding organic matter having a C:S ratio greater than 400 to soil. Organic residues with less than 0.15% S also stimulated immobilization (Stewart *et al.*, 1966a, 1966b).

Saggar *et al.* (1981b) also observed large increases in the incorporation of sulphate ^{35}S into organic S during incubation studies. The immobilized products were subsequently fractionated using 0.1 M NaOH-$Na_4P_2O_7$ extraction-separation techniques. It was found that during a short period (64 days) most of the incorporated ^{35}S (45-70%) was in the fulvic acid A (ester sulphate) fraction. These authors suggested that the organic S compound synthesized during the short periods are relatively mobile and contain significant amounts of microbial metabolites and relatively young materials. Fulvic acids are characteristically lower in molecular weight, but over long periods, these labile compounds were transformed into relatively resistant fractions such as humic acid A and B and humins (Saggar *et al.*, 1981b).

Apart from the incubation studies described above, little progress has been made in describing the long-term potential rates of sulphate immobilization in field soils under field conditions. This rate depends upon the rate and quality of carbon inputs into the soil. Factors affecting these long-term rates of organic S accumulation in soils, such as climatic, soil characteristics, plant species and addition of fertilizers are evident from periodic analysis of soil S content in the pastoral soil studies of Jackman (1964a, 1964b). From incubation studies we are lead to believe that the concentration of sulphate in soil solution and carbon are important in determining the extent of S mineralization and the nature of the S immobilized; i.e. C-S or C-O-

S. It is likely in field soils, however, that the availability of sulphate has a more direct effect on the rate of carbon input. For example, in the case of grazed pastures the pasture plant is the primary source of carbon entering the soil, either as plant litter and root plus their exudates, or, indirectly as dung from the grazing animal. The growth of the plant therefore and the rate of carbon fixation and organic S synthesis are limited in part by sulphate availability in soils. It is perhaps time to consider the plant as the primary origin of nutrient immobilization and the micro-organisms and soil animals as the main origin of mineralization. The fact that laboratory incubations have focused attention on the size of microbial population and carbon and sulphate supply as the most important factors influencing the extent of immobilization may be an artefact from studying the incomplete organic cycle. More S must be immobilized during periods of plant growth than after its decay. Not only is there more carbon in the living plant than its residues or the microbes growing on it, but the C:S ratio in plants is usually wider (>200) than in micro-organisms (<50) (Barrow, 1960c; Saggar *et al.*, 1981a; Chapman, 1987a, 1987b). In other words, the rate of immobilization is governed by the potential of plants to fix carbon. The C:S ratio or S content of the residues will, however, influence the speed at which mineralization occurs and the final form of the S immobilized .

It is clear that more detailed study of S forms in field soils, particularly around plant litter, roots and dung patches is required before we have a full understanding of these processes.

2.4 PLANT REQUIREMENTS

Sulphur is required by plants mainly for the synthesis of certain essential amino acids and proteins, a component of enzymes involved in photosynthesis and production of vitamins. Sulphide is a particularly important component of nitrogenase enzymes which catalyse nitrogen fixation of *Rhizobium* species (Lehninger, 1982), Flavours, odours and toxic agents in some plants can be linked to a range of S containing compounds. Sulphur is also important in improving the uniformity and quality of vegetables (Wainwright, 1984; Schnug, 1990). Details of the significance of sulphur in plant nutrition and forage quality and ruminant nutrition have been reviewed by Duke and Reiseneaur (1986) and Tisdale (1977).

A crop requirement for a specific nutrition is commonly defined as "the minimum content of that nutrient associated with maximum yield or the minimum rate of intake of the nutrient associated with the maximum growth rate (Spencer, 1975). However, in practice, the term requirement can be expressed in a number of ways, for example for a pasture, the amount of nutrient required for desirable pasture establishment, or, the long-term survival of perennial pastures, or, the maintenance of appropriate levels of yield, or, the production of suitable quality forage, and lastly, to sustain the fixation of maximum amounts of nitrogen in legume based pastures (Smith and Siregar, 1983). Feed quality or the percentage of S containing amino acids of pastures may be important in wool production because wool contains about 3.5 to 5% S, mainly as cysteine.

In general, plant S requirements can be divided into two parts: (a) *internal requirement*, which refers to the relationship between the concentration of elements in the plant and plant growth; (b) *external requirements*, a relationship between the concentration of an element in the soil or culture solution and plant growth. Within these two requirements, a concept of 'critical' value, (a minimum amount of nutrients per unit of soil or plant associated with 80-90% of maximum plant growth) has been established by many workers. These 'critical' values are the basis for ranking soil tests and plant analyses.

In recent years, interest in assessing plant S requirements has increased considerably because deficiencies of S have been reported with increasing frequency throughout the World (Walker and Gregg, 1975; Blair, 1979; Fox *et al.*, 1983; Tisdale *et al.*, 1986; Chaiwanakupt *et al.*, 1987; Syers and Curtin, 1987; Murphy and Borgan, 1988). The increase in S deficiencies may be generally classified into two categories: (1) decreased accretion and/or (2) increased depletion of soil reserves. The former may include the increased use of low S containing fertilizers and pesticides and less S input from atmospheric sources (Syers and Curtin, 1987).

The latter includes removal resulting from increases in crop yield, leaching and erosion losses. In addition, environmental and site factors may influence deficiency and sufficiency. These are distances from the sea or industry, temperature, crops and crop management, and inherent soil S status.

2.4.1 Assessing soil sulphur availability

Assessing plant S requirements for nutrients can be achieved by soil testing, glasshouse trials, plant analyses, field fertilizer trials and to a lesser extent by biological testing and visual deficiency symptoms (Reisenauer *et al.*, 1973; Reisenauer, 1975). The technique employed will depend on the resources available and state of knowledge concerning a particular combination of crops and soils and the nature of crop response to S.

Attempts have been made to establish environmental factors [e.g. distance from the sea and S content in the rainfall (Walker and Gregg, 1975; Ledgard and Upsdell, 1991), irrigation water, pH and S responses] for identifying S deficiency (Blair, 1979; Sinclair and Saunders, 1984). More data, however, are needed on the influence of these factors on the cycling of S before meaningful relationships can be developed to predict S deficiency which are not site specific.

Currently, both soil test and plant analyses remain the most common techniques being used to monitor the changes in soil and plant status occurring in the soil/plant environment as a result of applied cultural practices. Soil tests are a relative index of the fertility status of the soil, while plant analyses provide a means of determining whether the soil plus cultural practices are providing the plant with sufficient nutrient.

In general, data for assessing plant fertilizer requirements can be derived from either glasshouse or field fertilizer trials. Essentially, field trials using a range of S application rates remain the most reliable technique for assessing plant requirements although the cost of trial operation is rather high. Glasshouse trials, although a relatively low cost method, have many limitations (Smith and Siregar, 1983; Jones, 1986). The most serious of these limitations is that glasshouse trials often assess only the topsoil. In contrast, in the field trial some crops may be able to utilise the subsoil sulphate more effectively. Besides this, the low soil volume for the plant root system and the favourable moisture and temperature regimes maintained during the trial period tend to accentuate the deficiencies and thus overestimate the apparent S requirements. Alternatively, the preparation of some soils for potting can also increase the mineralization (Williams and Steinbergs, 1964; Sparling *et al.*, 1985) of soil organic S and thus mask the symptom of S deficiency. Therefore, whenever possible, it is desirable that the

results from pot trials be confirmed under field conditions for their reliability in making fertilizer recommendations (Asher *et al.*, 1983). However both pot and field trials are site specific. Careful consideration is needed in extrapolating results to other environments.

2.4.1.1 Plant analyses

Internal requirements are most commonly assessed by plant tissue analyses and to some lesser extent by visual symptoms of plant parts. Evaluation and interpretation, including factors affecting critical values in plant analyses for S have been reviewed by Dijkshoorn *et al.*, 1960; Eaton, 1966; Dijkshoorn and van Wijk, 1967; Bates, 1971; Metson, 1973; Jones, 1975; Andrew, 1975; Spencer, 1975; Smith and Siragar, 1983. A more detailed discussion of this area is not within the scope of this review.

In general, plant analyses used for diagnosing the S requirement of crops have included mostly determination of the following indices: total S, sulphate-S, the ratio of total N to total S and of HI-reducible S to total S (Metson, 1973; Spencer *et al.*, 1977a, 1977b; Maynard *et al.*, 1983b). The most commonly used indices are total S, N:S ratio and extractable S.

Another approach towards interpreting plant analyses is the use of DRIS (diagnostic and recommendation integrating system). The ratios of S and other nutrients are calibrated according to DRIS of Beaufils (1973). This method has been successful in diagnosing plant requirements for other nutrients but has not been tested extensively with S. Recently, Sinclair and Jones (1991) have shown that DRIS appears to hold promise as a tool for the interpretation of chemical analyses of clover samples from clover-based pastures in New Zealand. However, the authors considered that DRIS requires high quality data in terms of both the measurement of dry matter yield responses and chemical analysis of clover samples.

It is essential that proper evaluation and interpretation of plant test results take into account the crop environment. Crop environments include: the plant itself (species, age, parts analysed), environmental conditions (deficiency or toxicities of other elements, drought, temperature, insects, grazing animal, disease, plant defoliation and type of crop culture, i.e., field vs. potted soil vs. nutrient solution) and sample preparations (Jones, 1975; Spencer *et al.*, 1977a, 1977b; Smith and Serigar, 1983). For example, lower critical values have been often obtained in pot experiments than in the field (Jones, 1975; Smith and Siregar, 1983).

Evidence for the need to identify a target plant species for indicating limited soil **S** supplies was provided in a field investigation by Walker (1957) and Walker and Adams (1958). It was

suggested that the S status of clovers revealed the S requirement of the pastures better than that obtained by grasses. This is particularly relevant to legume based pastures where good legume vigour is required for nitrogen input.

The stage of plant growth can markedly influence S concentrations in plant tissue (McNaught and Christoffels, 1961; McNaught, 1970; Spencer *et al.*, 1977a, 1977b; Cornforth *et al.*, 1983). Such effects may make it difficult to interpret analytical data unless the approximate age of a plant is known.

Sulphur content may vary within plant parts (Jones *et al.*, 1972b; Spencer *et al.*, 1977a, 1977b). For instance, Jones *et al.* (1972b) reported the critical values of 0.23 and 0.08%, respectively, in the leaves and stems of Bur clover.

The deficiency of other elements may influence the magnitude of the critical values of plant S indices. For example, the correction of phosphorus and nitrogen deficiencies has been shown to lower the critical values of total S and sulphate-S in clover plants (Bouma *et al.*, 1969; Jones *et al.*, 1970; Spencer *et al.*, 1977a, 1977b; Cornforth *et al.*, 1983).

Interpretation of plant analysis is based on a 'standard value', 'critical level', or 'sufficiency level'. There are numerous sources for most of these values for a wide range of crops; e.g. Eaton (1966); Metson (1973); Maynard *et al.,* (1983b); Sinclair and Saunders (1984); Martin-Prevel and Gauther (1986); Reuter and Robinson (1986). To be reliable, it is essential that the material sampled from the field for plant analysis matches material used for the original derivation of the critical concentration. It is this aspect where plant tissue analysis is most commonly misused (Asher *et al.*, 1983; Blair, 1979).

It was suggested (Blair, 1979) that the tabulation of data, showing critical levels without specifying the methods of analysis and determination, is of little value for setting levels of adequacy. Errors in analytical procedures must be overcome before use of such indices can be made (Blair, 1979).

From personal experience gained in analysing herbage samples from the Wageningen International Exchange Programme, it is clear that there are large variations in laboratory results from different countries. This supports the above statements of Blair (1979) which reflects the need for standardized laboratory techniques before establishing 'critical', 'sufficiency' or 'standard' levels.

2.4.1.2 Soil analyses

Methods in soil S analyses and evaluation and interpretation of results have been reviewed by a number of authors e.g., Beaton *et al.*, (1968); Reisenauer *et al.*, (1973); Andrew (1975); Reisenauer (1975); Tabatabai (1982); Landers *et al.* (1983); Randall and Sakai (1983); Blanchar (1986).

Soils contain S in forms that range in their availability to plants; solution sulphate, adsorbed sulphate and organic S, which varies in lability from easily decomposed to extremely recalcitrant. It is probable that the lability of soil organic S occupies a continuous spectrum. However in soils it has been convenient to partition S into two pools; an active cycling pool and inert pool (Till and May; 1970a, 1971; Goh and Gregg, 1982a, 1982b; McLaren *et al.*, 1985). It is probable that much of the active pool consists of living organic matter such as micro-organisms and plant roots and litter. The size of the active pool can be quantified by using $^{35}SO_4^=$ as a radiotracer but it is not widely employed probably due to its technical complexity and high cost. Additionally the size of the cycling S pool may not relate to S availability.

The immediately-available pool, includes soil sulphate-S which is believed to be the main form taken up by plants and is frequently measured in assessing S requirements for plants because of its simplicity of measurement and low operation cost. Most soil testing procedures measure only this immediate sulphate pool. However the sulphate pool may represent only 20% or less of the S that becomes plant available in a growing season and it has been recognized that levels of soil sulphate are not always well correlated to crop yields (this is described in more detail in this Section).

Soil testing involves sampling, analysis and correlation of analyses with crop yield. The immediate goal of soil testing is to predict the likely crop growth response to S fertilizer in field situations. In the case of S nutrition, soil parameters such as total S and extractable S, have sometimes been successfully correlated with yield responses. However, most researchers have examined the value of extractable S (soluble sulphate and adsorbed sulphate) as a predictor of soil S status and plant growth responses to fertilizer application.

2.4.1.2.1 Depth of sampling and sample preparations

The amount of nutrient extracted may be influenced by the method of soil sampling and soil depth, soil preparation, soil-to-extractant ratio, extraction time and technique.

Depth of soil sampling may alter the relevance of a soil test result. Very acid soils may contain toxic concentrations of aluminium which limit root development and sulphate availability to plants (Metson, 1979a). However, there is evidence that appreciable amounts of S are obtained from subsoils (Bardsley and Kilmer, 1963; Blakemore *et al.*, 1969; Rorison, 1973; Gregg *et al.*, 1977; Risk and Boswell, 1988). Where soil properties do not limit root growth it can be advocated that soil samples should include these soil depths.

By using ^{35}S in their field studies, Gregg *et al.* (1977) showed uptake of subsoil S by pasture species. In a sandy soil and silt loam these authors calculated relative root activity using ^{35}S carrier-free isotope. Relative to 7.5 and 22.5 cm depths, pasture plants obtained 24% and 11%, respectively, of their S from subsoil below 22.5 cm depth in spring evaluation. The extent of the uptake zone reflected the depth of pasture rooting. In other experiments they showed S uptake occurred from greater depths (100 cm) in a sandy loam than in a silt loam (60 cm). However, like other authors using isotopically labelled nutrients to measure relative root activity, Gregg *et al.* (1977) failed to account for the fact that mineralization/turnover rates of native organic S and therefore the sulphate pool turnover rate and isotopic dilution would decrease with soil depth. Thus lower relative percentages of plant S were derived from lower depths than estimated by these authors. Saunders and Cooper (1975) and Nguyen *et al.* (1989a, 1989b) found no advantage in sampling to 15 cm relative to the normal 7.5 cm. Hoeft *et al.* (1973) and Westerman (1974) also reported no improvement in prediction of S response for pasture or for deep-rooted crops such as lucerne with the inclusion of soil samples below a 30 cm depth. The importance of subsoil S in plant nutrition appears to vary with climate, plant species and soil type. Thus no general rule can be applied to the importance of soil testing depth which must be evaluated depending upon local knowledge of soils, climate and crops.

Time of soil sampling has been shown to have a significant effect on the amount of extractable S. Ghani *et al.* (1990) showed that sulphate S concentration fluctuated from values as low as 1 mg S kg soil to 8 mg S kg soil during a one year study on Wakanui silt loam soil at Lincoln, Canterbury, New Zealand. In particular, they found that sulphate concentrations appeared to be decreased by substantial rainfall events which occurred a week before sampling. However, when rainfall was sufficient to cause sulphate leaching, occurred more than 1 week before sampling, and subsequent warm temperature facilitated S mineralization and returned sulphate-S concentrations to pre-rainfall events.

There is conflicting evidence with regard to the effect of air drying on the amount of extractable soil sulphate. Williams and Steinbergs (1964), Tabatabai and Bremner (1972a) and Searle and Sparling (1987) found that air-drying had no effect on the amount of extractable S. In

contrast, some researchers had reported increases in the amounts of extractable sulphate after drying soil (Freney, 1958; Williams and Steinbergs, 1959; Barrow, 1960c; Williams 1967; Kowalanko and Lowe, 1975). Oven drying of soils at high temperature (105 ^{o}C) markedly increased the amount of extractable S (Barrow, 1961a; Peverill *et al.*, 1975; David *et al.,* 1982). These increases have been attributed to the mineralization of soil organic S by microorganisms (Chaudhry and Cornfield, 1967b) and the physical breakdown of complex organic S into simple organic compounds and sulphate (Barrow, 1961a; Kowalenko and Lowe, 1975). David *et al.* (1982) found that drying soil increased the amount of extractable S only in the organic horizon soils. In some mineral horizons, a decrease in the amount of extractable S was found and this was attributed to the lower extractability of sulphate due to drying and possibly the crystallization of Fe and Al sesquioxides in B horizons which may have caused sulphate to be held in an unextractable form.

Storage of soil samples may have some effect on amount of extractable S. Searle and Sparling (1987) showed that the storage of moist or air-dried soil samples for up to 84 weeks resulted in considerable increases in the amount of sulphate. Probert and Jones (1977), in contrast, found no effect of soil storage.

In summary, to classify soil S availability a soil sampling depth appropriate to soil, crop and climatic conditions should be chosen and soil preparation and storage techniques should be standardized.

2.4.1.2.2 Extractants

The immediately available pool of S is believed to consist of sulphate ions in the soil solution plus adsorbed sulphate which is in exchange equilibrium with sulphate in solution. Apart from the addition of $SO_4^=$ through fertilizers, animal excretion, rainfall and pesticides, this immediately available pool is also generated through the mineralization of soil organic S (see Section 2.3.1).

A large number of methods have been proposed to estimate the size of the immediately available soil sulphate pool (Beaton *et al.*, 1966; Reisenauer *et al.,* 1973; Reisenauer, 1975; Tabatabai, 1982). For routine soil analysis most authors have resorted to simple chemical extractants involving anions that exchange with sulphate adsorbed to soil surfaces (Searle and Speir, 1988). The choice of chemical extractant is very much dependent on the properties of the soil to be analysed. Extractants can be divided into three groups:

1. Those removing readily soluble sulphate-S;
2. Those removing readily soluble plus portions of adsorbed sulphate-S;
3. Those removing readily soluble and adsorbed sulphate, plus portions of labile organic S.

a. Readily soluble sulphate-S

When the amount of adsorbed sulphate is negligible, an extractant which employs an anion that is not adsorbed strongly on the soil sulphate sorption sites, such as chloride (as either 0.01 M $CaCl_2$ or LiCl) is adequate (Williams and Steinbergs, 1959, 1962; and Bettany *et al.*, 1974). Calcium chloride has an advantage; not only are clear solutions easily obtained but extraction of organic S which occurs with water extraction is prevented (Fox *et al.*, 1964). Recent work by Bolan *et al.* (1991, personal communication, N.S. Bolan) suggested that $CaCl_2$ extraction of soils with low Ca exchange capacity may increase the surface positive charge on these soils resulting in slightly increased sulphate sorption. Amounts of sulphate in $CaCl_2$ extracts were found to correlate well with the amounts of S mineralized during soil incubations (Barrow, 1961a; Kowalenko and Lowe, 1975; Tsuji and Goh, 1979;), but correlate less well to plant uptake (Tsuji and Goh, 1975) and yield response in field conditions (Nguyen, 1982) where soils were $SO_4^=$ retentive.

b. Adsorbed plus soluble sulphate-S

Extraction of adsorbed sulphate requires the use of solutions containing anions such as OH^-, $H_2PO_4^-$ or HCO_3^- which can effectively compete with, and displace, sulphate from positively charged sorption sites on the soil surfaces. A disadvantage of using OH^- and HCO_3^- solutions is that large amounts of organic matter are extracted and this can cause interferences when sulphate is determined turbidimetrically or the organic S extracted gives rise to overestimation of sulphate values when it is determined by the reduction-distillation method. In this regard, $Ca(H_2PO_4)_2$ seems to be one of the best extractants, because phosphate ions displace the adsorbed sulphate, and Ca ions flocculate the soil colloids and clear extracts are readily obtained. The calcium ion also limits the extraction of organic matter (Ensminger 1954; Hesse, 1957; Fox *et al.*, 1964; Barrow, 1967a; Searle, 1979; and Tabatabai, 1982). Significant relationships between phosphate extractable S and plant S uptake have been reported for a wide range of soils (Fox *et al.*, 1964; Barrow, 1969a; Jones *et al.*, 1972a; Hoeft *et al.*, 1973; Tsuji and Goh, 1979; Saunders *et al.*,1988).

c. Readily available and adsorbed sulphate and portions of organic S

The extractants that remove readily available and adsorbed sulphate and significant portions of organic S include acetic acid-phosphate (Cooper, 1968; Hoeft *et al.*, 1973; Saunders and Cooper, 1975), $NaHCO_3$ at pH 8.5 (Bardsley and Kilmer, 1963) and 0.25 M KCl at 40 ^oC (Blair *et al.*, 1991). These extractants are not widely employed by researchers involved in relating soil S availability to S responses.

Regardless of the particular extractant used in assessing plant-available S, there is still little known about the chemical nature of the organic S in soil extracts (Beaton *et al.*, 1968) and because only empirical relationships between soil test values and crop yields or response are determined, there is no causal evidence to suggest that the forms of organic S extracted bear any relation to that part of the organic pool which supplies S to the growing plants (Hoque *et al.*, 1987).

d. Other methods

Apart from chemical extractants, anion exchange resin beads and anion exchange membrane have been employed in extracting soil sulphate-S or even soil phosphate (Kurmarohita, 1973; Searle and Speir 1988; Saggar *et al.*, 1991). Amounts of anion membrane extractable $SO_4^=$ agree well with those obtained by 0.01 M $Ca(H_2PO_4)$ extraction (Searle and Speir, 1988) . Its simplicity is of particular interest. The extraction provides a non-contaminating, non-destructive method to extract the soil sulphate without apparently affecting the microbial biomass, hence its application in S transformation studies may be more worthwhile. For example, resins were employed by Hedley and Stewart. (1982) to measure labile soil P prior to measuring microbial P in a soil P fractionation procedure.

Hoque *et al.*(1987) suggested that certain microbial assays could give meaningful indications of S mineralization rate. Microbial bioassay test values were significantly correlated with pasture response to applied S but it was concluded that much more research is needed to evaluate this approach to soil testing.

e. Use of radioactive ^{35}S

Although not suitable for routine soil analysis, there have been a few attempts to measure the pools of S available to plants by isotopic dilution techniques. Many of these studies were carried out in laboratory or glasshouse conditions (Nearpass *et al.*, 1961; Harward *et al.*, 1962;

Bettany *et al.*, 1974; Probert, 1976; Tsuji and Goh, 1979). Generally, 'A', 'E' and 'L' values (see review by IAEA, 1976; Vose, 1980 and Manzel and Smith, 1984; for definition of 'A', 'E' and 'L') have been correlated well with plant S uptake, growth response and other estimates of plant available S. The 'E' and 'L' value, which are estimates of the exchangeable pool of S in soil, normally increase with increasing applied S and time. Gregg (1976), however, failed to correlate the 'A' value and pasture yield response in field studies in New Zealand soils.

By means of radioactive studies, Till and co-workers (Till and May, 1970a, 1970b, 1971) and Goh and Gregg (1982a, 1982b) were able to determine the size of the S 'cycling pool' ('active' and 'inert' pool) in pasture soil. The 'active' cycling pool included S in entire living plants, S in residues, available soil S and S in animals. The 'inert' pool included S that does not enter the cycle (recalcitrant material, less mineralizable). The active cycling pools were variable among soils . However, further research is needed to verify the usefulness of radioisotopes in measuring cycling pools of nutrients in soils as an indication of soil S status.

2.4.1.2.3 Interpretation of soil tests

Strong correlations between soil tests using phosphate as an extractant and crop yield responses to applied S have been observed by investigators in both glasshouse and field trials (Spencer and Freney, 1960; Hoeft *et al.*, 1973; Probert and Jones, 1977; Lee and Speir, 1979; Saunder *et al.*, 1981; Scott, 1981; Lee *et al.*, 1985). Empirical relationships only exist between the amount of S extracted and plant yield or uptake in the glasshouse. As reviewed by Reisenauer *et al.* (1973), no one procedure has proved consistently superior in predicting responses to S fertilization. Whereas, results of other studies (Spencer and Glendinning, 1980; Cornforth *et al.*, 1983; Raymant, 1983; Jones, 1986; Hoque *et al.* 1987; Skinner, 1987 as quoted by Syers and Curtin, 1987; Vaughn, 1987; Nguyen and Rickard, 1988; Saunders *et al.*, 1988; Murphy, 1990 and Nguyen and Goh, 1991) have been far less encouraging. Apart from the need to ensure the soil sample taken reflects the plants effective rooting depth, the validity of empirical soil $SO_4^=$ tests for making S fertilization recommendations is limited due to the need to quantify several seasonally dependent sources of S input and loss which influence soil test values, particularly input of plant available S from mineralization of organic matter and S loss by leaching (Reisenauer, 1975; Saunders and Cooper, 1975; Spencer and Glendinning, 1980; Cornforth *et al.*, 1983), (see later discussion in Section 2.3.1 and 2.5.2.2).

In a temperate environment, like New Zealand with dramatic short-term changes in climate, Gregg (1976) suggested that adequate knowledge of the factors influencing the response patterns at each location along with a long term knowledge of fate of fertilizer S may be more

useful. In support of Gregg's statement, Cornforth *et al.*, (1983) have shown large fluctuations in soil test results in New Zealand soils. This variability was attributed to seasonal changes in leaching. It was suggested that soil sulphate test results cannot be used to predict S fertilizer requirements on their own. Interpretation depends on pasture age and fertilizer application history (which reflects the reserves of organic S in a soil), S leaching index and the season when the sample was taken. This test is best used to modify S fertilizer requirements estimated from a balance of S gained and losses from the system using a model by Sinclair and Saunders (1984), (see discussion in Section 2.4.2.1).

The limitations of various empirical methods of soil analysis have also been discussed by Reisenauer *et al.*, 1973; Reisenauer, 1975 and Oertli 1990. The authors again highlight the substantial difficulties in interpretation of such tests. One problem with these empirical tests is that they are not based on proven mechanisms of plant nutrition.

In conclusion it appears that results from empirical soil tests must be applied only to the type of soils or crops for which calibration of the method has been carefully tested (Oertli, 1990).

Oertli (1990) proposed new approaches in the use of soil tests in soil fertility management. Soils which react similarly to nutrients should be combined in classification units. For these units, detailed information on the reaction behaviour must be obtained. A few simple analyses might show how a specific field fits into this reaction pattern. It is difficult to see, however, how this 'new' approach differs to the current practice in New Zealand where clear patterns of growth and response to fertilizer have been established for different soil groups and types (During, 1984). In this context soil tests provide very useful information. Such information could probably be improved if one considered the question asked of soil tests. For example, for mobile nutrients like $SO_4^=$ does the test result indicate that during periods of plant growth the nutrient level will not meet plant demand. For perennial crops such as pastures it would seem logical to conduct a soil S test either immediately after or prior to the major spring-summer growth period. After is more suitable than before because climate effects on growth cannot be predicted. Adjustments to fertilizer application rate for the next year can be based on these soil test values. A limitation of this strategy is that dry summer soils are difficult to soil sample. Obviously, there are many factors other than soil test level that need to be considered in arriving at an accurate estimate of the S fertilizer requirement for a particular crop and soil type. Important among these are the effectiveness, cost and the availability of the fertilizer material.

2.4.2 Fertilizer S requirements

The amounts and types of S to be applied will depend on plant demand for S, the status of the plant environment; i.e. rate of mineralization of organic S, the contribution of S from subsoils, rooting habits, atmospheric and irrigation inputs, rainfall and leaching, temperature, solubility or availability of the fertilizer S, and interactions with other elements (Reisenauer, 1975; Asher *et al.*, 1983).

2.4.2.1 S recommendations for pastures in New Zealand

The practice for recommending S fertilizer for New Zealand pasture is discussed as an example of how soil testing procedures can provide information to assist fertilizer recommendation. The estimation of fertilizer S requirements for pastures in New Zealand are at present, based on the mass balance nutrient cycling concept developed by Sinclair and Saunders (1984). The Computerized Fertilizer Advisory Schemes (CFAS) model (Sinclair and Saunders, 1984) which is an 'external model' as shown in Figure 2.2 takes account of external inputs such as atmospheric S and losses by leaching and through animal products. The estimate of leaching loss depends on annual rainfall and the soil drainage (free or slow draining) and anion retention characteristics. Animal losses are estimated from product removal from the farm and the uneven return of nutrients in dung and urine to soil surface. A negative balance requires an input of S from fertilizer. The size of fertilizer input can be modified by considering the ability of soil to provide S from organic S mineralization and current available status. This ability is estimated as an empirical index calculated from pasture age and previous soil productivity (i.e. animal stocking rate), and the current amount of phosphate extractable S (0.01 M $Ca(H_2PO_4)_2$ at pH 4.0, 1:5 soil solution ratio, and 30 minutes extraction time of samples from the top 7.5 cm of pasture soil). Based on this model, fertilizer S required for maintenance of pasture production for the North Island, New Zealand, ranged from 13 to 28 kg ha^{-1} (Sinclair *et al.*, 1985)

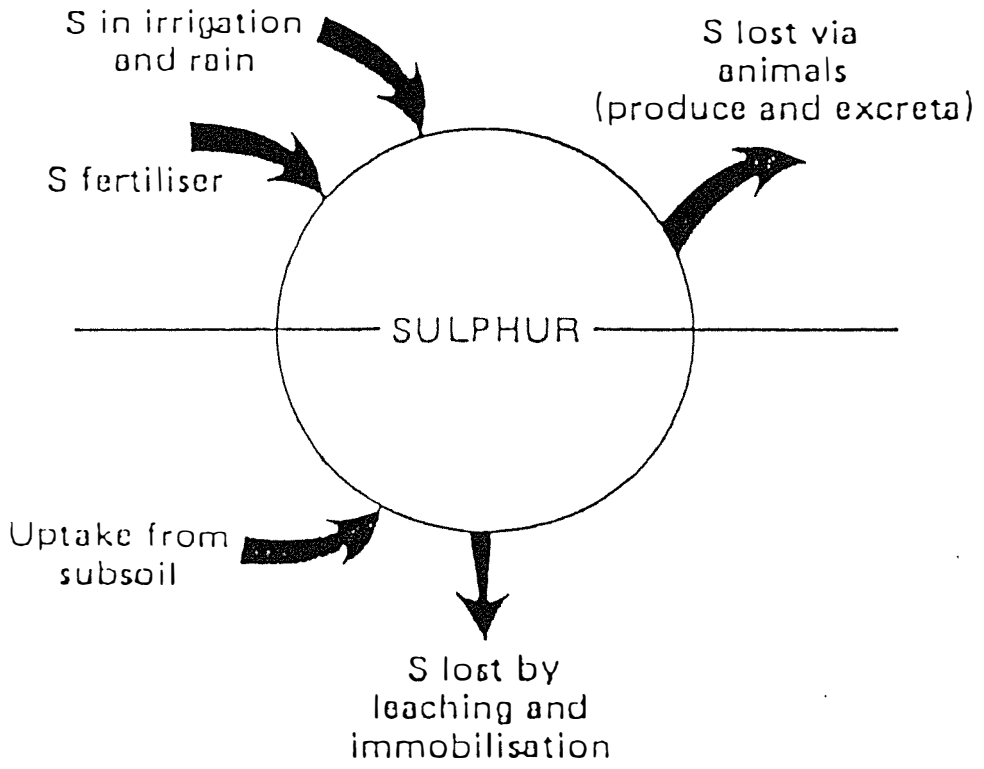


Figure 2.2 Simplified sulphur cycle showing gains and losses in the CFAS model (Sinclair and Saunders, 1984).

2.4.2.2 Form of fertilizers

Fertilizer S forms have been reviewed by Beaton (1987) and Higgins (1987). These major S fertilizers include either sulphate-S and/or S^0 in various mixtures and compounds in the nitrogen and phosphate fertilizers (Table 2.1).

In New Zealand, single superphosphate, applied at quantities adequate to overcome the widespread P deficiencies in legume based pastures has been the traditional S fertilizer for over 100 years and has simultaneously eliminated S deficiency in most situations (Walker, 1957; Walker, 1968; Walker and Gregg, 1975).

However, superphosphate may not supply adequate S to pasture plants on soils with low S retention and high rainfall (During, 1984). A slow release fertilizer such as S^0 is needed. This was achieved by adding S^0 to superphosphate (see later in this Section).

With recent increases in costs of fertilizers and their transport and application, there has been, in New Zealand pastoral farming, an increased interest in S-free fertilizers, such as ammonium phosphates, triple superphosphate, reactive rock phosphate and phosphate rocks partially acidulated with phosphoric acid. In the long term such fertilizers will require supplementation with some form of fertilizer S.

In New Zealand, alternative S fertilizers to superphosphate are primarily based on S^0 (Boswell, 1985). There are analytical advantages of adding S^0 to phosphate fertilizers. The S^0 added does not dilute the P content much and increases the total nutrient content (Rogers, 1985). S^0 has proven to be an effective S fertilizer of legume based pastures provided the correct particle size range is chosen with respect to a particular climatic zones (Sinclair *et al.*, 1985; Boswell and Swanney, 1986; Boswell *et al.*, 1988a, 1988b; Lee and Boswell, 1988; Swanney *et al.*, 1988; Chatupote, 1990 and Boswell and Swanney, 1991).

Various types of fertilizers containing S^0 which have been developed, manufactured and tested in New Zealand were described by Boswell (1985), Boswell and Swanney (1986), and Rogers (1985) and are listed in Table 2.1. These alternatives could be grouped into two groups: combined P and S fertilizers and high analysis S^0 fertilizers.

Table 2.1 Some standard and alternative S fertilizers in New Zealand (Boswell and Swanney, 1986).

Type	Content (%) S^0	$SO_4^=$	Total S	P
Standard S Fertilizer				
Single superphosphate (SSP)	-	11	11	9
Ammonium sulphate	-	24	24	-
Gypsum	-	18	18	-
Sulphurized SSP	9	9	18	9
Sulphurized SSP extra[1]	18	9	27	7
Screened 'agricultural' sulphur	100	-	100	
Alternative S^0 Fertilizer				
Sulphur/sodium bentonite	75-85	-	75-85	-
Sulphur/bentonite (imported)	90	-	90	-
Granulated ground S^0	90	-	90	-
Sulphur impregnated urea	20	-	20	-
'Thiovit'	85	-	85	-

[1], various S:P ratios are available as sulphurized SSP.

Of the combined S and P fertilizers, the sulphur super has two types depending on manufacturing methods and these are: molten mixed S^0 super (MMSS) and dry mixed screened S^0 into single superphosphate. In New Zealand, these have traditionally been used to apply more S to soils which are responsive to S but not P; e.g. brown-grey earths of Central Otago or areas where S leaching loss is severe (West Coast of the South Island, and yellow-brown pumice soils in the North Island, New Zealand). The molten mixed S^0 superphosphate has more finely divided S^0 particles than the dry mixed superphosphate (Sinclair and Boswell, 1983); e.g. up to 81% is less than 150 microns. In recent pasture trials (Boswell and Swanney, 1991) in a cool climate, the MMSS (27% S) has proved to be a more effective form of S fertilizer than screened agricultural S^0 probably because of the combination of readily available $SO_4^=$ and slower release S^0. Possibly finer particle size.

The S^0/sodium bentonite fertilizer was developed in New Zealand by the Department of Scientific and Industrial Research (DSIR) (Rothbaum *et al.,* 1980; Boswell *et al.,* 1988a, 1988b). The pelletized particles are hard, stable, largely dust-free, and more importantly, do not present an explosive hazard. On wetting the clay expands 14 times its volume and assists in fracture of the S^0 into fine particles (Boswell and Swanney, 1986). Commercial products are now being manufactured (Boswell *et al*., 1988a, 1988b) but tend to be more costly per unit S than MMSS.

2.4.2.3 Efficient use of fertilizer S

Increasing costs of farm inputs and variable prices of farm products make it essential that more efficient use be made of fertilizer added to pastoral systems. Fertilizer may account for more than 40% of an annual cost in pasture farming (Kane, 1983) in New Zealand. A cost which many indebted pastoral farmers cannot bear. Improved efficiency of use may be helpful in reducing long-term fertilizer costs.

Gregg (1976) and Sinclair and Saunders (1982) suggested the following practices in maximizing fertilizer efficiency:

1. Reducing the downward movement of S beyond the A horizon. A well-granulated sulphate fertilizer S is necessary in less S retentive soils and should be applied in a period of low rainfall. Superphosphate S applied in autumn to soil of high leaching index has a very low efficiency. The slow-release S^0 is more desirable where intense rainfall is expected soon after application
2. Form of fertilizers and time of application should be matched with climatic

condition. For example, in a dry and cool area a very finely divided S^0 form is preferable to coarser and slower release S^0.

3. Small frequent applications of sulphate-S are necessary. This may minimize a luxury uptake of S by plant which would otherwise inflate loss through animal transfer.

It would appear that various forms of S^0 are more effective than sulphate-S in decreasing the leaching loss of S. Gregg (1976) and Sinclair and Saunders (1984) did not consider the role of stimulating S immobilization into soil organic matter as a longer term route to improve the efficiency of S use although suggested by Gregg and Goh (1982a, 1982b) in considering the fate of fertilizer S added to plant and soils. Effective use of non-sulphate fertilizers (S^0) requires knowledge of their rate of conversion to plant available sulphate and factors affecting the conversion. At present, S^0 is becoming an increasingly popular fertilizer as described above. S^0 is an ideal slow-release fertilizer since its release rate can be readily controlled by adjustment of particle size (see Section 2.5.1 on S^0 oxidation). By manipulation of particle distribution of S fertilizer or S in combination, it becomes possible to tailor the sulphate release to meet crop needs under a given set of environmental conditions (Boswell and Swanney, 1988; Chatupote, 1990).

Research and practice towards the efficient use of fertilizer S in New Zealand are progressing. Recommendations for the application of sulphate and S^0 containing fertilizers according to such factor as climate and S leaching index have been made (Gregg, 1976; Sinclair and Saunders, 1984; Sinclair *et al.*, 1985; Boswell and Swanney, 1988). These recommendations are based on field fertilizer trials as mentioned above. Figure 2.3 summarizes current recommendation schemes for S^0 particle sizes in New Zealand pastoral soils.

2.5 THE FATE OF FERTILIZER SULPHUR IN SOIL

As discussed in the previous section, a number of fertilizers, exhibiting widely different physical and chemical forms, have been developed to correct S deficiency (Beaton, 1987; Higgins, 1987). They can be categorized into two distinct groups: those which are in chemical forms directly available for plant uptake (sulphate forms) and those that require prior oxidative conversion to sulphate (non-sulphate form), usually elemental S (S^0).

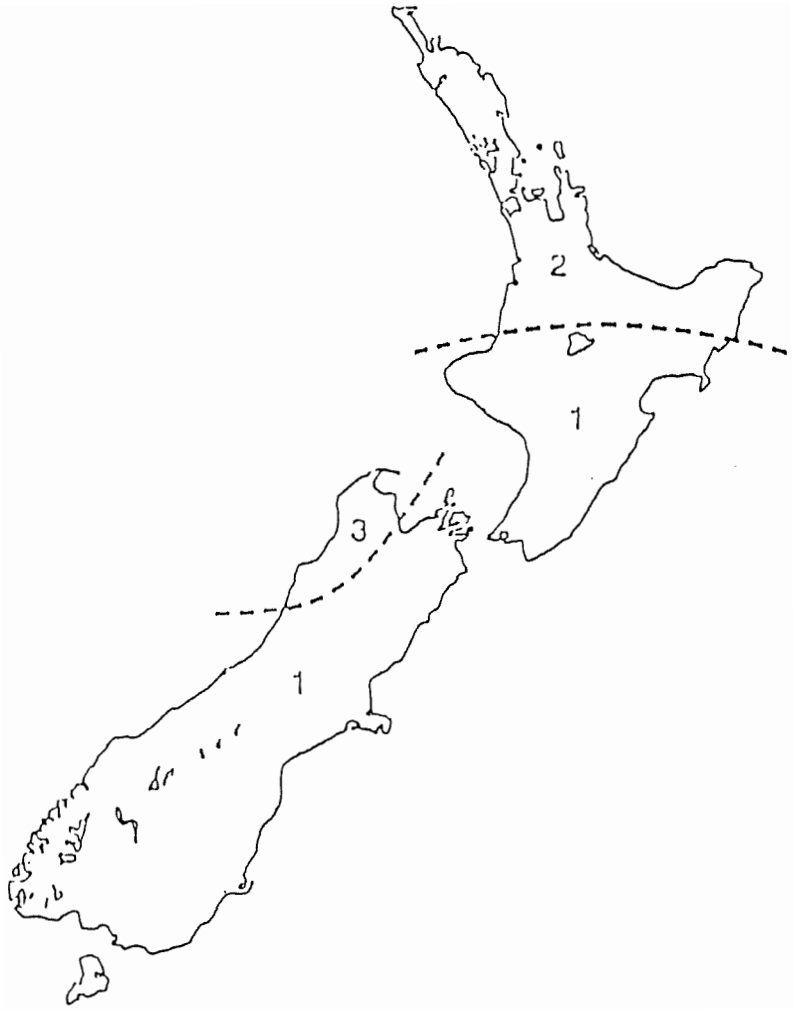
Current knowledge indicates that for any form of S fertilizer to be effective in plant nutrition, it must be converted into the sulphate form. Likewise many other fates of S fertilizer In soli

systems require that fertilizer S is first converted to sulphate. Prior to discussing the fate of S fertilizer in soil, the conversion of S^0 to sulphate will be discussed.

2.5.1 Oxidation of S^0 in soil

As mentioned above S^0 must be transformed to sulphate before being adsorbed by plant roots. Although some abiotic chemical oxidation may occur, biological oxidation is far more rapid at normal soil temperature and moisture (Alexander, 1977). Numbers and types of microorganisms that are responsible for S^0 oxidation are listed elsewhere (Lee, 1983; Wainwright, 1984; Konopka *et al.*, 1986). Species of the genus *Thiobacillus* have long been regarded as the most important S^0 oxidizers in soils (Wainwright, 1984). There are, however, other heterotrophic bacteria, fungi and actinomycetes that are thought to be important in S^0 oxidation in soils but their contribution in the S^0 oxidation is still unknown in many soils (Vitolin and Swaby, 1969) and have been overlooked by most researchers except for Germida and co-workers who found considerable heterotrophic S^0 oxidation in Canadian prairie soil (Grayston and Germida, 1990) and who have studied the influence of crop rhizospheres on the populations and activities of heterotrophic S^0 oxidizing micro-organisms.

Thiobacilli are chemolithotrophic bacteria and gain their energy from the oxidation of S^0. Several species occur in soils. The most important appear to be *T. thioxidans* which are active below pH 5 and *T. thioparus* which is dominant in the pH range 5-6.5 (Vitolin and Swaby, 1969). It is expected that the pH at the S^0 surface may well be lower than this restricting the activity of many heterotrophs thus making the chemolithotrophics important as far as fertilizer effectiveness is concerned where large concentrations of S^0 are present in small volumes of fertilized soil. A general discussion of pathways and mechanisms of S^0 oxidation has been reviewed (Trudinger, 1979; Wainwright, 1984 and Chatupote, 1990).



Recommended S^0 particle size ranges

Region	Annual application	Biennial application	Four-year application
		Size (µm)	
1	< 150	< 250 (50% <150)	< 500 (25% <150, 50%<250)
2	< 250	< 500 (50% <250)	< 1000 (25% <250, 50%<500)
3	< 250	< 500 (50% <250)	< 1000 (25% <250, 50%<500)

Figure 2.3 Map of New Zealand showing broad climate zones which affect recommended fertilizer S^0 particle size ranges (Boswell and Swanney, 1988).

The rate of this process depends on the interaction of three types of factors namely: (a) the substrate, i.e. the S; (b) microorganisms and (c) their environment (Weir,1975; Wainwright; 1978). The substrate factors include S^0 surface area (particle size) and the environmental factors include soil type and properties, soil pH, temperature, soil moisture and aeration, rate and placement, microbial population and inoculation and nutrient supply (including fertilizer grade). Favorable conditions for S^0 oxidation in soils (Lee, 1983; Janzen and Bettany, 1987a, 1987b; Goh, 1988, Lee *et al.,* 1988a, 1988b) are those typical of most microbially mediated processes:.

1. Moderate temperature (23-30 ^oC). The rate increases as the temperature increases. Oxidation can still proceed at 4 ^oC and 55 ^oC but is very slow.
2. Soil moisture should be near field capacity. However, at the field capacity in some soils S^0 oxidation may be restricted by O_2 supply (Moser and Olson, 1953).
3. Oxidation rate is not critically limited by soil pH as it can occur between pH 2-9. Addition of lime or basic phosphates to acid soil generally increases the oxidation rate (Chatupote, 1990).
4. High numbers of S^0-oxidizing organisms are present in soils. Inoculation of soils with S^0-oxidizing organisms increases the oxidation rate only in soils with a low population of these organisms. Addition of S^0 stimulates the rapid growth of chemolithrotrophic oxidizing organisms in most soils.
5. Most importantly, S^0 is finely divided. Surface area which is determined largely by particle size, is the most important characteristic affecting the rate of oxidation of S^0. Oxidation increases systematically as surface area increases.

A wide range and type of oxidation rates have been reported (Li and Caldwell, 1966; Attoe and Olson, 1966; Shedley, 1982; Janzen and Bettany, 1987a, 1987b). Since S^0 oxidation is exclusively a surficial reaction, many authors (Janzen and Bettany, 1987a, 1987b; McCaskill and Blair, 1987; Watkinson, 1988, 1989) have found that oxidation rates appear to be essentially constant per unit S^0 surface area (termed specific oxidation rate, K, $\mu g\ cm^{-2}\ day^{-1}$) for constant environmental conditions. If the oxidation rate is expressed as μg S (S oxidized) cm^{-2} (surface area of S^0 exposed to oxidation) day^{-1}, this expression is thus useful for comparing the oxidative ability of different soils under different conditions.

Various methods for measuring the rate of S^0 oxidation in soil have been used, for example, measuring the decrease in soil pH (Li and Caldwell, 1966), the increase in extractable sulphate (Li and Caldwell, 1966; Janzen and Bettany, 1987a, 1987b; Chapman,1989) and the decrease in residual S^0 (Barrow, 1968; Lee *et al.*, 1987; Watkinson *et al.*, 1987; Lee and Boswell, 1988;

Chatupote, 1990). Since some portion of sulphate produced during the incubations may be rapidly converted into organic forms (Freney *et al.*, 1971) and soils vary in their ability to buffer pH change, a measure of residual S^0 is the most appropriate method for a wide range of soils. This can be achieved with extraction using solvents such as toluene, chloroform and acetone (Barrow,1968; Watkinson *et al.*, 1987; Nguyen, 1988; Chatupote, 1990). Based on solvent extraction a wide range of specific oxidation rates (K) have been reported, for New Zealand and Australian soils ranging from 48-76 μg S cm^{-1} day^{-1} (Watkinson *et al.*, 1987), 11-19 μg S cm^{-1} day^{-1} (Chatupote, 1990) and 54 μg S cm^{-1} day^{-1} in an incubated Australian soil (McCaskill and Blair, 1987).

These oxidation rates which were measured in long term incubations, glasshouse or field trials are much faster than sulphate release rates measured for short incubation periods (Janzen and Bettany, 1987a, 1987b) where slow rates are presumably the result of a microbial growth lag phase. The specific rates of oxidation measured in New Zealand pasture soils by Watkinson *et al.*, (1987), Lee and Boswell (1988) and Chatupote (1990) are sufficiently rapid to make S^0 an effective fertilizer for most soils in New Zealand provided the particle size of S^0 is chosen carefully (Sinclair *et al.*, 1985; Boswell and Swanney, 1988). Field trials have shown that for reasonable annual release rates of sulphate in most areas of New Zealand, the S^0 should be 100% less than 0.150 mm and 50% less than 0.075 mm. Depending upon the frequency of desired S^0 application this size specification can vary (Boswell and Swanney, 1988) (see previous discussion in Section 2.4).

The advantage of S^0 fertilizer is that prior to oxidation it is insoluble in water and not susceptible to leaching loss as are sulphate based fertilizers. This property is one that may be exploited to improve the efficiency of S fertilizer use in New Zealand and partly provides a focal objective for this thesis.

There is currently no information in the literature concerning the fate of S^0 in soil other than its conversion to sulphate; the rate of transformation to organic soil S has not been measured.

2.5.2 The fate of sulphate-S

Once sulphate enters the plant available pool many related processes operate. These ions may be leached to ground water, adsorbed on soil colloids, taken up by plants, assimilated into microbial biomass, or reduced to S^{-2} and precipitated as metal sulphide or emitted as reduced S gases e.g. H_2S (Freney *et al.*, 1971; Sachdev and Chhabra, 1974; Gregg, 1976; Brown, 1985; David and Mitchell, 1987). It is likely that in most aerobic pasture soils, reduction to sulphide or H_2S is insignificant in terms of affecting the supply of S to plants.

In this Section the immediate fate rather than long term fate of fertilizer sulphate is discussed because, as mentioned earlier, the long term fate will involve recycling of S via decaying organic matter. The focus of this Section is literature that reports on the quantitative fate of sulphate fertilizers compared to S^0.

Fertilizer S taken up by plants may be returned to the plant available pool through the immediate release of sulphate from or through the decay of plant litter and animal excreta. Similarly fertilizer S incorporated into microbial tissue may later be returned to the plant available pool. Fertilizer requirements may therefore be a function of the rate of S cycling between the soluble soil sulphate pool and organic forms of S as well as the overall plant S demand. Although increased rates of nutrient cycling may lead to more efficient use of nutrients per unit of plant biomass produced, in animal grazed systems this is likely to make that nutrient more susceptible to losses from leaching particularly from soluble $SO_4^=$ deposited in concentrate zones such as urine or dung S.

2.5.2.1 Uptake by pasture

As sulphate is a mobile nutrient in soil solution, plant uptake appears to be adequately modelled through considering actual plant water use and the soil solution concentration in the effective root zone (Barber, 1984 and Sakadevan, 1991). This suggests that most plant uptake occurs through convective flow and not by diffusion, although at a very few soil solution concentrations in strongly anion retentive soil, diffusion may be a more important process (Barber, 1984). This process is discussed again when S cycling processes are considered in Section 2.6.

In more practical application terms, the amount of fertilizer S utilized by plants depends on the stage of pasture improvement (amount of organic S accumulated in the pasture soil), pasture yield, time of application and fertilizer rate and placement (Gregg, 1976; Goh and Gregg; 1982b; Gregg and Goh, 1982; Sakadevan, 1991).

The amount of fertilizer S taken up by plants also depends on the form and particle size of fertilizer S. A study by Jones *et al.* (1968) comparing plant uptake of ^{35}S labelled gypsum, (< 0.1 mm) and pelleted elemental $^{35}S^0$ (0.5 to 0.9 mm) in lysimeters using three different soils, revealed that the initial percentage of plant (*Bromus molis*) derived S from fertilizer taken up was greater with gypsum (34-57%) followed by fine S^0 (25-43%) and pelleted S^0 (1-2%). Six months later, the percentages were higher for fine S^0 (40-58%) followed by pelleted S^0 (20-

40%) and gypsum (4-8%). It was also observed that the percentage of S taken up by plants from ^{35}S gypsum were higher in light textured soil (sandy loam) than loam and clay soil, which were more anion retentive or had faster S immobilization rates.

Field studies in pastures in the South Island, New Zealand, (Gregg and Goh, 1982), gave total plant recovery of ^{35}S fertilizer gypsum ranging from 6-33% over 600 days of pasture growth and these were consistent with other published results (Walker and Adams, 1958b; Jones, 1964; May *et al.*, 1968; Jones *et al.*, 1970; During and Cooper, 1974; Sheard *et al.*, 1978). Low recoveries were associated with large leaching losses and/or surface run-off. Higher recoveries (4-11%) occurred in the first 50-75 days, but by the end of the experiment the percentage recoveries during any one growth period were less than 1%. As discussed below the major portion (more than 30%) of the labelled gypsum was immobilized in the soil organic S. Saggar *et al.* (1990a, 1990b) have also shown that whereas increased S application to improved pastures may increase soil organic S, S leaching losses also increase.

Differences in the ability of pasture species to take up S have been observed (Gregg and Goh, 1982) where larger amounts of labelled S were recovered from the improved pasture as compared to unimproved pasture. The improved pasture also had lower S leaching losses but this will be a function of fertilizer application rate versus immobilization potential as observed by Saggar *et al.* (1990a, 1990b). Grasses had total recoveries greater than clover and this was attributed to higher dry matter production of grasses (Gregg and Goh, 1982). Care should be taken in extrapolating this data further because in pure swards in lysimeter studies the total recovery of S was greater for subclover (20%) than soft chess (11%) and filaree (7%) as reported by Shock *et al.*, (1983). Obviously a number of factors, probably including species, plant age, rooting depth and soil moisture content in the profile, interact to produce the differences in plant S uptake observed by various authors. Currently insufficient measurements have been made to be able to rank the importance of each factor.

The relative uptake of fertilizer S between roots and plant tops has been shown by Gregg and Goh (1982). On improved clover-ryegrass pastures, on average six times more labelled S was taken up in plant tops in comparison to roots, but only three times in the unimproved pasture.

In Australian pastures, Kennedy and Till (1981b) showed that percentages of plant S derived from (%SDFF) ^{35}S gypsum over a period of 400 days was higher with higher application rates (30% at 125 kg ha^{-1} and 55% at 250 kg ha^{-1}). The %SDFF was also affected by the distance from fertilizer. The closer the plant, the higher the %SDFF, but larger values for %SDFF lasted only for the first two months of plant growth and thereafter levelled off at 10%. Notably, overall

these %SDFF values were higher than those calculated by Gregg and Goh (1982) for New Zealand South Island conditions and may be attributed to lower amounts of rainfall and leaching and greater soil anion retention properties in the Australian soils. Plant uptake of S must also be considered along with the potential of S to leach in various soil environments.

2.5.2.2 Leaching losses

It appears that leaching of S remains the largest loss of S from most NZ pasture soils (Sinclair and Saunders, 1984; Saggar *et al.* 1990a, 1990b). As mentioned earlier (Section 2.2.2.2) sulphate is not strongly adsorbed by many New Zealand pasture soils because they have high organic matter contents which tends to carry net negative charge and are well fertilized with phosphate which out-competes sulphate for anion sorption sites (Bolan *et al.,* 1986 and see also discussion in Section 2.2.2.2). Laboratory, glasshouse and lysimeter studies have been employed most frequently in investigations of S movement in soil rather than field studies. Rare exceptions for pasture soils are the studies of Smith (1979), Heng (1991) and Sakadevan (1991). Laboratory and glasshouse studies (Gregg, 1976) have been primarily useful in determining:

1. The relative influence of soil properties on the extent of S leaching;
2. The relative susceptibility of various S fertilizers to leaching.

However, many laboratory and glasshouse studies have shown that the movement of sulphate in soils is likely to be affected by a wide range of soil properties; adsorption (Section 2.2.2.2), climatic, management, plant and animal factors (Chao *et al.*, 1962a, 1962b; During, 1984; Muller and McSweeney, 1974; Gregg, 1976; Gregg and Goh, 1978, 1979, Goh and Gregg 1982a; Saggar *et al.,* 1990a, 1990b).

Losses of sulphate in laboratory and glasshouse studies are unlikely to reflect actual losses in the field. Sieving, bulking and repacking alter some natural soil physical properties such as soil structure, porosity and stratification particularly in pasture soil. The amount, velocity and pattern of soil-water movement, the soil solution $SO_4^=$ concentration and hence the extent of S leaching is altered (Harward and Reisenauer, 1966). Air drying soils enhances S movement in soil (Peverill *et al.*, 1975). Whilst soil repacked pots may cause more or less solute/soil interaction than that which occurs in field soils, many investigators have reported that larger amounts of sulphate were detected after air drying (Barrow, 1961b; Williams, 1967). 'Edge-effects', associated with the soil container interface may promote 'preferential' water movement (Till and McCabe, 1976) and increase S leaching loss.

Tabatabai (1987) considered that both physical and chemical soil properties affect the movement of the sulphate ion within the soil; the physical properties determine the rate and pattern of water movement and diffusion of ions, where the chemical properties determine the exchange and reactions of the ion with the soil constituents, $SO_4^=$ buffer capacity and susceptibility to leaching loss. The combination of these properties determines the final distribution of sulphate within the soil profile. Based on the information available, some previously discussed by Tabatabai (1987), the main characteristics of the losses of sulphate-S with drainage water are as follows:

1. Losses of $SO_4^=$ are reduced in the presence of growing plants, and are less with perennial crops such as pastures than with annual crops. This is partly because fallow or cultivation induces mineralization of soil organic S and fertilizer S is applied to annual crop seedbeds when plant root length is small and plant demand for S is negligible.
2. Leaching losses of $SO_4^=$ are greatest when monovalent ions such as K and Na predominate; next in order are divalent ions such as Ca and Mg; and leaching losses are the least when soils are acid and appreciable amounts of Fe and Al hydrous oxides are present.
3. Under comparable soil and cropping conditions the amount of $SO_4^=$ removed from the profile is generally directly related to the amount of drainage (Sinclair *et al.,* 1985). Application of phosphate to sulphate retentive-soils will cause sulphate to be desorbed and can increase leaching losses (Aylmore and Karim, 1968; Boswell, 1983; Bolan *et al.*, 1988). It is often observed that when phosphate and sulphate are applied simultaneously, as single superphosphate, the phosphate is absorbed in the upper topsoil whilst sulphate moves down the profile and may be adsorbed in the subsoil (Aylmore and Karim, 1968; Ensminger, 1954). Recently, similar conclusions have been drawn by Saggar *et al.* (1990a, 1990b) from their study of some New Zealand pastoral soils.
4. Sulphate losses increase with liming (Bolan *et al.*, 1988) or amendment with phosphate because this increases soil negative charge and liming may induce mineralization of soil organic matter (Freney and Williams, 1983; Syers and Curtin, 1987). When the soil content of Fe and Al hydrous oxides is negligible or the soil has little other sources of positive charge, the mobility of sulphate can be as high as that of chloride (Aylmore and Karim, 1968).
5. In cropping situations sulphate losses are less when the S fertilizer is banded than when broadcast i.e. when S is presented in a favourable situation for plant uptake.

6. Sulphate content of drainage water is higher from soils amended with fertilizer S (Smith, 1979; Smith *et al.,* 1983 and Heng, 1991).

As discussed earlier, the extent of leaching loss is commonly inversely related to the rate of plant growth but also the nature of the plant may influence the drainage volume. Shock *et al.* (1983) showed that using lysimeters the amounts of ^{35}S labelled sodium sulphate lost in leachates were positively related to the amount of ^{35}S applied and volume of leachate, and as expected were negatively related to the extent of S uptake by plants. In addition, there were species differences, greater leachate volume was lost from lysimeters containing filaree than containing soft chess or subterranean clover.

The nature of fertilizer also has a significant effect. Using lysimeters cropped with soft chess (*Bromis mollis)*, Jones *et al.* (1968), showed that leaching varied with type of fertilizer, particle size and soil type. Initially, larger leaching losses of ^{35}S labelled fertilizers occurred in the gypsum than the S^o treated plots. From 80-90% of applied gypsum, about 50% of fine S^o and 10-15% of pelleted S^o was accounted for in leachates in a 4-year period. Most of the gypsum lost was accounted for the first year while S^o was recovered in subsequent years. Higher amounts of labelled S were lost from fine particulate S^o than coarser particles. Loamy soil held gypsum against leaching much better than sandy and clay soils.

Most of the above studies have been conducted using sieved soil in repacked lysimeters. Undisturbed or intact soil cores have also been used in leaching studies by Peverill and Douglas, (1976), Peverill *et al.* (1977), McLay *et al.* (1988), Williams *et al.* (1990a, 1990b), Sakadevan, (1991). In contrast to repacked soil columns or lysimeters, it has been shown that preferential flow through natural soil macropores such as earthworm channels or continuous cracks can markedly increase the rate of leaching of surface applied fertilizer S and urine S or K (McLay *et al.*, 1988; Williams *et al.* 1990b). Leaching trends for S in undisturbed soil cores were similar to P, in which there were high initial losses and about 40-70% of S, applied as gypsum, was lost (Peverill *et al.*, 1977), showing that in conditions of macropore flow physical soil parameters may be more important than soil anion retention characteristics (Williams *et al.* 1990b).

Direct measurement of field leaching losses have been undertaken by only a few researchers (e.g. Hingston, 1959; Jones *et al.*, 1968; Gillman, 1973; Rhue and Kamprath, 1973; During and Cooper, 1974; Smith, 1979; Heng, 1991; Sakadevvan, 1991).

Smith (1979), in a tile drained (45 cm depth) plots on a pastoral soil (Typic Fraglaqualfs) In North Island, New Zealand, found that on the unfertilized and non-Irrigated plot 7.5 kg $SO_4^=$ S

ha^{-1} $year^{-1}$ were lost through tile drainage. An additional 6.7 kg $SO_4^=$ S ha^{-1} $year^{-1}$ (total 14.2 kg S ha^{-1} $year^{-1}$) were lost during irrigation in the summer of 1977. About 10% of S from the applied fertilizer as superphosphate (45 kg S ha^{-1}) was lost through the drainage system. Similar results were also obtained on the same soils by Heng (1991) who found that winter drainage losses of S could be reduced by applying S^o rather than superphosphate in autumn.

Saggar *et al.* (1990a, 1990b) constructed a nutrient transfer model using a mass balance approach to predict the fate of applied S and P in a hill country pasture. The model indicated that large amounts of S, 8-77% of S added as superphosphate over a period of 8 and 12 years of application were lost by leaching and surface run-off. Losses were greater with increased superphosphate application rates and greater on yellow-grey earth-yellow-brown earth intergrades with low sulphate retention capacity than on yellow brown loam-yellow brown earth intergrades with high sulphate retention characteristics.

Other authors have calculated field leaching losses indirectly through the recovery of isotopically labelled fertilizer (Goh and Gregg, 1982a) or by input-output mass balance calculations (Saggar *et al.*, 1990a, 1990b). Using radioisotope ^{35}S labelled fertilizers, such as gypsum, provides the most promising results in studies of the fate of S in field soils. Till and his colleagues (May *et al.*, 1968; Till and May, 1970a, 1970b and 1971), Gregg and Goh (1978, 1979), Goh and Gregg (1982a) followed the fate of ^{35}S in Australian and New Zealand pasture soils, respectively.

Gregg (1976) and Gregg and Goh (1978, 1979) concluded from their field studies with ^{35}S labelled gypsum that leaching losses of the labelled ^{35}S are affected mainly by rainfall, sulphate retention capacity, pasture production, form and rate of fertilizer, fertilizer placement. This can be summarized as follows:

1. The pattern of S movement is influenced by the water holding capacity of soil. For soil under similar rainfall and sulphate retention capacity, the penetration of ^{35}S labelled fertilizer was greater in a soil with a lower water holding capacity.
2. Greater leaching of sulphate has been observed in low sulphate retention soils than higher sulphate retention soils. Similar findings also had been obtained from the field (Hogg, 1965; Hogg and Toxopeus, 1966; Gillman, 1973) but these were also dependent upon the rate of S application (Hedley *et al.*, 1990; Saggar, 1990a, 1990b). Laboratory studies by Bolan *et al.* (1986) and McLay *et al.* (1988) generally support these findings.
3. Spring fertilizer S applications result in less leaching loss on a sandy soil than autumn application (Gregg, 1976; Gregg and Goh, 1978, 1979).

4. Gregg (1976) found the percentage of S losses from the increasing rates of sulphate fertilizer were similar which confirmed the results from leaching columns or lysimeter studies (Chao *et al.*, 1962a; Cooper and Hogg, 1966) but recent field studies by Saggar *et al.* (1990a, 1990b) and Hedley *et al.* (1990) show a definite influence of application rate.
5. The type of pasture species (Gregg, 1976) and the extent of pasture improvement also influence S leaching loss.

2.5.2.3 Immobilization of applied fertilizer sulphur

Some discussion on immobilization has been presented in Section 2.3.1. Immobilization of fertilizer S or applied sulphate-S can be divided into two categories, namely, long term (more than a year) and short term immobilization. In the long term processes, this can be quantitatively estimated from increases in soil organic matter (Walker and Adams 1958a, 1959; Walker *et al.*, 1959; Jackman, 1964a, 1964b; Walker, 1968). In the short term, however, immobilization can be investigated through the change in ^{35}S specific activity of soil fractions after application of ^{35}S labelled materials. Some aspects have already been discussed in Section 2.3.1. In this Section, more emphasis is focused on field investigations.

The long-term immobilization process is behind the success of New Zealand's grazed pasture system (Walker *et al.*, 1959, Walker, 1968) and current dairy pastoral productivity. Large amounts of organic S (and more importantly N) have been accumulated in New Zealand pasture soil fertilized with single superphosphate relative to virgin or unfertilized soil (Walker, 1968; Nguyen and Goh, 1990). Once an equilibrium state is reached between the formation (immobilization) and decomposition (mineralization) of this organic S, the immobilized S, annually, contributes large amounts of available S for pastures growth (Sinclair, 1983). It can be considered that in well developed and well fertilized pasture soils immobilization is an important every-day-storage S process. In contrast, this process may be considered as a temporary or permanent loss of available S in less developed pasture soils. Walker (1968) and Walker *et al.* (1959) pointed out that additions of S and P to grass-clover pasture soils increased soil organic S dramatically. After 25-30 years of pasture establishment and fertilization with single superphosphate, organic S accumulated at the rate of 7-18 kg S ha^{-1} $year^{-1}$ (Walker *et al.*, 1959a, 1959; Jackman, 1964a, 1964b). Similar results in building up soil organic S were also obtained recently by Lambert *et al.* (1988) and Saggar *et al.* (1990a, 1990b) for North Island (New Zealand) hill country soils and Hingston, (1959), Barrow (1969b), Lewis *et al.* (1987) for an Australian soil. The increase in organic S in the pasture may mainly derive from accumulation of plant residues, dung and urine from grazing animals. The cycling

of litter, dung and urine from grazing animals in the grazed pasture soil are discussed in Section 2.6. Saggar *et al.* (1990a, 1990b) have shown that in hill country soils the accumulation of soil S is not spatially consistent because soil S may accumulate on areas receiving proportionally more animal excreta than in other areas less well suited to animal camping, e.g. on steep slopes exposed to unfavourable climatic conditions soil organic S may be continuously depleted irrespective of S application rate.

Incorporation of ^{35}S labelled fertilizer into soil organic S has been investigated by Till and May (1970a, 1970b) and Gregg and Goh (1978, 1979). In New Zealand pasture soil, Goh and Gregg (1982a, 1982b) and Gregg and Goh (1982) showed that after application to the soil the ^{35}S gypsum was converted rapidly into soil organic forms. Within 17 to 75 days after application, 17 to 40% of the applied S was in the organic form in the topsoil (0-15 cm). Larger amounts of labelled S were converted in improved pasture compared to unimproved pasture. In general, the amount of incorporation increased linearly with time during the first 50-100 days and thereafter the trend levelled off (steady) throughout the experiment period. However, in similar study carried out in an Australian pasture soil (May *et al.*, 1968; Till and May 1970a, 1970b), about 80% of the applied S was incorporated in the top 10 cm. Gregg (1976) considered that the lower amount of incorporated ^{35}S in New Zealand soils may be due to larger leaching losses of S down to the lower soil profile. Leaching loss was negligible in the Australian soil. No attempt had been undertaken to fractionate organic S fractions in these two studies.

The fate of applied fertilizers S was characterized in more detail in a series of field experiments examining aspects of the residual value of S fertilizer applied to pasture grown on a S efficient basaltic soil (McLachlan and De Marco, 1971). McLachlan and De Marco (1975) showed that under a pasture regime, most of the applied S accumulated in organic forms with greater accumulation of carbon-bonded S. There was an increase in the carbon-bonded S fraction with increasing level of S applied. About two-thirds of the applied S was incorporated into carbon-bonded S. HI-reducible S accumulation was only weakly related to the level of S applied. Furthermore, the authors showed that this carbon-bonded S was remineralized to available S through the HI-reducible S (Freney *et al.*, 1971) when the soils were brought under cultivation for cropping.

Recently, in New Zealand, Nguyen and Rickard (1988), Nguyen *et al.,* (1989c) and Nguyen and Goh (1990) also found that after 35 years of application of three rates of S from superphosphate (0, 21 and 42 kg S ha^{-1} $year^{-1}$) to irrigated pasture there were large accumulations of organic S in soil, 50-60% as carbon-bonded S and 40-50% as HI-reducible

S. At a rate of superphosphate application of 188 or 376 kg ha^{-1} $year^{-1}$ (or 21 and 42 kg S ha^{-1} $year^{-1}$), the incorporation into soil organic S reached an equilibrium plateau of 400 mg S kg^{-1} soil. There was no further increase in the organic fractions by increasing annual inputs from 21 to 42 kg S ha^{-1}. This was presumably because biomass production was limited by secondary factors which limited the fixed carbon available for S immobilization. The authors also showed that the proportion of organic S as carbon-bonded decreased with depth whereas the reverse occurred with HI-reducible S. However in well developed New Zealand pastures, over 59-82% of organic S in the top 0-7.5 cm of soil is in the form of carbon-bonded S, while the remaining proportion is in HI-reducible S form (Perrott and Sarathchandra, 1987).

In summary, it is clear from the review of current literature that deficiencies of information exist in the study of $SO_4^=$ leaching from structured field soils and at the beginning of this study in 1986 there was a complete lack of information comparing the fate of S^o and $SO_4^=$ in pasture soil particularly the rate of incorporation into soil organic matter which is the most important reserve of plant S in soils.

2.6 SULPHUR CYCLING IN GRAZED PASTURES

2.6.1 Background

A pasture ecosystem represents a grouping of components; soils that support the plants, animals that graze these pasture plants, residues of these plants and animals, microflora and microfauna, which decompose these residues, and atmospheric gases, all linked together as functional entities by food webs controlling the flow of energy and the flow of mineral nutrients (Wilkinson and Lowrey, 1973).

In grazed grasslands, nutrients cycle through various soil, plant and animal pools by numerous interactive pathways and processes as shown in Figure 2.1. In grazed systems, S moves from soil to plant and back to soil, either directly in plant residues, or indirectly via the excreta of grazing animals. Sulphur enters the system in rainfall, dry deposition or fertilizers and is subjected to immobilization into organic S by plants and microbial processes both in soil and animal's rumen (Freney, 1986; Goodrich and Garret, 1986) and then this organic S is re-mineralized to sulphate in the soil. The input requirement of fertilizers is influenced by the relative rates of transfer of nutrients in the various recycling processes, e.g. excessive immobilization (Jackman, 1964a, 1964b) or leaching increases the levels of immediate inputs required to sustain the cycling pool. The amount of soluble sulphate in the soil at any time is

the net result of the complex interactions shown in Figure 2.1. The major factors affecting the amounts of dissolved sulphate in most grazed pastures in New Zealand are plant uptake, adsorption to and desorption from soil minerals, mineralization and immobilization of organic S, and leaching losses. It is immediately obvious that to understand the fate of S in pasture systems and make decisions to improve the efficiency of fertilizer use, a thorough understanding of factors influencing the rate of S transformation is required.

2.6.2 Uptake of S by pasture plants

Soil sulphate-S taken up by plants moves to plant roots mostly by mass flow. Mass flow is the main transport mechanism for mobile ions such as $SO_4^{=}$, NO_3^{-}, Cl^{-}, Ca^{+2}, Mg^{+2}, Na^{+}, (Barber, 1962, 1984). This involves ions moving in the transpiration stream of water and the rate depends on the rate of actual evapotranspiration, ion concentration in the soil solution and plant requirements (Nye and Tinker, 1977, Barber, 1984). Diffusion takes place when the rate of supply by mass flow is less than the rate of uptake by plant; i.e. a concentration gradient is created toward the root. Diffusion is the main transport process when ion concentration in soil solution is low or soil adsorption capacity is high (Barber, 1962; Lewis and Quirk, 1967). Diffusion is also reversible when mass flow supply exceeds uptake at the root (Barber, 1962). Root interception is considered more important only when soils have low moisture contents that restrict diffusion of nutrients through a soil. This involves all nutrients contacted by growing roots regardless of whether they are in solution or adsorbed onto soil particles. Other mechanisms, direct exchange and particle diffusion, are of limited importance.

Since in fertilized soils, most sulphate moves to plant roots by mass flow, the most important factors governing the plant uptake of S are soil moisture content (plant available water), sulphate concentration in soil solution and the actual evapotranspiration from the sward. Models developed from these concepts accurately predicted pasture S uptake in a range of soil fertility conditions (Sakadevan, 1991). For most New Zealand soils where pasture production ranges from 17 to 0.5 t DM ha^{-1} $annum^{-1}$ the S uptake may range from 60 kg S ha^{-1} $annum^{-1}$ to below 8 kg S ha^{-1} $annum^{-1}$ depending on soil fertility, climate and sward composition. The spatial variability of S uptake can be short range depending upon the influence of slope, aspect, soil moisture content and the influence of stock grazing and camping behaviour on soil fertility. For example, in central North Island hill pasture on yellow-brown earth-yellow-grey earth intergrades the mean annual pasture production ranged from 17.2 t ha^{-1} on campsites to 12.4, 9.5, 7.3 t ha^{-1} on easy, moderate and steep slopes, respectively. Pastures at each of these sites varied in S concentration from 0.40% on campsites to 0.22% on steep slopes giving approximate S uptake values ranging from 69 kg S

ha^{-1} $year^{-1}$ on campsites to 16 kg S ha^{-1} $year^{-1}$ on steep slopes (Saggar *et al.,* 1990a, 1990b).

When the concentration of sulphate in soil solution is increased, irrespective of whether S is the key nutrient limiting pasture yield, plant S uptake tends to increase (Goh and Gregg, 1982b; Boswell, 1983; Rennenberg, 1984; Boswell and Swanney, 1988; Nguyen *et al.*, 1989a, 1989b, 1989c; Saggar *et al.*, 1990a, 1990b). According to Rennenberg (1984), it was considered that the plant root cells are not equipped to prevent an uptake of excess sulphate. Therefore root cells do not appear to cope with excess sulphate in the soil by avoidance of its uptake. For example, Saggar *et al.* (1990a, 1990b) showed that pasture S concentration increased with increasing extractable soil sulphate (phosphate extraction). The S concentration of pastures on the easy slope and steep slope with 40-50 and 20-40 mg of extractable S kg^{-1} soil respectively were about 0.35-0.44% and 0.22-0.30%, respectively.

In New Zealand pasture soils, P and S are the major fertilizing nutrients applied to soils to promote pasture yields. Pasture production increases with increasing rate of single superphosphate applied (Gillingham, 1980; Gillingham *et al.*, 1980; During, 1984; Rowarth *et al.*, 1988; Saggar *et al.*, 1990a, 1990b). Commonly, applications of approximately 250 kg ha^{-1} of superphosphate (12% S) provide adequate P and S for pasture production (Crouchley and Sinclair, 1982; Williams and Morton, 1985; Nguyen *et al.*, 1989a, 1989b) on many sedimentary soils.

2.6.3 Return and decomposition of plant litter

Pasture S has two fates 1) ingestion by the grazing animal and 2) uneaten plant material senesces (either through age or saprophytic attack) and is returned to the soil as litter which is decomposed by soil organisms. The amount of litter returned depends on grazing animals and grazing management and pasture production (King and Hutchinson, 1976).

In well managed New Zealand pastoral farming, about 80 to 90% of the pasture production is ingested by the grazing animal and about 10 and 20% of pasture production remains after normal grazing (Gillingham and During, 1973; Sinclair and Cornforth, 1981). The amount of litter remaining on pasture has been investigated by Gillingham (1980) and Gillingham *et al.,* (1980) in hill country and was estimated to be about 2.5-3.0, 1.2-1.9 and 1.1-1.6 t ha^{-1} $year^{-1}$ on campsites, moderate and S in litter, the amount of S return to pasture may amount to 3.2-6 kg S ha^{-1} $year^{-1}$.

Some have considered that S is immobile within the plant (Bouma, 1967). Thus, the S content of the standing dead pasture may be expected to be the same as that of green pasture. However losses of S from dead material by leaching may occur (Till and colleagues, unpublished data; quoted by Boswell, 1983). They found that S in the dead material was only 60% of the S content of green material (A.R. Till and colleagues, unpublished data; quoted by Boswell, 1983). They found that S in the dead material was only 60% of the S content of green material. Losses of S by leaching from the plant litter can be considered as part of litter decomposition processes. This may represent an early flush of S release from litter which is dependent on rainfall.

The mineralization of the mineralizable organic components depends on the associated micro flora and fauna of the decomposer system. Other factors affecting mineralization were discussed in Section 2.3.1. Other factors affecting litter decomposition may consist of physical influences affecting the degree of contact between litter and decomposer macro and micro organisms. Initially, trampling by stock and breakage by frost may be important in allowing contact of litter with soil. Subsequently, soil insects, earthworms and soil micro-organisms are responsible for mixing and decomposition.

Re-utilization of S from plant litter and fertilizer S incorporated in plant residues has been investigated in controlled conditions (Till and Blair, 1978; Goh and Gregg, 1980; Till *et al.*, 1982; Boswell, 1983; and Chapman, 1987b). These results showed that 7-40% of ^{35}S labelled litter can be re-utilized by clover and ryegrass plants. Over 5-30 days there was fast release of labile organic forms of nutrients from the litter followed by a slower rate as the organic constituents are mineralized. Furthermore, Chapman (1987b) showed that about 7% of ^{35}S labelled ryegrass litter appeared in soil microbial biomass after 34 weeks of the incubation with soil.

The nature of the plant part and species may influence the rate of litter decomposition. For example Goh and Gregg (1980) showed that recovery of ^{35}S by pasture uptake from ^{35}S labelled clover root and grass tops were higher than from ^{35}S labelled grass root and clover tops, respectively. No causal effects of this were discussed and it is difficult to explain the apparent difference between the availability of root and shoots in different species. Nodulated clover roots may be high in N and in sulphide groups present in the nitrogenase of rhizobia.

Till and Blair, (1978) and Till *et al.* (1982) also showed that dried ^{35}S labelled clover root added to soil had a higher S release rate than ^{35}S labelled clover tops and further suggested that in grazed pastures, where litter tends to be younger and growing plants are trampled and

enter the litter pool, significant amounts of nutrients may be returned to the system in rapidly decomposed forms. In field studies on decomposition of unlabelled clover and grass litter, Boswell (1983) found similar results in the short term. The decomposition followed two steps: a rapid initial process when soluble material was removed and there was mineralization of the more mobile components; followed by a slow process in which tissues more resistant to decomposition were mineralized. The initial rates of release of S were greatest from subclover followed by dung pellets (discussed later) and by grass litter. Initial field decomposition rates of subclover were about 50% greater than grass litter. In the longer term, however, the release of S was similar for grass and clover litter which was more rapid than dung. A similar study in a controlled environment using ^{35}S labelled ryegrass and clover litter, dung and urine, has shown that the rate of S release from litter and dung at 0-37 days was 16.15 mg S g^{-1} S day^{-1} and 4.5 mg S g^{-1} of S day^{-1}, respectively (Boswell, 1983). In general, uptake of S from plant litter was, again, higher than S from dung but much less than S from urine. More than 80% of S released from plant litter was rapidly immobilized into soil organic S. Unfortunately, there was no attempt to characterize further fractions of the immobilized S in his study.

Little is known about the rate of S returns from root litter. When relating their results to field conditions, Goh and Gregg (1980) suggested that less than 10% of the labelled S incorporated into the plant root would be made available to the living plant within one year. Others suggested, however, that recycling of nutrients from the roots of pasture plants may be of more importance than recycling of shoot litter S in a permanent pasture. Garwood (1967) and Evans (1971) found that in perennial ryegrass (*Lolium perenne L.*) pastures most of the roots were annual. These findings suggest an almost complete turnover of root dry matter production per annum either as litter or harvested by root feeding arthropods and other microfauna. In grazed pasture, it has been estimated (Goedewaagen and Schuurman, 1950) that 4000-5000 kg ha^{-1} of dry matter are added annually in the root mass alone. Additionally, dry matter resulting from root exudation (Shamoot *et al.*, 1968) may amount to 200-1000 kg ha^{-1}. Thus the annual addition of the underground plant residues may be in the range 5500-6000 kg ha^{-1}. Assuming that S content in the root is about 0.2%, then approximately 10-12 kg S ha^{-1} may be returned to the soil annually by underground residues.

In general, it can be expected for a pasture growing 15,000 kg of dry matter ha^{-1} $year^{-1}$, with a S concentration in mixed herbage of 0.3% and 70% of the pasture being utilized by grazing animals, that 13.5 kg S ha^{-1} will be returned in uneaten litter. With root dry matter turnover of 4000 kg ha^{-1} $year^{-1}$ with 0.2% S then a total of 21.5 kg S is returned in plant remains.

2.6.4 Ingestion and excreta return by the grazing animal

Grazing animals exert positive and negative effects on pasture production and this has been reviewed by Barrow (1967b), Petersen *et al.*, (1956), Curll (1982) and Lantinga *et al.* (1987). Grazing animals may affect pasture production through grazing, treading, scorching by excreta return and rejection of herbage (Curll, 1982, Lantinga *et al.*, 1987). Plant photosynthetic area is reduced by grazing, particularly when pasture cover drops below 1200 kg dry matter ha^{-1} and less fixed carbon and ATP (photophosphorylation) will be available for translocation to the roots. Root enlongation and root respiration may therefore be slower (Davidson and Milthorpe, 1966a, 1966b), and the supply of ATP to drive active nutrient uptake and other metabolic processes may be reduced.

In this Section, however, emphasis is placed on the animal influence on the fate of plant nutrients.

The amount of S removed in animal products, e.g. wool, beef and fat lamb, are very small compared with the amounts removed or redistributed in returns of animal excreta and play little part in determining the requirements for available nutrients (Till, 1975). Only 10-15% of ingested S (During, 1984; Wilkinson and Lowrey, 1973; Till, 1975) is used by animals in the production of body tissues, milk and wool and the remainder, 85-90%, is returned to the soil in the animal excreta.

2.6.4.1 Sulphur in dung and urine

The manurial influence of grazing sheep on pasture was observed by Sears and Goodall (1948). They found that both sheep dung and urine applied separately, influenced the sward content and pasture growth, giving a clover-dominant sward in the former treatment and a grass-dominant sward in the latter. Neither dung nor urine alone achieved the production level attained from mixed deposition under grazing. Others have reported contradictory results. For example, Watkin (1954), Weeda (1967) and Lantinga *et al.* (1987) reported results showing little or no benefit to pasture production from N circulation through cattle urine and dung. Similar conclusions were also obtained by Skrijka (1987) who found that sheep dung had no effect on pasture yield. Recently, in New Zealand, Morton and Baird (1990) found that the return of N from dung and urine affected insufficient area to influence pasture growth even at high stocking density. However, in pastures grazed by sheep (Sakadevan, 1991) pasture yield increased by 59% after urine application in a lysimeter study. In addition, about 40% of total K uptake ha^{-1} was derived from areas affected by cow urine (Williams *et al.*, 1990a). Similar

results were also reported by Morton and Baird (1990). Obviously, the influence of excreta return on pasture production is dependent upon several other factors such as temperature, extent of drainage, sward composition and inherent soil fertility status.

Sulphur cycling via grazing animals is considered to avoid the slow processes of decomposition of plant residues and effectively increases the turnover rate of S in the plant-soil cycle (Wilkinson and Lowrey, 1973; Till, 1975). This increase in the rate of S cycling occurs because:

1. A large proportion (50-80%) of S in the excreta, particularly urine, is in the immediately plant available sulphate form (Till, 1975).
2. The passage of plant material through the animal, and the partitioning of the S between urine and dung in favour of urine, means that some plant derived S is mineralized more rapidly than if the plant litter is returned directly to the soil (Barrow, 1961b, 1967b; Till, 1975).

The amount of dung excreted by animals varies from 18 to 25 kg fresh weight animal^{-1} day^{-1} for cattle and 1.2 to 2.5 kg animal^{-1} day^{-1} for sheep (Boswell, 1983). It was observed that during grazing, ewes defecated 4-7 times and urinated 3-5 times daily. The average daily production per ewe amounted to 0.5 kg dung and 0.6 kg urine (Skrijka, 1987). The average area of an individual sheep dung patch was 0.025 m-^{2} (Morton and Baird, 1990).

For both sheep and cattle the faecal excretion of S is approximately constant per unit of dry matter eaten (Barrow and Lambourne, 1962) with approximately 0.1 g of S being excreted in the dung per 100 g of feed eaten. However, of the total S returned in the excreta, the proportion of S in urine varies from 6-90% (Walker, 1957; Barrow and Lambourne, 1962; Till, 1975; Doyle and Moir, 1979) depending upon pasture S content. The proportion of S in urine on non-S-deficient pasture being about 50-60% of excreted S.

Most of the S in dung is in organic combination although some inorganic S is present (Walker, 1957; Doyle and Moir, 1979). Most of the organic S in dung is in a form that is resistant to rapid mineralization (Walker, 1957; Barrow, 1961b; During and Weeda, 1973; Boswell, 1983). Bird (1971) reported that of the ^{35}S infused into the rumen of sheep, 87-94% of dung S was carbon-bonded S and 4.1-5.4% was in ester-$SO_4^=$ while inorganic sulphate represented only 0.5-4% of dung S. Therefore in many situations the nature of S in the dung may reflect rumen microflora activity rather than the form of plant S ingested.

The S in urine is in a form which is immediately available to plants or in a form which is readily hydrolysed to an available form (Barrow, 1967b; Walker, 1957; During and McNaught, 1961; During, 1984; Boswell, 1983). The amount of S excreted in urine has been measured at about 0.42 g sheep^{-1} day^{-1} (Langlands *et al.*,1973) but this again depends on the S intake and feed quality (Barrow and Lambourne, 1962). S content in urine ranges from 3 to 13% depending on the status of the diet (Kennedy, 1974). The average area of individual sheep urine patch was 0.03 m^{-2} (Morton and Baird, 1990).

2.6.4.2 Distribution of dung and urine

The area of pasture influenced by animal excreta was investigated by Petersen *et al.*, (1956), MacLusky (1960), MacDiarmid and Watkin (1971, 1972a, 1972b), Kennedy and Till (1981b) and Morton and Baird (1990). The area influenced by ^{35}S labelled sheep urine was studied by During and Martin (1968) and Kennedy and Till (1981b), respectively. MacLusky (1960) showed that dung affected the growth over an area about six times that actually covered. This agreed with the findings of MacDiarmid and Watkin (1971, 1972a). MacDiarmid and Watkin (1971, 1972a) concluded that grasses growing up to 15 cm from the edge of a cow dung patch can derive N from the region under the patch owing to the lateral spread of grass roots. They found an increase in available soil N at 6 inches beyond the edge of the dung. Assuming a mean area of dung patch of 0.05 m^2, this was equivalent to an affected area of about 0.25 m^2. Further, it was shown that the percentage area of paddock covered by cow dung patch after 24 hour's grazing ranged from 0.31 to 0.68%, depending principally on stocking rate (MacDiarmid and Watkin, 1972a, 1972b). At stocking rates of approximately 20 stock unit per ha, it was calculated that at any grazing time approximately 5% of the pasture could be affected by cow dung-patch nutrients and it would take 2.5 years for an area equal to that of the paddock to be affected by dung patches and ten years for the same area to be covered by dung patches. This was similar to an empirical calculation performed by Petersen *et al.*, (1956) who estimated that it would take 13 animal-years of grazing (4745 cow-days) to cover 100% of the pasture with dung.

However, there appears to be no existing similar information on the area of soil that is influenced by sheep dung return.

Lotero *et al.* (1966) observed that the greatest growth response occurred in the centre, and decreased towards the periphery of the roughly circular area affected by urine. According to Kennedy and Till (1981b), it was found that the area influenced by a urine patch extended as far as 20 cm; with time and with more rain may be further. There was both lateral and vertical

movement of S from the urine patch and this was in agreement with the result reported by During and Martin (1968). On average, recovery by pastures of ^{35}S from urine was less than 5%. Initially (after 7 days), the percentage of plant S derived from ^{35}S urine (%SDFU) was highest (21%) in the innermost zone and decreased toward the outer zone. After 10 days, there was not much difference in %SDFU among zones (average 5-7%).

Since the animal in the pasture system is mobile, cycling of nutrients through animals will be a function of this mobility (Wilkinson and Lowrey, 1973). Factors affecting the time-space distribution of excreta include stocking rate, camping, grazing pattern, type of animal and the amount and frequency of excretion (Wilkinson and Lowrey, 1973). The problem of uneven excreta return has been discussed by Sears (1950), Petersen *et al.* (1956), Hilder (1964), Gillingham and During, (1973), Till (1975), Gillingham (1978), Boswell (1983), Rowarth (1987), Williams (1988), Morton and Baird (1990) and Saggar *et al.,* (1990a, 1990b). S ingested over a large area is voided to a small area, thus concentrating the S at that site, such as a campsite. Losses by leaching can therefore be accentuated (Petersen *et al.*, 1956; Gillingham, 1978; Boswell, 1983; Williams *et al.,* 1988; Hedley *et al.*, 1990; Saggar *et al.*, 1990a, 1990b; Goh and Nguyen, 1990). Hence the cycling of S through animals is spatially less effective than through plant root and shoot litter, or in other words, nutrients are transferred to an area where the nutrients are unproductive (an area already rich in nutrients or raceways and yards).

The unevenness of dung distribution on a flat paddock was shown by Hilder (1964), in a study of grazing sheep in Australia. It was found that the campsite which comprised 3% of the total area, contained about 22% of the total dung and that 10% of the total area contained 34% of the total dung. Similar conclusions were also reported in New Zealand by Gillingham (1980) studying hill country pasture land where the added effects of land slope, aspect to sun and prevailing climatic conditions influence animal grazing and camping behaviour (Saggar, 1990a, 1990b). Gillingham (1980) found about 10.3, 1.2 and 0.15 t ha^{-1} $year^{-1}$ of sheep dung were deposited on campsite (0-25^{o} slope), moderate (25-45^{o} slope) and steep slopes (>45^{o} slope), respectively. Boswell (1983) showed that at the campsites total soil S throughout the soil profile and leaching loss of S from soil were generally higher than that in the non-campsites. However, more than 50% of urine S was retained in soil by immobilization processes rather than redistributed by plants grown on campsites.

The spatial distribution of urine is unknown but it is expected to follow dung distribution (Till, 1975).

According to Till (1975)

> "An estimate of the likely importance of redistribution can be made by considering an improved pasture stocked at 10 sheep ha^{-1} and fertilized with S at 25 kg ha^{-1} $year^{-1}$. In such a system the return of S in excreta would be about 4 kg ha^{-1} $year^{-1}$ in dung and 6 kg ha^{-1} $year^{-1}$ in urine. If the dung distribution is 34% on 10% of area (Hilder, 1964), this would give deposition (and perhaps effective loss) of 1.4 kg dung S or 5.6 percent of the annual application"

If dung and urine are similarly distributed the loss would be 2.4 kg S and equivalent to 9.6% of the annual S application.

2.6.4.3 Excreta decomposition

With the exception of pioneering work by Barrow (1961b) and recent work by Boswell (1983), there has been little published information on the rate of release of S from dung. Barrow (1961b) showed that the amount of S mineralized was closely related to the S content of the dung and the proportion of S mineralized was less than with plant material. Dung was more resistant to decomposition than was plant material (Barrow, 1961b; Boswell, 1983). Barrow (1961b) also reported that there was likely to be net immobilization below a dung S content of 0.22% while similar results were likely to occur with plant litter with S content below about 0.12%.

Dung mineralization rates are more rapid in crushed samples than pad samples (Bromfield and Jones, 1970; Rowarth, 1987) and in the presence of arthropods such as earthworms (Barley, 1964) and dung beetles (Bomemissza and Williams, 1970) which can be expected to enhance the mineralization rate through the action of communition of material and transporting it to the vicinity of microorganisms (MacFadyen, 1978). Similarly dung decomposition rates are slow in dry conditions as compared to cold moist conditions. For example, Hilder (1966) reported sheep pellets remaining for 10 weeks in cold moist conditions in Armidale, Australia. However in New Zealand, Rowarth (1987) reported that dung samples (by physical disappearance) decomposed within 20 days in cool moist winter periods, while longer periods (up to 60 days) were observed during summer.

The fate of ^{35}S labelled urine and dung and decomposition of unlabelled dung was studied by Boswell (1983) in controlled conditions in the field by the litter bag technique. Results can be summarized as follows:

1. Uptake of S from urine in camp site soil was initially higher than from non-camp site soil.
2. The mean rate of release of S from dung (0-37 days) was about 4.5 mg S g^{-1} S day^{-1} which was much lower than from litter.
3. There was immobilization of soil S after S had been released from dung. This agreed with the results reported by Barrow (1961b).
4. Movement of S from dung and urine to soil organic S was as rapid as from plant litter. In particular, about 68% of urine S was in organic S within 6 days. In general, more than 80% of the S released was converted into organic forms. Unfortunately, there was no partitioning of organic S fractions in the study.

The uptake of ^{35}S labelled sheep excreta was also investigated by Kennedy and Till (1981b) under field conditions. Results showed that the percentage of plant S derived form urine and dung (Excreta, %SDFE) were as high as 94% and 54% respectively. About 20% and 10% of the S from the urine and dung were taken up by plants. Recovery of S by pasture plants from both sources dropped rapidly with time, especially from urine. It was calculated that the excreta from 20 sheep ha^{-1} could provide 20% of the S requirement of the above ground plants. Furthermore, it was found less than 40% of total activity were recovered by pastures after 384 days. Leaching loss and lateral spread were suggested as responsible for the low recovery.

Both dung and urine have also been shown to affect soil properties where they were returned, particularly at campsites. They have been shown to increase cation exchange capacity, organic matter, N, P, exchangeable potassium, calcium and magnesium (MacDiarmid and Watkin, 1972a; During and Weeda, 1973) and soil pH (Doak, 1952; Watson and Lapins, 1969; During and Weeda, 1973). As the soil pH increases, greater desorption of sulphate may occur (Ensminger, 1954; Kamprath *et al.*, 1956; During and Weeda, 1973; Bolan *et al.*, 1988) and mineralization of soil organic S may increase (White, 1959; Barrow, 1960b, 1960c; Williams, 1967). This favours accelerated local losses of soil S by leaching (Boswell, 1983; Sinclair and Saunders, 1984; Saggar *et al.*, 1990a, 1990b) and leads many researchers to consider that nutrient cycling through grazing animals is an inefficient process resulting in considerable loss of effective nutrients from productive areas of paddocks (Hedley *et al.*, 1990; Saggar *et al.*, 1990a, 1990b; Goh and Nguyen, 1990)

To summarize, the general fate of S in a paddock being grazed by animals can be represented by litter, dung, urine and amounts retained in animal tissue. Of the total S in pasture plants, about 10-30% are in litter, 25-30% as dung, 30-40% as urine and about 10-15% held In animal

products. Of the total S in litter, dung and urine returned to the soil, it appears that approximately 5-10%, less than 5% and 20-30%, respectively, will be re-utilized in the short term by pasture plants. The remainder (about 60-75% of total S returned to the soil) is mostly immobilized into soil organic matter. From this review, the urine deposition is expected to stimulate the greatest increase in plant S uptake or leaching in the short term. Deposition of S in carbon rich, plant litter and particularly dung is expected to have low plant recoveries and in the short term may be responsible for promoting immobilization of soil sulphate.

2.7 SUMMARY AND CONCLUSIONS

Sulphur plays an important role in the nutrition of higher plants, particularly in protein synthesis. The primary nitrogen fixation step in legume based pastures is dependent on adequate S nutrition. In an aquic temperate climate (USDA taxonomy) such as most of New Zealand, S fertilizers are required to maintain adequate soil S status for pasture production.

Sulphur can be a highly mobile nutrient in soil as well as very biologically active. The persistence of plant available S in soils is therefore dependent on rainfall, drainage, sulphate retention characteristics of soils and the extent to which added S is converted to organic S reserves. Although considerable laboratory and glasshouse research has studied the influence of soil properties on S uptake, immobilization and retention and the mineralization of organic S, few of the findings can be extrapolated with confidence to effectively explain the cycling of S under field soil conditions on naturally structured soils.

Mineralization and immobilization are the key processes that govern the amount of immediately plant available S and the fate of fertilizer S in soils. Leaching or surface run-off under field conditions lead to inefficient use of sulphate based fertilizers e.g. superphosphate. Incorporation of fertilizer S into organic S may conserve S against leaching losses. Even though the use of slow release forms of S such as elemental S (S^0) is considered to reduce these initial leaching losses in some climatic situations in New Zealand. Little information is available on the comparative fate of sulphate and S^0 based fertilizers in soils.

Radioisotope techniques have proved to be useful for tracing the fate of applied S and measuring the flux of S between various soil pools. Their use in tracing the fate of S^0 is a comparatively recent development. S^0 labelling techniques require further development so that uniformly labelled S^0 in various particle sizes can be conveniently produced and blended with P sources to simulate commercially available fertilizers.

The general objective of this thesis is to develop radioisotope techniques to study the fate of sulphate based and S^o fertilizers in field soils and having developed these techniques to investigate the factors which influence the fate and plant availability of these fertilizers, in order to provide information that can be used to formulate and improve fertilizer recommendations for pastures.

CHAPTER 3

EXPERIMENTAL SITES AND TECHNIQUES

3.1 METHODS FOR RADIOACTIVELY LABELLING FERTILIZER SULPHUR

3.1.1 INTRODUCTION

The radioactive isotope of sulphur-^{35}S has been used extensively to measure the agronomic efficiency of S fertilizers, to follow the fate of applied fertilizer S (Gregg and Goh, 1978, 1979, 1982; Shedley *et al.*, 1979; Goh and Gregg, 1982a, 1982b; Boswell, 1983) and to determine the nature of the sulphur cycle in soils (Till and May, 1970a, 1970b, 1971; Goh and Gregg 1982a, 1982b; David and Mitchell, 1987). The ^{35}S isotope is particularly well suited for use in longer term soil/plant studies because it has a long half-life (87.4 days) and upon decay emits a low energy β particle (β max 0.167 MeV) which is easily detected by liquid scintillation counting systems and is safe to use.

Most studies have used ^{35}S labelled $SO_4^=$-S fertilizers but few studies have used ^{35}S labelled elemental S (S^o). Except for Shedley (1982), no technique for manufacturing labelled S^o fertilizer has been published. However, Shedley (1982) labelled S^o by dissolving ^{35}S labelled and unlabelled S^o in carbon disulphide. Labelled S^o crystals were obtained after carbon disulphide was evaporated. However, it is hazardous to work with carbon disulphide. A safer labelling technique needed to be developed.

3.1.2 Calculating the required ^{35}S enrichment in fertilizer materials

The amount of ^{35}S to be added to the fertilizer material depends on the fate of S in the soil and plant system being studied, length of study, dilution of the label by soil and herbage S, sample size taken for analysis and the efficiency of the radioactivity counting system (IAEA 1976). Previous research with a particular soil and plant system is helpful so that both the amount of S that is likely to exchange with the isotope and the rate of exchange can be estimated. Based on the above, the optimum amount of radioisotope to be mixed with fertilizer material can be estimated and will be expected to give a satisfactory count rate in samples taken from the various plant and soil S pools. Calculations, in detail, are shown in Appendix 3.2.

A minimum count rate between 500-1000 cpm ml^{-1} for ^{35}S in a 0.04 M $Ca(H_2PO_4)_2.H_2O$ soil extract (1:10; soil:extraction solution ratio) was used for calculating the total amount of activity required to label superphosphate or S^o for 150 day experiments.

3.1.3 Labelling S^o fertilizer

Both forms of radioactive sulphur, $^{35}SO_4^=$ and $^{35}S^o$ (carrier-free) were supplied by Amersham International Plc., England. All subsequent operations were carried out in a fume hood.

3.1.3.1 Labelling microfine S^o

Microfine particles (less than 0.010 mm) of $^{35}S^o$ labelled S^o were prepared by dissolving agricultural grade S^o (< 0.500 mm particle size) in toluene (commercial grade) and mixing this with carrier-free $^{35}S^o$. Aliquots were then pipetted onto a layer of 15 g of finely ground soil in a glass-petri dish and evaporated to leave crystalline S^o on the surface of the soil particles. This method of labelling produced microfine S^o (< 0.010 mm particle size) which was used in the field experiment in Chapter 5.

3.1.3.2 Labelling S^o of different particle sizes

To produce labelled S^o of different particle sizes (< 0.075 mm to 1.00 mm or greater), aliquots of the carrier-free $^{35}S^o$ dissolved in toluene were added to samples of unlabelled S^o (< 0.500 mm particle size and agricultural grade) placed on thin aluminium foil in flat-bottomed porcelain crucibles (approximately 1.00 ml of toluene was added per 3.5 g ground S^o). The mixture was carefully melted on an electric hot plate (with remote switching) at 115^o-120^oC. Five to eight minutes of heating were required to melt all the S^o. To reduce volatilization of S^o during melting, a watch-glass containing 2.00 ml acetone was placed on top of the crucible for cooling and condensing the remaining toluene. The melt was agitated carefully to ensure even mixing, removed from the hot plate and immediately cooled in the air draught of a fume hood. After overnight cooling in a fume cupboard, the solidified S^o was crushed with a pestle and mortar and sieved to different particle sizes using nylon sieve cloth. There is loss of some isotope in S^o of the incorrect particle size. This can be remelted and crushed again if necessary. This $^{35}S^o$ was used in field and glasshouse experiments in Chapter 7 which were conducted for 180 days.

3.1.4 Labelling sulphate containing fertilizers

Single superphosphate (SSP) containing sulphate labelled with $^{35}SO_4^=$ was prepared by adding carrier-free $^{35}SO_4^=$ to the sulphuric acid used to acidulate phosphate rock (Nunn and Dee 1952; Siems, 1953; Young *et al.*, 1985; Bolan *et al.*, 1987). Calculations are shown in detail in Appendix 3.3. The stoichiometric equation for the reaction between apatite rock and sulphuric acid is shown in equation 1.

$$Ca_{10}(PO_4)_6F_2 + 7H_2{}^{35}SO_4 + 3H_2O \rightarrow 3Ca(H_2PO_4)_2.H_2O + 7Ca^{35}SO_4 + 2HF \quad (1)$$

A mixture of 1:1 Nauru Island:Christmas Island A grade phosphate rock was acidulated with 65% (w/w) sulphuric acid labelled with $^{35}SO_4^=$ in a food mixer at an acid:rock ratio of 0.62. The diluted sulphuric acid was preheated to 60 ^oC before mixing (Bolan *et al.*, 1987).

Labelled ^{35}S gypsum was prepared by reacting a slight excess amount of $CaCO_3$ with an equivalent amount of $^{35}SO_4^=$ labelled 50% W/W H_2SO_4 as shown in equation 2.

$$2CaCO_3 + 2H_2{}^{35}SO_4 \rightarrow 2Ca^{35}SO_4.2H_2O + 2CO_2 \quad (2)$$

The labelled $Ca^{35}SO_4.2H_2O$ precipitate and superphosphate were dried at room temperature to avoid formation of the hemihydrite ($CaSO_4.H_2O$, plaster of Paris). In the production of superphosphate and gypsum all the radioisotope was conserved in the product.

3.2 EXPERIMENTAL SITES, SOIL AND PLANT PREPARATIONS

3.2.1 Experimental sites

The research programme undertaken involved both field and glasshouse trials and was designed to examine the short-term fate of fertilizer S in naturally structured samples of pasture soil. All field experiments were conducted on permanent pasture on Massey University farms located within 15 km of the University campus. Site and soil descriptions are presented in the Material and Methods Sections of the appropriate chapters.

3.2.2 Isolating undisturbed soil cores for field and glasshouse experiments

Galvanized steel cylinders (15 cm diameter, 15 cm long, wall thickness 0.5 mm) were used to cut and contain soil cores for field and glasshouse experiments. The fifteen centimetre depth was chosen as the appropriate depth for the cores because many investigators (Gillingham *et al.*, 1980; Williams, 1988; Williams *et al.*, 1990a, 1990b) have shown that ryegrass/white clover pastures, particularly in yellow-grey earth soils, utilize insignificant amounts of nutrients from soil below a depth of 10 cm. The majority of pasture roots (85% of total weight) were found in

this zone and routine soil samples for diagnostic purposes are commonly taken from the upper 7.5 cm portion of the soil profile (Saunders *et al.*, 1988; Nguyen *et al.*, 1989a, 1989b). The diameter of the soil cores was 15 cm which was considered to be adequate to provide a representative sample of the pattern of soil macropores, i.e. naturally structured samples, in the soil used (Bouma, 1980; Williams *et al.*, 1990a, 1990b).

The cores were driven into areas of uniform ryegrass/white clover pasture at each field site. Each cylinder was pushed into a depth of about 12.5 cm; a 2.5 cm high rim was allowed to avoid the loss of rain water or liquids that were later applied to the surface during experiments. Care was taken to avoid areas of sward which had obviously been recently affected by urine or dung patches. For field experiments, cylinders in each replicate were about 10 cm apart and were fenced to prevent access by cattle. For glasshouse experiments these soil cores were dug out and carefully transported to the laboratory.

3.2.3 Sample preparations

3.2.3.1 Sheep dung sample preparation

All dung samples were dried and milled in a similar manner to herbage samples which is described below.

3.2.3.2 Soil sample preparation

Soil samples from non-radioactive experiments were air-dried at room temperatures up to 25 ^{o}C. To stop microbial activity during drying and to prevent lysis of soil micro-organisms, all soil samples from radioactive experiments were frozen and freeze-dried before milling.

All soil samples were ground in a laboratory hammer mill. To prevent contamination between samples, the hammer mill was cleaned after each sample. After milling at least 95% of the ground soil passed through a 0.5 mm sieve.

3.2.3.3 Herbage sample preparation

Herbage was dried in a forced-draught oven at 65 ^{o}C for 24 hours and weighed to constant weight. All herbage and root material was ground in a small coffee grinder and stored in sealed plastic bags awaiting analyses.

3.3 CHEMICAL ANALYSES OF SAMPLES

3.3.1 Total S In plant material

3.3.1.1 Alkaline hypobromite oxidation method

Finely ground samples of dried herbage (0.02-0.05 g), were digested with sodium hypobromite (NaOBr) solution in digestion tubes (2.5 cm in diameter and 20 cm long) on a thermostatically controlled electric aluminium digestion block. The method was based on that published by Tabatabai and Bremner (1970a). The amount of freshly prepared sodium hypobromite (NaOBr) used in this digestion varied between 4.00 ml and 8.00 ml depending on the amount of sample. In the original method 3.00 ml of NaOBr was recommended for digestion of plant sample, containing 0.010-0.050 mg of S, in a 50 ml round bottomed, digestion-distillation flask on a sand bath at 250-260 ^{o}C. Many incomplete digestions were observed, however, and the results were highly variable when using 3.00 ml of NaOBr in digestion tubes in the aluminium block. Modifications to the method were made to make it suitable for use on an aluminium block digester. A greater volume of NaOBr was added stepwise and the digestion temperature was gradually increased as described below.

1. A 1.00 ml aliquot of NaOBr was slowly added to 0.02 g of finely ground sample, containing 0.04 to 0.08 mg of S, in a dry digestion tube. The tube and contents were stored overnight in a fume hood.

2. A further 1.00 ml of NaOBr was slowly added and the tube placed into a cool digestion block and the temperature raised to 150 ^{o}C (over a period of approximately 20 minutes). The digestion was continued reducing the volume of the digest by two thirds.

3. The tubes were briefly removed from the hot plate and a further 1.00 ml of NaOBr was added, the tube contents were mixed for a few seconds using a vortex mixer. After replacing the tube in the block, the temperature was raised to 200 ^{o}c and the tube heated until one third of the volume in the tube remains. The tube was again briefly removed from the block, allowed to cool for about 5 minutes and a further 1.00 ml of NaOBr added and again the contents were mixed thoroughly.

4. When the temperature reached 250-260 ^{o}C, the digesting tube is placed into the digestion block and heated until their contents were evaporated to dryness. Heating was continued for an additional 25-30 minutes after taking the tube contents to dryness.

The tubes were removed from the digestion block and allowed to cool, and 1.00 ml of formic acid (98-100%) added. Then, the tubes' contents were diluted to a desired volume with deionized water, mixed thoroughly with a vortex mixer and left until all the sediment had settled. Aliquots of the clear supernatant were then removed for S and ^{35}S analysis as described in Section 3.3.6 and Section 3.3.8, respectively. A 0.20 ml aliquot was taken for measuring ^{35}S radioactivity.

This modification of the digestion method was evaluated using test herbage samples supplied from the International Plant Analytical Exchange, Wageningen Agricultural University, Netherlands. The result are shown in Appendix 3.1. The relationship between the analytical results, (average 36 to 229 mmoles kg^{-1}) obtained using this method 'Y' and the median value obtained by several international analytical laboratories 'X' was Y = 1.7 + 1.02X; R^2=99.2, indicating that results from this modified method agreed well with other laboratories. Although the modification method involves several handling steps, the benefit of this semimicro-digestion is that up to 100 to 150 digests can be performed in one working day using four digestion blocks, each taking 32 tubes.

3.3.1.2 LECO sulphur analyzer

The method involves the combustion of the sample in a stream of oxygen and SO_2 evolved is analysed by idiometric titration. The LECO semi-automated total S analyzer consisted of an

induction furnace (model 765-000) and sulphur titrator (model 532-000) and gas purifying train (model 516-000). Details were described by Bremner and Tabatabai (1971), Jones and Isaac (1972). This method was used for determination of total S in herbage from non-radioactive treated plots.

Results of total S in herbage using the LECO analyzer were similar to those using the alkaline hypobromite method discussed above (data not shown).

3.3.2 Soluble and extractable S in soil samples (CaCl-S and CaP-S)

The soluble and extractable S was determined by the method described by Williams and Steinbergs (1959), Searle (1979), Landers *et al.* (1983), Tabatabai (1982). Soil samples (5.00 g) were weighed into 50 ml screw capped centrifuge tubes and shaken with 40 ml of 0.04 M calcium dihydrogen phosphate [$Ca(H_2PO_4)_2$, CaP] at pH 4, for extractable S, **CaP-S** (Searle, 1979), and 0.01 M $CaCl_2$ for soluble S, **CaCl-S** (Williams and Steinbergs, 1959). The samples were shaken on an end-over-end shaker for two hours. The suspension was centrifuged at 10,000 rpm to remove suspended particulates. The supernatant was then filtered through Whatman No.42 filter paper and kept frozen in a plastic vial before the concentration of HI reducible S and the ^{35}S activity in the filtrate were determined as described in Section 3.3.6 and Section 3.3.8 respectively. Both the CaP-S and CaCl-S fractions may contain some soluble organic S. A 1.00 ml aliquot of each extract was taken for measuring ^{35}S radioactivity.

3.3.3 Total S in soil (TT-S)

The total S content of soil was determined by using a modification of the dry oxidation procedure, described Landers *et al.* (1983). The sample (0.50 g) was combined with 1 scoop (about 0.50 g) of mixed oxidant (25 g $NaHCO_3$ and 1 g Ag_2O) in a Pyrex glass tube which was 1.00 cm in diameter and 15 cm long. The contents were mixed thoroughly with a Vortex mixer. Another scoop of the mixed oxidant was layered on the top of the sample as a trap for SO_2 gas. The tube was then placed on sand material in a clay or aluminium container. The container was placed in a cold muffle furnace and the temperature was raised to 550 ^{o}C and heated for 3 hours. After cooling, the dry mixture was dissolved in 10 ml of 3 M HCl. Depending on the amount of radioactivity remaining, larger samples can be used with proportional increases in the amount of mixed oxidant. The Pyrex tube diameter and the volume of acid to dissolve the oxidized sample should also be increased. The diluted digest mixture was mixed thoroughly and left to stand overnight before the concentration of ^{32}S and the activity of ^{35}S sulphate S were determined as described in Section 3.3.6 and Section 3.3.8, respectively. A 0.2 ml of aliquot of the diluted digest was taken for measuring radioactivity.

3.3.4 Total S^o in soil samples (TT-els)

A method described by Chatupote (1990) was used. Soil samples of about 40 g containing 20-40 mg S^o, were mixed with 200 ml acetone in glass bottles and were shaken on an end-over-end shaker overnight at approximately 20 ^oC. The acetone-soil suspensions were allowed to settle. Aliquots containing 10-50 micrograms of S^o were placed in distillation tubes and evaporated to nearly dryness and the S^o content was determined as described by Nguyen (1988). In this method the S^o was converted to hydrogen sulphide by a tin and hydrochloric acid reducing mixture using the modified reduction and distillation procedure of Johnson and Nishita (1952) and the resulting hydrogen sulphide was assayed colorimetrically as methylene blue manually.

Aliquots of 1-2 ml of acetone were evaporated in a scintillation vial (Chatupote, 1990) before measuring $^{35}S^o$ activity as described in Section 3.3.8.

3.3.5 Organic S (Org-S) in soil samples

Total organic S was determined by the difference between total soil (TT-S) and inorganic soil S (CaP-S) as follows:

Org-S = (TT-S) - (CaP-S) or
= (TT-S) - (CaP-S) - (TT-els); for S^o treated plot

The error in this calculation caused by the organic content of the CaP-S extract (3-10 ppm S) is small compared to the amount of organic S (200-600 ppm S) and its natural spatial variability.

3.3.5.1 HI-reducible S (HI-S)

This was determined by the method described by Landers *et al.* (1983) by reacting a sample (usually 0.2-0.5 g) with 6.00 ml of reducing mixture, consisting of hydriodic acid, formic acid and hypophosphorous acid in the ratio of 4:2:1 in the modified Johnson and Nishita apparatus (8 unit distillation apparatus). Each sample was refluxed at 110 ^oC for 30 minutes. The reduced sulphur (H_2S) was determined colorimetrically by the methylene blue method. Radioassays of the ^{35}S activity in this fraction were determined after the methylene blue was developed as described in Section 3.3.6. A 1.0 ml aliquot of the HI-reducible S trapping solution was taken for measuring radioactivity (Section 3.3.8).

3.3.5.2 Ester-$SO_4^=$ (Est-S)

Ester-$SO_4^=$ S was determined by subtracting the CaP-S from the HI- reducible fractions (HI-S). For the samples from the S^o treated plots, the amount of S^o remaining in soil was also

subtracted from the total HI-reducible S fraction. Unoxidised S^o still remaining in the sample was also reduced to hydrogen sulphide. Hence

Est-S = (HI-S) - (CaP-S) or
Est-S = (HI-S) - (CaP-S) - (TT-els); for S^o treated plots

3.3.5.3 Carbon-bonded S (Cb-S)

The carbon-bonded S was determined as the difference between the total S and the HI reducible S (HI-S).

Cb-S = (TT-S) - (HI-S)

3.3.6 HI-reducible S in digested and extracted samples

The determination of sulphate S in aliquots from herbage digestion (Section 3.3.1.1), soil extraction (Section 3.3.2 and Section 3.3.3) and fertilizer extracts (Section 3.3.7.2) was performed using a modification of the method of Johnson and Nishita (1952) described earlier. Prior to the introduction of an automated reduction and distillation procedure in 1987, reduction and distillation was achieved using an 8 unit distillation apparatus and the hydrogen sulphide produced was determined manually by the methylene blue method. Since 1987, the total S in sulphate solutions has been analysed by using an automated reduction and distillation unit (CSIRO Division of Forest Research, Method No. PS17). The hydrogen sulphide produced after reduction in the acid mixture was determined colorimetrically and automatically by forming bismuth sulphide (Dean, 1966).

3.3.7 Determination of total S in fertilizers

3.3.7.1 S^o containing fertilizers

Solubility of S^o at 25 ^oC in toluene was about 2.018 g S 100 g^{-1} of saturated solution and 2.084 g S 100 ml^{-1} of 100% acetone solution, respectively. The toluene extract can be directly added into a scintillation cocktail which is also toluene based. Acetone, on the other hand, produces some quenching if it was not evaporated before adding to the cocktail solution (Chatupote, 1990).

A method developed by Chatupote (1990) was employed. Approximately 0.050 g of $^{35}S^o$ labelled S^o fertilizer (10-100% S) was mixed with 200 ml acetone in a 250 ml glass bottle and shaken on an end-over-end shaker overnight. After diluting, aliquots of 1-2 ml were evaporated in scintillation vials (Chatupote, 1990) before measuring ^{35}S activity as described in Section 3.3.8.

A suitable aliquot of each extract, containing 20-50 microgram of S, was used for determining the total S^o using the direct determination of S^o as described by Nguyen (1988).

3.3.7.2 Sulphate containing fertilizers

Total S in sulphate fertilizer materials was determined using a modified method described by AOAC (1984). About 0.100 g of fertilizer was mixed with 200 ml of 1:1 HCl:H_2O and shaken on an end-over-end shaker overnight. A 0.1 ml aliquot was used for measuring ^{35}S activity by mixing with 12 ml of the cocktail solution and 1 ml of deionized water (Section 3.3.8).

Aliquots of the HCl extract containing 10-50 microgram of S, were used for determining total S as described in Section 3.3.6.

3.3.8 RADIOASSAY OF ^{32}P AND ^{35}S ACTIVITIES

The radioassay of weak beta-emitters is best performed by liquid scintillation counting. A Beckman Liquid Scintillation System, model 3801, Bench-top and microprocessor-controlled, was used for the radioassay. The instrument is also equipped with an automatic quench correction programme.

The automatic quench correction programme was used for single and dual label counting (Beckman Liquid Scintillation System: Operation Manual) of ^{35}S and ^{32}P radioactivity in samples from experiments described in Chapter 4 and 5.

3.3.8.1 Liquid scintillation counting

The scintillation cocktail used in these studies was prepared by mixing 8 g of PPO (2,5-diphenyloxazole), 0.2 g of POPOP (1,4-di-[2-(5-phenyloxazole)]-benzene), 1340 ml of toluene and 600 ml of Triton-X 100 (Faires and Boswell, 1981). The mixtures were stirred overnight by a magnetic stirrer and kept in a dark glass bottle. Twenty ml glass scintillation vials were used for counting. The various aliquots of digest (described above) were mixed with volumes of deionized water, where appropriate, to give aqueous phase:scintillation cocktail ratios of 1:12. Commonly total liquid volume was 13 ml except when larger sample aliquots were required to give sufficient counts. The mixtures were mixed thoroughly and left overnight in a dark room in order to limit chemiluminescence before measurement of radioactivity.

3.3.8.2 Establishing quench curves

The liquid scintillation device is based on the direct relationship between the amount of light being emitted from the vial being linearly related to the number of beta particle emissions from the samples. In practice, a number of factors (beta particle not activating the fluor, chemicals deactivating fluor molecules, or light from the fluor being adsorbed by other chemicals in the

mixture) act to reduce the amount of light being emitted per beta emission. The phenomenon is referred to as "quenching" and a sample in which it occurs is said to be "quenched." All samples prepared in the laboratory are quenched to some degree. Therefore, the number of counts recorded must be converted to a value that correctly reflects the number of beta emissions-disintegrations that actually occurred in the sample. In these experiments, the quench curves were generated using the "H-number" method, for single and dual isotope counting (Beckman Liquid Scintillation System: Operation Manual).

Separate sets of quenched samples were prepared for each counting system (e.g. soil extracts, herbage extracts, etc.) and both isotopes ^{35}S and ^{32}P. The following quench curves were generated:

1. Sulphate S extracts - after extraction with CaP-S and $CaCl_2$ extraction

$$\mathbf{Y} = 96.09 + 0.086\mathbf{H} - 0.0006\mathbf{H}^2, \qquad R^2 = 0.93$$

2. HI-reducible S after methylene blue development

$$\mathbf{Y} = 95.20 + 0.079\mathbf{H} - 0.0004\mathbf{H}^2, \qquad R^2 = 0.94$$

3. Herbage S digests (NaOBr-digestion)

$$\mathbf{Y} = 98.33 + 0.059\mathbf{H} - 0.0005\mathbf{H}^2, \qquad R^2 = 0.95$$

4. Total S soil extracts ($NaHCO_3$ + Ag_2O digestion)

$$\mathbf{Y} = 97.46 + 0.082\mathbf{H} - 0.0006\mathbf{H}^2, \qquad R^2 = 0.94$$

5. Herbage P digests

$$\mathbf{Y} = 94.7 + 0.074\mathbf{H} - 0.0005\mathbf{H}^2, \qquad R^2 = 0.97$$

6. Extractable P (Olsen P)

$$\mathbf{Y} = 95.0 + 0.062\mathbf{H} - 0.0008\mathbf{H}^2, \qquad R^2 = 0.94$$

Where

$\mathbf{Y}$ = Counting efficiency (%)
$\mathbf{H}$ = H-number

After counting, all data were normalized to the day when the S labelled fertilizers were applied to experimental plots. Details of calculations of relevance to ^{32}P and ^{35}S activities in soil and herbage samples are described in the appropriate sections.

CHAPTER 4

EVALUATION OF THE ROLE OF SHEEP DUNG IN THE SHORT TERM IMMOBILIZATION OF SOIL AND FERTILIZER SULPHUR

4.1 INTRODUCTION

Any study which attempts to measure the immediate fate of fertilizer under pastoral field conditions needs to consider the whole nutrient cycle and factors, which in the short term, may influence the direction of nutrient flow. Once sulphate enters the plant root and is assimilated into shoot S, the above ground nutrient flows (as modelled by Saggar *et al.*, 1990a, 1990b) are similar whether S is applied as S^0 or sulphate-S fertilizer (Figure 4.1). Thus factors which are likely to play important roles in determining whether S^0 is conserved more efficiently in soil than sulphate-S must influence the S transformations which generate plant available sulphate in the soil or affect the rate at which the sulphate pool is depleted by microbial immobilization, leaching and plant uptake. Some of these factors, which have a large influence on short term nutrient flow, are: climate, plant species, soil type and structure and defoliation frequency (grazing interval). These factors can be easily simulated and their effects studied in small plot or undisturbed soil core experiments. The effects of excretal return from the grazing animal are less easy to simulate. Some researchers, however (Smith R.G and M.J. McLaughlin, personal communications), have used the return of dung (or dung products) on field plots and glasshouse pots to simulate excretal return. In general, however, little information is available on the immediate effect of sheep dung on the uptake of S by pasture plants.

The return of animal excreta is not easily simulated in small scale experiments. This is partly because on the field scale the grazing animal returns excreta, containing large amounts of growth-limiting nutrients, unevenly across a field. The irregular pattern of return depends on topographical and climatic influences upon the animals' grazing and camping behaviour (Hilder, 1964; Gillingham, 1980; Gillingham *et al.*,, 1980; Rowarth, 1987; Rowarth *et al.*, 1985, 1988, 1990; Saggar *et al.*, 1990a, 1990b). Furthermore large nutrient (particularly nitrogen) returns in excreta applied to a small trial plot would have a major influence over nutrient availability and pasture production, probably confounding or obscuring the influence of the rate of a fertilizer S treatment. The return of S to soils as animal excreta, however, remains a major pathway of S in the S cycle of grazed pastures. Experiments conducted with sheep to measure the effect of their excreta on pasture production have shown that the return of sheep dung gave an 18% increase in dry matter production (Sears and Goodall, 1948), whilst Watkin (1954) found that sheep dung made no contribution to pasture production, and only observed an effect in combination with a high rate of nitrogen fertilizer. Site specific results such as these are expected because the responsiveness of pasture growth to dung return will partly reflect the availability of nutrients in the dung and current soil nutrient status.

In a study in New South Wales, Australia, using ^{35}S label gypsum, it was calculated that the excreta of 20 sheep per hectare could provide 20% of the S requirement of pasture plants over a grazing period of 120 days (Kennedy and Till, 1981a). Of the S excreted by sheep approximately 60% is excreted in the urine and 40% in dung (Till, 1975). Dung has a C : S ratio at >200 : 1. The return of excreta to a pasture soil may influence the immediate fate of fertilizer S in the following manner:

1. Increased pasture and root growth, stimulated mainly by the excretal nitrogen, could increase the plant uptake of soil and fertilizer S, or accelerate the conversion of sulphate S to organic S forms (Barrow, 1967b; Curll, 1982; Boswell, 1983; Goh and Nguyen, 1990; Haynes and Williams, 1991).

2. The carbon added to soil as undigested herbage in dung has been shown to decay slowly (Barrow, 1961b; Boswell, 1983) in soil and therefore could act as a carbon source used by micro-organisms to immobilize free sulphate-S from the soil solution. Goh and Nguyen (1990) have suggested that excretal returns could stimulate soil micro-and macro-flora and fauna such that S^o oxidation may increase.

Research on the spatial distribution of excreta has been undertaken for cattle (Petersen *et al.*, 1956; Richard and Wolton, 1976) and for sheep (Tallis and Donald, 1964; Donald and Leslie, 1969; Gillingham and During, 1973; Gillingham, 1978; Gillingham, 1980; Gillingham *et al.*, 1980; Thorrold *et al.*, 1985). Recently, in New Zealand Morton and Baird (1990) have shown that the spatial distribution of sheep dung in relation to stocking rates was best described by a negative binomial function. There was significantly more aggregation of dung patches at lower stocking densities than at higher stocking densities. These authors considered the returns of nitrogen from dung and urine affected an insufficient area to influence pasture growth. In addition, Rowarth, (1987) and Rowarth *et al.* (1985, 1988 and 1990) have shown the major mechanisms controlling the movement of P from sheep dung into soil was the rate of physical break-down of sheep dung rather than the leaching of P from the dung sample. During the winter/spring period, the physical break-down take place within a month, as a result of high rainfall and biological activity, whereas in the summer/autumn period the dung persisted for approximately three months. During a short period (17 and 8 weeks for autumn and spring, respectively) dung P was less available to pasture than monocalcium phosphate. There is no information describing the influence of sheep dung on the subsequent fate of soil and fertilizer S in the zone of excreta-affected soil. Although Kennedy and Till (1981a) and Boswell (1983) have studied the fate of dung S alone.

Thus, before proceeding with the main experiments to examine the immediate fate of fertilizer S in pastoral soils grazed by sheep, preliminary experiments have had to be carried out to determine:

a. the likely area of pasture influenced by sheep dung return and
b. the influence of dung return on the short term fate of fertilizer S.

These experiments also served to evaluate radioisotope handling and measuring techniques.

4.2 EXPERIMENTAL OBJECTIVES

1. To determine the area of soil influenced by sheep dung by observing the uptake of ^{35}S and ^{32}P isotopes by pasture at different radial distances from radioactively labelled sheep dung pellets.

2. To determine the influence of sheep dung on the growth of unfertilized pasture and pasture fertilized with S^0 or sulphate-S fertilizer (superphosphate).

4.3 MATERIALS AND METHODS

4.3.1 EXPERIMENT 1, AREA OF PASTURE INFLUENCED BY SHEEP DUNG

4.3.1.1 Design of the experiment

The experiment was conducted on a permanent clover/ryegrass pasture growing on a Tokomaru silt loam (Typic Fragiaqualf) at Keeble farm, three kilometers southwest of the Massey University campus. Some soil properties are presented in Table 7.1 and Section 7.3.1. The pasture was mown to approximately 3 cm height at the beginning of the experiment and the trial area was fenced off to prevent access by cattle. On June 27, 1985, three rates of dung weighing 0.5 g(D1), 1.0 g(D2) and 2.0 g(D3) (dry weight basis), labelled with ^{35}S and ^{32}P (as described below), were randomly applied to the surface of the pasture soil. Steel pins were used to mark the position of the dung. Each dung application was replicated five times. Each plot was approximately 1.0 square meter. There were a total of 15 circular plots in this experiment.

Plots were harvested twice at 30 and 60 days after dung application. A jig of concentric rings, 10 cm(R1), 15 cm(R2), 25 cm(R3) and 30 cm(R4) in radius, was centred around the steel pins remaining in each plot. The pasture within each concentric ring was harvested separately using electric hand shears. All pasture samples were dried at 80 ^oC, weighed and ground to pass through a 1.0 mm sieve.

Weekly rainfall, temperature data and sunshine hours were used to calculate the amount of drainage water (Scotter *et al.*, 1979) over the period of the experiment (Appendix 4.7).

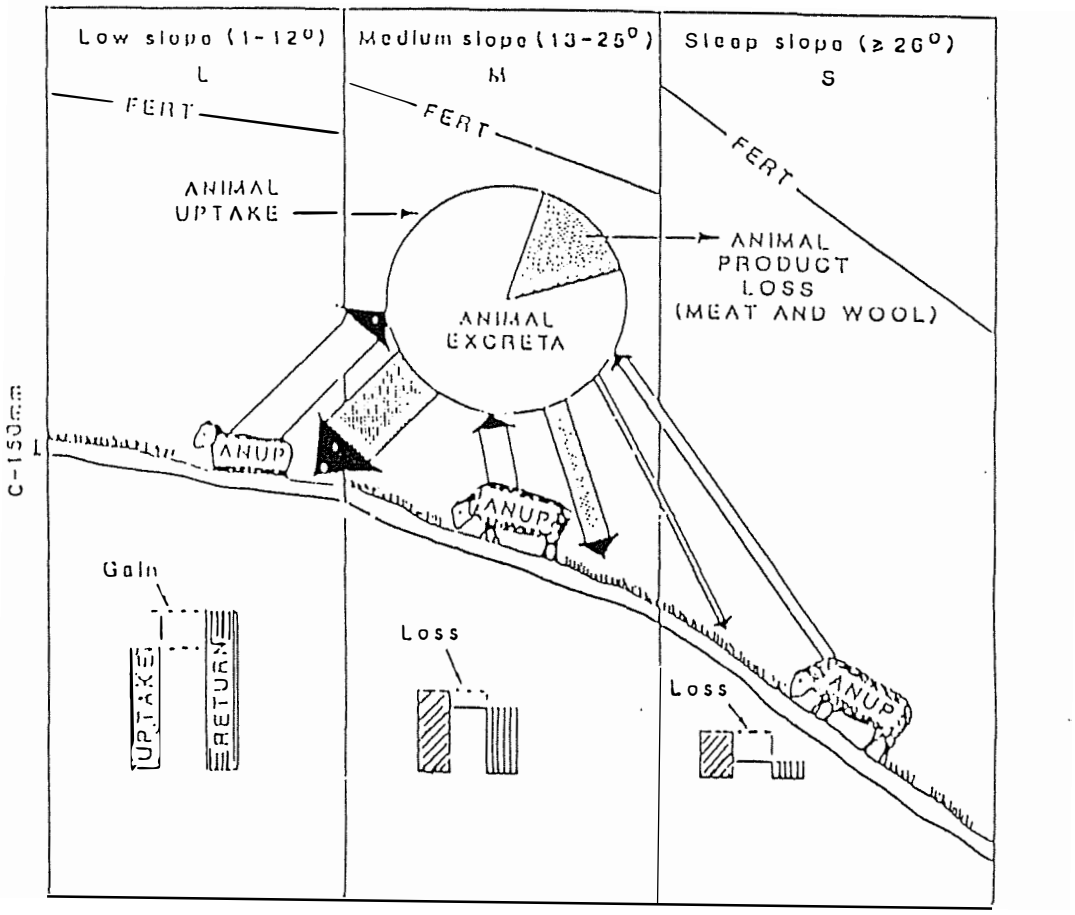


Figure 4.1 A schematic illustration of nutrient transfers in a grazed hill-country pasture (Saggar et al., 1990a).

4.3.1.2 Labelling of dung with ^{35}S and ^{32}P

Aqueous solutions of carrier free ^{35}S sulphate and ^{32}P orthophosphate were obtained from the Radiochemical Centre, Amersham, U.K.

Three milliliters of the ^{35}S sulphate solution (approximately 26.337 MBq ml^{-1}) were mixed with 8.0 ml of ^{32}P solution (containing approximately 7.225 MBq ml^{-1} ^{32}P) and the mixture was then diluted with deionized water to 20.0 ml. Aliquots of the mixture (0.9 ml per gram of dung) were thoroughly mixed with pre-weighed samples of finely ground sheep dung (0.5, 1.0 and 2.0 g) in glass capsules (0.5 g dung is equivalent to 2-3 sheep dung pellets). The labelled dung samples were incubated at room temperature for two weeks before being applied to the field plots. During the incubation period, deionized water was applied to the dung in the glass capsules to keep the dung at a 30-40% moisture content.

4.3.1.3 Total and extractable S and P in dung

The S and P fractions in the incubated dung samples were measured using three replicates of dung samples (Table 4.1). Almost 90% of the ^{35}S and 54% of the ^{32}P in the dung were in a soluble form. Calcium phosphate extractable sulphate accounted for approximately 10% of the total dung S and Olsen extractable P accounted for 43% of total P in the dung. This proportion agreed with other values reported in the literature (Kennedy and Till, 1981a; Bromfield, 1961 and Barrow and Lamboume, 1962). The dung sample contained 0.3% of total S and 1.27% of total P. Based on the specific activity of the soluble fractions it was calculated that the ^{35}S isotope had exchanged with about 10.2% of the total S in the dung whereas the ^{32}P had exchanged with 80% of total P in the dung (Section 4.3.5)). Therefore the incubation technique resulted in dung P being more evenly labelled with isotope than dung S.

Losses of both radioactive tracers during the incubation were negligible.

Table 4.1 Mean S and P concentrations, total radioisotope activity and specific activity (SA) of ^{35}S or ^{32}P in dung and extracts of dung (results expressed on dung dry weight).

S forms	Concentration of S or P in dung mg kg^{-1}	Total activity of ^{35}S or ^{32}P MBq g^{-1} dung	% of total activity %	S.A[a] MBq mg^{-1} S or P	Exchangeable pool[b] as % of total S or P %
TOTAL POOL					
Total S	3000 (9.02)[c]	3.570 (0.059)	100.0	1.190	-
Total P	12706 (12.87)	2.588 (0.035)	100.0	0.204	-
EXCHANGEABLE POOL					
Cap-S	275 (4.22)	3.195 (0.055)	89.94	11.618	10.20
Olsen-P	5454 (7.30)	1.392 (0.035)	53.79	0.255	80.00

[a] *calculated as described in Section 4.3.4c*

[b] *calculated as described in Section 4.3.4a*

[c] *numbers in parenthesis are standard errors of means*

4.3.2 EXPERIMENT 2, EFFECT OF SHEEP DUNG ON GROWTH AND YIELD OF PASTURE

4.3.2.1 Design of the experiment

This experiment, designed to examine the effect of sheep dung on the growth of an unfertilized permanent pasture and a pasture fertilized with superphosphate and elemental S (S^0), was conducted at the same field site described in Chapter 5. Treatments consisted of two dung rates: 0 and 375 kg dung ha^{-1} and three fertilizer treatments: control (no fertilizer), superphosphate (SSP, 11.9% S) and very fine agricultural grade S^0 (99% S, <0.5 mm in diameter) applied at 30 kg S ha^{-1}. Treatments were replicated four times. Fertilizers were broadcast evenly on the plots which were 2.5 m by 1.5 m. The plots were then subdivided into two areas of 2.0 m by 1.5 m and 1.5 m by 0.5 m. The smaller area received dung applications. Pasture on the plots was mown to a height of 2.5 cm one week prior to dung and fertilizer applications. Ground sheep dung (total S = 2640 mg S kg^{-1} of dung and calcium phosphate extractable S = 244 mg kg^{-1} of dung) was applied at the rate of 375 kg ha^{-1} at harvest (equivalent to an amount of dung return from 25 sheep ha^{-1}, assuming that there were 500 g of dry excretion per sheep per day (Cleland A., personal communication). Sheep dung was reapplied every 30 days after herbage harvesting. Superphosphate, S^0 and KCl (at 40 kg K ha^{-1}) were applied on October 14, 1985. Finely ground monocalcium phosphate was applied on the S^0 plot at a rate (25 kg P ha^{-1}) equivalent to the amount of P in the superphosphate treatment. Sheep dung was first applied on November 18, 1985, after the first harvest. Plots were harvested five times with a 30 day interval between mowings. Electric hand shears were used to cut the pasture at 2.5 cm above the ground level inside a quadrant (40 x 40 cm) which was randomly placed within each plot. The pasture in each plot was harvested separately. All pasture was dried at 80 ^oC and weighed. A subsample (approximately 25 g) was taken for grinding and subsequent chemical analysis.

4.3.3 Chemical analyses

4.3.3.1 Total S and P in pasture samples

The determination of total S and ^{35}S activity in pasture samples were described in Section 3.3.1.1 and 3.3.1.2 (for experiment 1 and 2, respectively)

The total P and ^{32}P activity in plant samples were determined after a Kjeldahl-type digestion. The P concentration in the digested solution was measured using the method of Murphy and Riley (1962). Absorbances were measured at 712 nm using a Pye Unicam SP 1800 spectrophotometer. A 1.0 ml aliquot of the diluted digest solution was taken for measuring ^{32}P activity using a dual label counting program as described in Section 3.3.8

4.3.3.2 Total S and P in dung samples

Total S, P, ^{35}S and ^{32}P activities in dung samples were determined using the digestion method of Dick and Tabatabai (1977). Total ^{35}S and ^{32}P activity in the digest solution were measured simultaneously using a dual label counting program as described in Section 3.3.8.

4.3.3.3 Extractable S in dung samples

Wet dung samples (moisture content between 30-35%) were mixed with 10 ml 0.04 M $Ca(H_2PO_4)_2$ in 50 ml polyethylene tubes. The samples were then shaken on an end-over-end shaker for 2 hours, centrifuged at 10,000 rpm for ten minutes (SS34 head, RC2B Sorvall centrifuge) and filtered through No. 42 Whatman filter paper. A 4.0 ml aliquot of the extract was used for determining sulphate-S concentration by a modified method of Johnson and Nishita (1952) as described in Section 3.3.6. A 1.0 ml aliquot of the extract solution was taken and its ^{35}S activity was measured, as described in Section 3.3.8.

4.3.3.4 Exchangeable P in dung samples

Wet dung samples (moisture content between 30-35 %) were mixed with 10 ml of 0.5 M $NaHCO_3$ (Olsen *et al.*, 1954), shaken on an end-over-end shaker for 0.5 hour , centrifuged at 10,000 rpm (as above) for 10 minutes and filtered through No. 42 filter paper. A 1.0 ml aliquot was used for determining the concentration of P as described by Murphy and Riley (1962). Also 0.1 ml of extract solution was used for determining the ^{32}P activity as described in Section 3.3.8.

The amount of exchangeable S and P calculated to be in the dung were reported on a dry weight basis.

4.3.3.5 Radioassay of ^{35}S and ^{32}P activities

The method has been described in Chapter 3, Section 3.3.8. A dual counting program was used.

4.3.4 Presentation of results (method of calculation)

All radioactivity data were normalized to the day when the dung treatments were applied (day 0; June 27, 1985), using the formula:

$$A_0 = A_t * \exp(t * 0.693/T) \quad \text{Bq}$$

where

A_0 = radioactivity at day 0
A_t = radioactivity at time **t** (days)
t = time from day 0
T = half life of the isotope (days): ^{35}S=87.5 days; ^{32}P=14.3 days

a. Extractable plus recently immobilized pool of S or P in dung

These are the percentage fractions of dung S or P that exchanged with each radioisotope added (i.e. the exchangeable S or P pool in dung plus that which was recently immobilized into an organic form).

$$EI = TS/ES * 100 \quad \text{percent}$$

where

EI = percent of activity that had been exchanged and recently immobilized
TS = specific activity of total pool in dung (total activity / total nutrient content, Bq/mg)
ES = specific activity of the extracted fraction (activity in extract / amount of nutrient in extract, Bq/mg; extractions were described above).

b. Percentage recovery of radioactivity in plant materials

Rc = **Ac*Dm/B***100 percent

where
Rc = recovery percentage
Ac = activity of radioactive nutrient per gram of plant (Bq g^{-1} plant)
Dm = total dry matter of plant (g)
B = total radioactivity applied to dung (Bq)

c. Specific activity of radioactive nutrients in pasture and dung

SA = **Ac/Sc** Bq mg^{-1} nutrient

where
SA = specific activity of the radioactive nutrient, ^{35}S or ^{32}P
Ac = activity of radioactive nutrient in plant or dung (Bq g^{-1} of plant)
Sc = nutrient content (mg S or P g^{-1} plant)

d. Percentage of plant S and P derived from dung

Pasture plants can derive nutrients from the extractable pool of nutrients in the dung and an unknown amount of organic nutrient in the dung which will mineralize during the field trial. During the pre-incubation the dung S and P were incompletely labelled with ^{35}S or ^{32}P. Boswell (1983) has shown that the mineralization of organic S from radioactively labelled sheep dung in moist soil was extremely slow, about 4.5 mg S day^{-1} g^{-1} dung S during a period of 0-37 days. Two dung specific activity values were used to calculate the percentage of plant nutrients derived from the dung. This is because a degree of uncertainty exists about the specific activity of the plant available pool in dung. Firstly, the specific activity of the extractable pool of S and P in the dung after pre-incubation and before addition to the soil was used. This represents a situation where no mineralization of unlabelled dung S and P occurred after dung addition to the soil and the highest specific activity of the S and P pools available for plant uptake. Secondly, a specific activity calculated using the total amount of S and P in the dung was used which represents the highest amount of mineralization of organic nutrient and the lowest specific activity of the S and P pool available for plant uptake.

%NDFD = **SAp/SAd***100 percent

where
%NDFD = percent of plant S and P derived from dung
SAp = specific activity of radioactive ^{35}S or ^{32}P in plant (Bq mg^{-1} of S or P)
SAd = specific activity of radioactive ^{35}S or ^{32}P in the extractable pool in the dung or the total nutrient specific activity of the dung (Bq mg^{-1} of S or P)

4.3.5 Statistical analyses

Analysis of variance was performed on data obtained from the treatments (three rates of dung application with pasture sampled at four radial distances away from the dung) arranged in a split plot design. The SAS (SAS Institute Inc., 1985) and Minitab (Minitab Inc., 1989) programmes were employed to process the data.

4.4 RESULTS

4.4.1 EXPERIMENT 1, AREA OF PASTURE INFLUENCED BY SHEEP DUNG

An area of pasture significantly influenced by a dung spot should either have a higher pasture yield, higher S and P uptake than surrounding area or have derived a significant amount of S and P from the dung instead of the soil. The following Section (Section 4.4.1.1) presents results used to evaluate these effects.

4.4.1.1 Pasture yield

There was no effect of dung weight nor the proximity of pasture to the dung on the yield of pasture (Table 4.2). This indicates an adequate supply of native soil S, probably derived from the previous superphosphate applications made annually to the trial site. Pasture yields from the second harvest, however, were greater than those of the first harvest. This was probably due to the more favorable conditions of both temperature and moisture during the second growth period. Pasture yields at both harvests during the winter period were much lower than those of the second experiment (Section 4.4.2) which was carried out during the spring-summer season (Appendix 4.7). The slower winter growth rate may have contributed to the lack of pasture response to the dung.

4.4.1.2 S and P concentrations in and their uptake by pasture

The S and P concentrations of mixed pasture from all plots ranged from 0.17% to 0.30% and 0.28% to 0.30%, respectively. At the first harvest, the highest dung rate (D3) produced higher pasture S and P concentrations than the lower dung rates (D1 and D2), as shown in Table 4.3. At the second harvest, pasture from the lowest dung rate (D1) had a lower mean S concentration than that of the higher rates. In general, S concentrations in the pasture at both harvests, and P concentrations in the pasture at the first harvest, increased with the increasing rate of dung applied. The concentration of both elements in the pasture at increasing distances from a dung patch (R1, R2, R3 and R4), however, showed no significant change. The S content in pasture from the second harvest was higher than that of the first harvest. Furthermore, as a consequence of the higher pasture yield at the second harvest, the total uptake of S by pasture in the second harvest was more than twice that of the first harvest.

Although the concentrations of S in the pasture at the first harvest were low, ranging from 0.17-0.21%, the total uptake of both elements showed no effects of dung rate (D) or proximity (R) of pasture to the dung at either harvest (Table 4.3). The application of higher dung weights yielded higher cumulative (first plus second harvest, 1+2) uptake of S. There were no significant interactions between dung rate and total uptake of both nutrients at different distances from the dung.

4.4.1.3 Uptake of ^{35}S and ^{32}P by pasture

The pattern of isotope uptake must be considered prior to calculating the amount of plant S and P derived from the dung.

4.4.1.3.1 Pattern of ^{35}S uptake

The total activity of ^{35}S recovered per unit area of pasture at both harvests increased with increasing amounts of dung added (Figures 4.2B and 4.2C, and Appendix 4.1), but increasing amounts of dung added had no significant effect on the percentage of added ^{35}S recovered in pasture at both harvests (Figures 4.3B and 4.3C, and Appendix 4.2). Even though the harvested surface area was smaller in the central ring, the total recovery of ^{35}S and therefore the percentage of added ^{35}S recovered in pasture of the central ring at both harvests were markedly higher than that recovered in each of the outer rings (as shown in Appendix 4.2). At the second harvest, larger percentages of ^{35}S were recovered by the pasture in areas R2, R3 and R4 than at the first harvest (data in Appendix 4.2 and Figure 4.3B compared to Figure 4.3C). This is partly due to the higher dry matter yield and S content of pastures in these areas at the second harvest. There may also have been more lateral movement of $^{35}SO_4^=$ away from the dung by the second month. The soil used in this study has little capacity to retain sulphate-S (P-retention = 20-30%, see Appendix 7.14). During and Martin (1968) and Kennedy and Till (1981b) had shown that there was rapid lateral movement of ^{35}S out of the center zone where ^{35}S labelled gypsum was applied to pasture soil.

The total recovery of isotope in the pasture expressed as the amount of activity per square centimeter of harvested pasture showed significant interactions between dung rates and distances of pasture from the dung, as shown in Figures 4.2B and 4.2C, and Appendix 4.1. The percentages of added ^{35}S which were recovered in the pasture, however, showed no significant interaction between the rate of dung added and the distances of the pasture from the dung, as shown in Figures 4.3B and 4.3C, and Appendix 4.2.

4.4.1.3.2 ^{35}S specific activity and percent of plant S derived from dung (%SDFD)

The specific activity of ^{35}S in the harvested pasture (Appendix 4.3) and the specific activity of either the extractable-S pool or the total pool of S in the dung (Table 4.1) were used to estimate the percent of plant S which was derived from the dung. The results presented in Figures 4.4B and 4.4C and Appendices 4.3 and 4.4 show that at both harvests the percentage of plant S derived from dung (%SDFD) increased with increasing dung application rate and also increased as proximity to the dung increased. The specific activity of the ^{35}S in pasture and %SDFD were influenced significantly by an interaction between dung rate and distance (Appendices 4.3 and 4.4, Figures 4.4B and 4.4C).

The percentages of plant S that were derived from dung in the second harvest were, in general, about 50% of the first harvest (Figures 4.4B, 4.4C and Appendix 4.4).

In addition to the percent of plant S derived from dung calculated using the specific activity of the extractable sulphate pool (Figure 4.4, Appendix 4.4), the %SDFD was also calculated using the specific activity of the total pool (Appendix 4.3) because some of the non-exchangeable pool remaining in the dung might be mineralized in the field after application. Table 4.1 shows the activity of the ^{35}S in the exchangeable pool represents almost 90% of the total activity and this fraction represents 10% of the total dung S. There were ten-fold differences between the values produced by using the different specific activities in the %SDFD calculations (Appendix 4.3). It appears unlikely that much organic dung S would mineralize during the span of this experiment because only about 0.03-0.04% of sheep dung S mineralized during the first 100 days of an experiment reported by Boswell (1983). Therefore, the %SDFD, calculated using the specific activity of the extractable pool, may be a more valid estimate than that calculated using the specific activity of the total dung S.

It should be pointed out here that the percentages of S derived from dung shown in Appendix 4.4 were the average value for particular zones away from the dung (0-10 cm(R1), 10-15 cm(R2), 15-25 cm(R3) and 25-30 cm(R4)). In the 0-10 cm zone the value was less than 0.5%. In fact, the %SDFD might be greater at a closer proximity to the dung. Unfortunately, it was impossible to harvest pasture closer to the dung. A mathematical procedure, however, was used to predict the %SDFD at distances less than 10 cm away from the dung. Isotopic uptake data from the D3 treatment were used to derive a relationship for the cumulative amount of plant S derived from the dung as distance from the dung increased. This relationship was integrated for distance intervals of 1 cm instead of the original 10, 15, 25 and 30 cm distances. In each case, the predicted total cumulative ^{35}S derived from the dung was within 1% of the values measured at the 10, 15, 25 and 30 cm distances. The results are shown in Figure 4.5A and Figure 4.5B. Using these figures, the average amount of pasture S derived from dung during the first 30 days in a zone 1 cm away from the dung is about 2%, if the specific activity of the extractable pool was used and 17% if the specific activity of the total pool was used.

4.4.1.3.3 Pattern of ^{32}P uptake

Data on P uptake was available only from the first harvest because thereafter the ^{32}P activity in the pasture was too low to determine with any accuracy. The total recovery per unit area and recovery percentage of ^{32}P were lower than those of ^{35}S (Appendices 4.1 and 4.2, Figure 4.2A compared to Figuures 4.1B and 4.1C). Most of the activity remained in the center rings (R1) and the amount recovered by pasture growing in the center ring increased with the dung rate (Appendix 4.2 and Figure 4.3A). However, the dung rates had no effect on the total percentage recovery of ^{32}P by plants from all rings. More activity was recovered in the second ring of the higher dung rate; showing that a significant interaction occurred between dung rate and distance of pasture from dung (Figure 4.2A).

4.4.1.3.4 *^{32}P specific activity and percent of plant P derived from dung (%PDFD)*

The specific activity of ^{32}P in pasture (Appendix 4.3) was used in the same manner as ^{35}S to calculate percentages of plant P derived from dung (%PDFD). The calculation showed that the %PDFD were much higher than percent of plant S derived from dung, %SDFD, (Appendix 4.4, Figures 4.4A, 4.4B and 4.5C). The dung application D1 gave lower %PDFD than higher dung rates (D2 and D3). The %PDFD in the center ring (R1) was much higher than those of the outer rings, as shown in Figure 4.4A.

There were only small differences between the %PDFD calculated using either the specific activity of the exchangeable or total P pool, as shown in Appendix 4.3 and Figures 4.5A and 4.5B. Approximately 42% of total P was in the extractable pool and 53% of the total ^{32}P activity was in this pool (Table 4.1). It has been suggested that the fraction of organic S and P in dung contributes little to plant nutrition in the short term (McAuliffe *et al.*, 1949a, 1949b; Bromfield, 1961; Boswell, 1983; Rowarth, 1987; Rowarth *et al.*, 1985, 1988, 1990). Thus, the %PDFD calculated using the ^{32}P specific activity of the extractable P pool was considered to be a more valid estimate.

4.4.2 *DISCUSSION, EXPERIMENT 1*

Prior to conducting this experiment it was envisaged that all other conditions being equal, the area of pasture soil influenced by dung will mainly depend on the following factors:

1. The average maximum radial distance that roots, effective in nutrient uptake, extend from pasture species. The radial distance that roots extend horizontally in a soil is a function of the pasture species and physical soil properties that influence root ramification through soil (i.e. site dependent).

2. The radial rate of movement of nutrients away from the dung patch into the soil is influenced by the amount of soluble nutrient present in the dung or the weight of dung deposited. In this respect, nutrients which are not strongly adsorbed by soil surfaces and have larger coefficients of diffusion through soil may be expected to move to greater distances from the site of dung deposition. Therefore sulphate was initially expected to have a greater radial distance of diffusion than phosphate and lead to a greater recovery by plants. As outlined below this expected result did not occur.

The average amount of^{35}S and ^{32}P activity in the pasture in each ring, $^{35}S(R1)$, $^{35}S(R2)$, $^{35}S(R3)$, $^{35}S(R4)$, $^{32}P(R1)$, $^{32}P(R2)$, $^{32}P(R3)$, and $^{32}P(R4)$, expressed as the percent of total activity taken up, $\Sigma^{35}S$ or $\Sigma^{32}P(R1+R2+R3+R4)$, from each dung application (D1, D2, and D3) is shown in Table 4.4. After the first 30 days, the largest amounts of ^{35}S activity

taken up by the pasture were highest in the center zone (R1). However, the percentage of ^{35}S activity taken up (or the %SDFD at 30 days, Figure 4.4B) in the outer zones, R3 and R4, were smaller than the percentage of ^{32}P taken up (or %PDFD at 30 days, Figure 4.4A). Unexpectedly, the plant uptake of ^{32}P had less activity in the central zone but more in the outer zone than that of plant ^{35}S activity. This suggested that lateral diffusion of the ^{35}S was slower than that of ^{32}P during the first 30 days. Despite the fact that the amount of ^{35}S activity in the labelled dung present in an extractable S pool (CaP-S) was about 40% higher than that the activity of ^{32}P in a similar pool (Olsen P) (Table 4.1). In addition, by the second harvest (60 days after application) the amounts of ^{35}S in the pasture in the outer rings were about twice as much as the amount of ^{35}S taken up in the first 30 days but were 30-50% less than the amount of ^{32}P taken up after the first 30 days. This also provides more evidence for the unexpected slow lateral movement of labelled sulphate S (see Table 4.4). This result was unexpected and required the diffusion theory, discussed above, to be examine closely (Appendix 4.5).

The calculation conducted in Appendix 4.5, however, demonstrated that the initial rates of P and S diffusion away from the dung should be similar despite the smaller diffusion coefficient for P in soil because of a much greater gradient in P concentration between dung and soil than S gradient. Thus the differential rates of diffusion did not explain the observed differences in ^{32}P and ^{35}S recovery by plants

A large drainage event (140 mm) occurred during the experimental period (Appendix 4.7). Some of the exchangeable $^{35}SO_4^=$ in the dung could have been leached beyond the 10 cm soil depth. As most of the root activity is in the top 10 cm of the soil profile (Hedley *et al.*, 1992, personal communication) leaching would have reduced the recovery of sulphate by the plants. In contrast, the ^{32}P present as exchangeable P in the dung would be strongly adsorbed by the soil surface and would not be susceptible to leaching. A greater amount of the added ^{32}P may remain in the active root zone and this may have caused the ^{32}P uptake pattern to have a slightly wider spread than the ^{35}S uptake at the first harvest (Table 4.4).

Therefore, the results suggest that the relatively lower recovery of ^{35}S than ^{32}P activity, taken up by plants at greater distances away from dung, may result from: (a) a greater leaching loss of ^{35}S than ^{32}P or (b) that roots at greater radial distances from the mother plant take up P more actively than S.

Although S concentration in pasture increased with dung application rates, there was no significant effect of dung rate on plant yield nor on the radial distribution of pasture S concentration around the dung. Consequently, total uptake by the pasture did not increase as a result of dung application. This agreed with the results from the second experiment (see Section 4.4.2).

The %SDFD in the zone closest to the dung patch was less than 2% (Figure 4.5A). Furthermore, the influence of dung S, measured as %SDFD decreased toward the outer rings.

As mentioned above, the lateral movement of sulphate S was slow, thus in the short term (30 days) the area of pasture significantly influenced by the dung is expected to be less than 5.0 cm away from the dung.

Although the percentage recovery of ^{35}S in plant at both harvests was higher than that of ^{32}P, the %SDFD was much lower compared to that of plant P. As discussed earlier, larger amounts of soil sulphate, including $^{35}SO_4^=$ were probably leached during the heavy rain events (Hogg and Cooper, 1964; Hogg and Toxopeus, 1966; Gregg and Goh, 1978, 1979) or was transformed into organic forms before being utilized and assimilated by pasture plants (Freney *et al.*, 1971; Gregg, 1976).

More soil S was taken up by plants in the innermost zone. This was probably caused by a synergistic effect of other elements particularly dung N. Generally, it can be seen that dung application did provide some S to plants but only in the zone closest to the dung and this did not have a significant effect on total S uptake. This also suggested that the soil used in this trial had sufficient available S. Extractable S in the top 3 cm was approximately 15 mg S kg^{-1}, a level at which pastures are not expected to show S deficiency (Nguyen *et al.*, 1989a, 1989b).

4.4.3 EXPERIMENT 2, EFFECT OF SHEEP DUNG ON GROWTH AND YIELD OF PASTURE

4.4.3.1 Pasture yield

Pasture yields (group means) at four harvests for different dung and fertilizer applications are shown in Table 4.5A. Neither the application of dung nor fertilizers produced a significant yield response at any harvest, and there were no significant interactions between dung application and the type of fertilizer used (S^0 or superphosphate). Compared to the first experiment carried out in winter, pasture yields were much higher, which was probably due to the more favourable temperature during the spring and summer seasons (Appendix 4.7).

4.4.3.2 Plant S concentration

There was no significant effect of sheep dung on plant S concentration (Table 4.5B). Throughout the season, pasture on control plots had a significantly lower S concentration than that of the fertilized plots. At the second harvest only, superphosphate fertilized plots yielded significantly higher pasture S concentrations than elemental S fertilized plots. Thereafter, no significant differences between the two treatments were observed, nor did any significant interaction between dung and type of fertilizer occur.

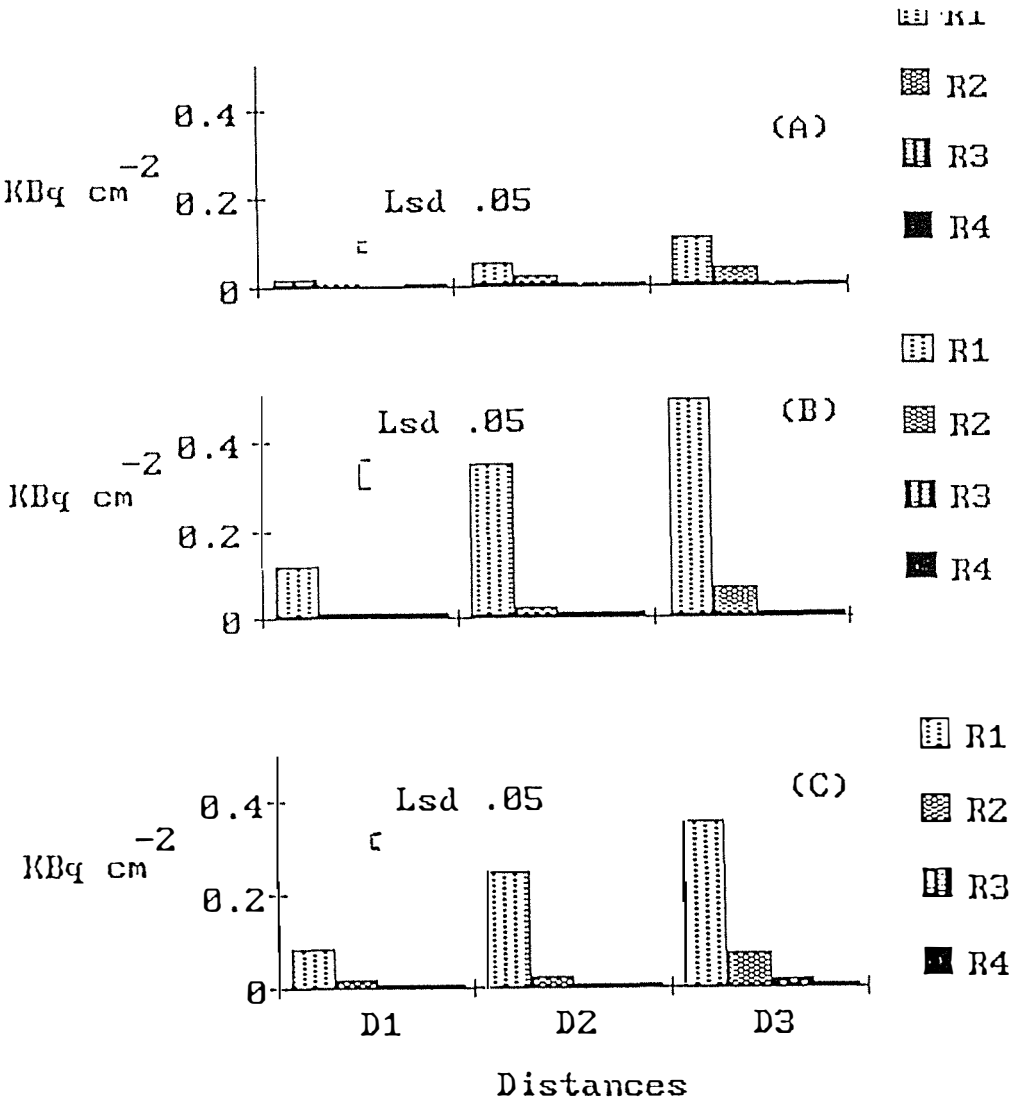


Figure 4.2 Radioactivity per unit area in harvested pasture at four radial distances (R1, R2, R3 and R4) from radioactively labelled dung applied at three rates (D1, D2 and D3); A = ^{32}P and B = ^{35}S at the first (30 days) harvest and C = ^{35}S at the second harvest (60 days).

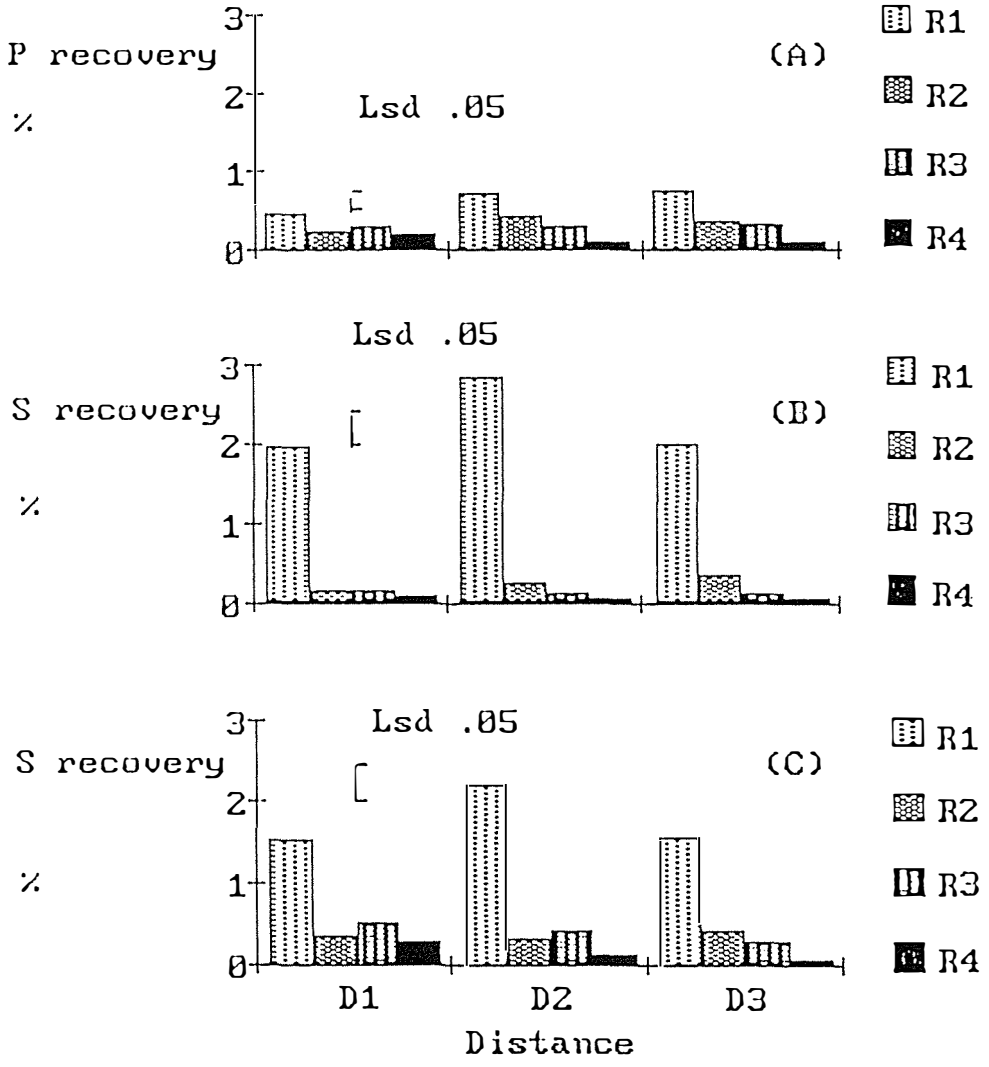


Figure 4.3 Total percent recovery of ^{32}P and ^{35}S in pasture harvested at four radial distances (R1, R2, R3 and R4) from radioactively labelled dung applied at three rates (D1, D2 and D3); A = ^{32}P and B = ^{35}S at the first harvest (30 days) and C = ^{35}S at the second harvest (60 days).

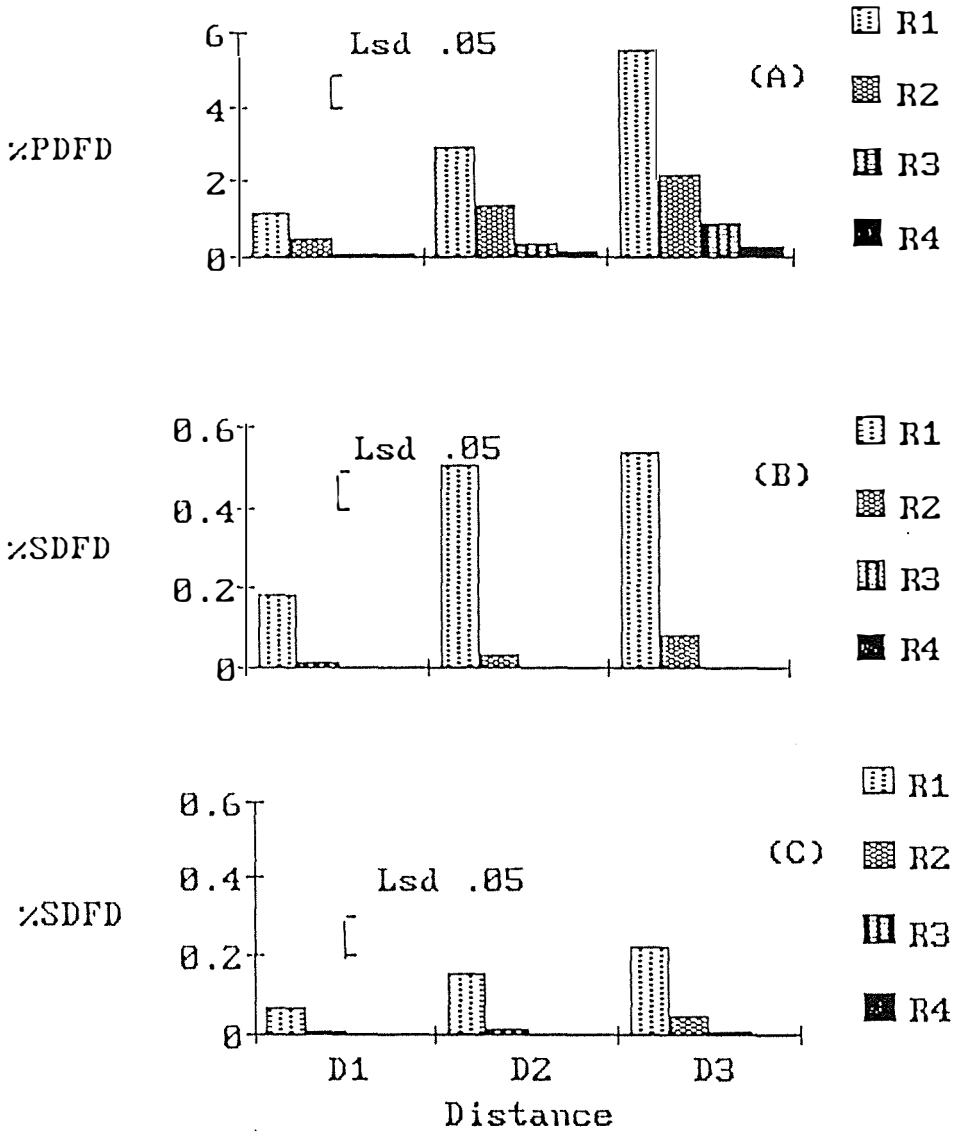


Figure 4.4 Percentage of pasture P and S derived from dung at four radial distances (R1, R2, R3 and R4) from dung applied at three rates (D1, D2 and D3); A = %PDFD and B = %SDFD at the first harvest (30 days) and C = %SDFD at the second harvest (60 days) (calculated using specific activity of dung extracts).

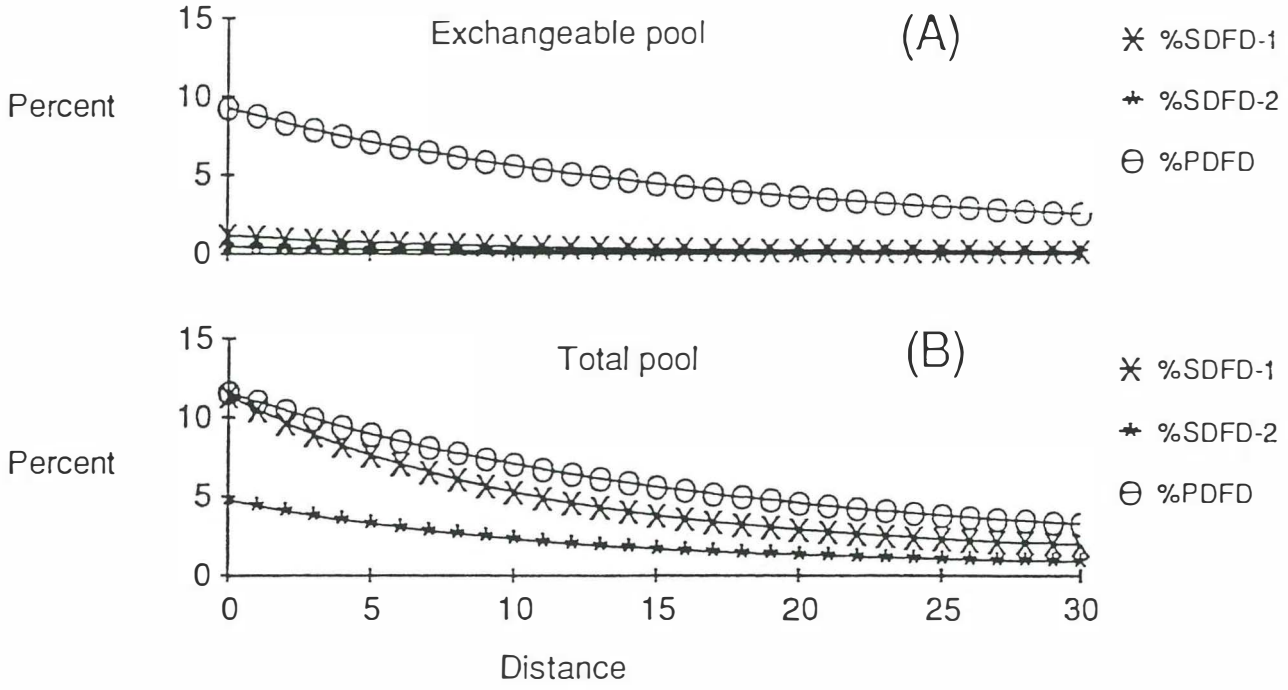


Figure 4.5 Percentage of pasture P and S derived from dung (%PDFD and %SDFD) at increasing distances from dung (R1, R2, R3 and R4); %SDFD-1 and %PDFD were calculated from the first harvest data (30 days) and %SDFD-2 was calculated from the second harvest data (60 days) (calculated using the specific activity of the (A) exchangeable P and S and (B) the total dung P and S.

Table 4.2 Pasture dry matter yield as influenced by dung application rates and radial distances of pasture from dung.

	Treatments	...Harvest... 1	2	Cumulative yield
			..... gm m^{-2}	
	Dung rates[a]			
	D1	27.8	49.2	77.1
	D2	26.1	53.4	79.5
	D3	27.4	53.7	81.2
	Distances[b]			
	R1	29.4	46.3	75.8
	R2	27.9	49.0	76.9
	R3	25.2	56.3	81.5
	R4	25.8	56.8	82.7
Lsd 5%	Dung	ns	ns	ns
	Distances	ns	ns	ns
C.V. %		25.5	23.6	15.1

ns = not significant at 5% level

[a] *averaged over all four distances and five replications*

[b] *averaged over all three dung rates and five replications*

Table 4.3 Pasture S and P concentration, S and P uptake of pasture and cumulative S uptake as influenced by dung application and radial distances of pasture from dung.

Treatments	Harvest 1 S concentration %	Harvest 1 S uptake mg m^{-2}	Harvest 2 S concentration %	Harvest 2 S uptake mg m^{-2}	Cumulative S uptake mg m^{-2}	Harvest 1 P concentration %	Harvest 1 P uptake mg m^{-2}
Dung rates[a]							
D1	0.18	50.3	0.25	123.9	174.2	0.26	73.3
D2	0.17	45.5	0.27	143.9	189.4	0.28	74.3
D3	0.21	58.9	0.28	150.0	209.0	0.29	78.5
Distances[b]							
R1	0.21	60.9	0.26	122.6	183.4	0.27	79.4
R2	0.17	48.6	0.26	130.7	179.3	0.28	77.0
R3	0.18	47.3	0.27	150.7	197.8	0.28	71.2
R4	0.19	49.7	0.27	153.2	202.8	0.29	73.5
Lsd 5% Dung	0.03	ns	0.01	ns	23.0	0.02	ns
Distances	ns	ns	ns	ns	ns	ns	ns
C.V. %	21.8	35.3	5.8	26.4	18.9	9.9	26.3

ns=not significant at 5% level

[a] *averaged over all four distances and five replications*

[b] *averaged over all three dung rates and five replications*

Table 4.4 The percent distribution of ^{35}S and ^{32}P taken up by pasture at different radial distances from ^{35}S and ^{32}P labelled dung applied at various rates.

	Dung			
Distances	D1	D2	D3	Means
PART A (FIRST HARVEST)		..... % ^{35}S		
R1	83.1	88.7	80.1	83.4
R2	6.6	7.4	13.5	10.6
R3	7.1	3.2	5.1	4.7
R4	3.2	0.6	1.3	1.3
PART B (SECOND HARVEST)		..... % ^{35}S		
R1	55.7	68.9	64.8	64.9
R2	13.4	11.3	17.9	15.0
R3	19.9	14.3	13.5	14.7
R4	10.8	5.5	3.7	5.3
PART C (FIRST HARVEST)		..... % ^{32}P		
R1	40.6	45.6	48.2	46.6
R2	20.6	27.3	23.1	24.1
R3	20.3	18.8	21.6	20.7
R4	18.4	8.2	7.1	8.6

4.4.3.3 Plant S uptake

Amounts of plant S uptake are shown in Table 4.5C. At the second harvest which was taken 30 days after dung application, the dung treatment resulted in significantly lower S uptake than the no dung treatment. This may be due to immobilization of the available S during the initial decomposition of dung by organisms. There was no significant effect of dung in the following harvests. Overall, the control treatment had lower S uptake than the fertilizer treatments, although the differences were not significant. There was no significant interaction between dung and type of fertilizer.

4.4.4 DISCUSSION, EXPERIMENT 2

In this experiment an amount of dung, similar to that returned by sheep after one grazing was applied uniformly to unfertilized pasture plots and plots fertilized with S^o and superphosphate. Similar to experiment 1, dung application had no effect on dry matter yield. Fertilizer application did affect the pasture S concentration and uptake throughout the period but again did not influence pasture yield. The increase in S concentration and uptake without any increase in yield due to fertilizer application represents the luxury uptake of S. The crease in S content and total S uptake (Tables 4.5B and 4.5C) in fertilized plots above the control ranged from 6-41% and 12-36%, respectively, but this did not increase dry matter yield on fertilized plots (Table 4.5A). As discussed in Section 4.4.2, the trial area had a sufficient soil S status.

Considering the contribution of dung S to pasture plants in this experiment; amounts of less than 1.0 kg S ha^{-1} were applied to the experimental plots (375 kg ha^{-1} of dung, total S = 0.26%). The total available S added was probably less than 0.1 kg S ha^{-1}. Therefore, this amount should not have any effect on pasture as compared to the more readily available fertilizer forms, such as superphosphate and slowly released S^o. Watkin (1954) and Skrijka (1979) have shown that the sheep dung had no effect on pasture yield. Recently, in New Zealand, Morton and Baird (1990) have shown that the return of N from dung and urine (which contained more N than S) affected an insufficient area to influence pasture growth even at high stocking density (1800 sheep ha^{-1} day^{-1}). Furthermore, Smith (1976) and Ledgard *et al.,* (1991) have shown that, for well developed, regularly fertilized, permanent pastures, fertilizer S can be withheld for 2-3 years before there is a measurable S deficiency. Presumably a similar period for S responsiveness to occur is required after dung and urine S is withheld.

Table 4.5 Pasture dry matter yield (PART A), sulphur concentration (PART B) and total pasture S uptake (PART C) as influenced by dung and fertilizer applications at five harvests.

Treatments		Days after fertilizer application 30	60	90	120	150
PART A, DRY MATTER YIELD				..... g m^{-2}		
Dung application[a]						
w/o dung		ND	199.5	201.2	119.1	178.8
w/ dung		ND	194.5	194.6	131.6	189.0
Fertilizers[b]						
Superphosphate		182.6	190.0	190.4	121.2	185.8
Elemental S		203.0	208.1	209.2	125.1	180.0
Control		228.9	193.0	193.7	129.7	177.9
Lsd 5%	Dung	-	ns	ns	ns	ns
	Fertilizers	ns	ns	ns	ns	ns
C.V. %		14.2	9.9	9.5	18.5	19.0
PART B, S CONCENTRATION				..... %		
Dung application[a]						
w/o dung		ND	0.47	0.41	0.48	0.44
w/ dung		ND	0.44	0.41	0.46	0.40
Type of fertilizers[b]						
Superphosphate		0.46	0.54	0.44	0.49	0.45
Elemental S		0.44	0.42	0.44	0.49	0.44
Control		0.33	0.39	0.36	0.42	0.35
Lsd 5%	Dung	-	ns	ns	ns	ns
	Fertilizer	0.09	0.04	0.06	0.03	0.06
C.V. %		14.4	9.9	15.0	7.5	14.0
PART C, S UPTAKE				..... mg m^{-2}		
Dung application[a]						
w/o dung		ND	94.0	83.3	60.1	81.4
w/ dung		ND	84.1	79.9	62.7	81.8
Type of fertilizers[b]						
Superphosphate		84.0	102.2	83.9	60.1	81.4
Elemental S		87.3	87.9	91.4	61.5	86.1
Control		75.0	77.1	69.5	53.5	63.2
Lsd 5%	Dung	-	8.9	ns	ns	ns
	Fertilizers	ns	11.4	15.1	10.9	18.8
C.V. %		12.6	12.3	17.7	18.5	23.4

ns = not significant at 5% level; ND = not determined

[a] *averaged over three fertilizer types and four replications*

[b] *averaged over two dung rates and four replications*

Table 4.6 Calculated percentage of a paddock annually influenced by dung considering camping and noncamping behaviour of sheep.

	noncamping	camping[a]		
		campsite	noncampsite	total
		..... percent of paddock		
Radius of the area of influence per patch Rp + Rd = Rt[b]				
6.4 + 10.0 = 16.4 cm	27.76(2.31)[c]	5.00	18.60(1.55)	23.60
6.4 + 8.0 = 14.4 cm	21.40(1.79)	5.00	14.34(1.19)	19.34
6.4 + 6.0 = 12.4 cm	15.87(1.32)	5.00	10.63(0.88)	15.63
6.4 + 4.0 = 10.4 cm	11.16(0.93)	3.68	7.48(0.62)	11.16
6.4 + 2.0 = 8.4 cm	7.28(0.60)	2.40	4.80(0.40)	7.20
6.4 + 0.0 = 6.4 cm	4.22(0.35)	1.39	2.83(0.23)	4.22
Annual S return from dung[d] kg ha^{-1}	9.85	3.25	6.60	9.85
Readily plant available fraction[e] kg ha^{-1}	0.985	0.325	0.66	0.985

It is assumed that average radius of dung patch=6.4 cm[f]; 6 excretions/day/animal[g]; and 15 sheep/ha/year and dung patches were evenly distributed over the area

a *33 percent of the total dung output located in 5% of paddock (Hilder, 1964)*

b *Rp=radius of dung patch*
Rd=extended radius of influence
Rt=total radius of influence

c *numbers in parenthesis are percent of paddock influenced monthly by dung*

d *calculated from mass of dung of 0.1 kg/excretion and 0.3% total S*

e *calculated from mass of dung of 0.1 kg/excretion and 0.03% Cap-S*

f *Herriott and Wells (1963)*

g *Sears and Newbold (1942); Herriott and Wells 1963 and Skrijka, 1987*

4.4.5 Area covered by dung and Influenced by dung sulphur

A simple calculation of the percentage of a paddock annually and monthly covered by sheep dung and dung S when both camping and non-camping behavior of sheep are considered is presented in Table 4.6 (calculations for the number of sheep and amount of dung are presented in Appendix 4.6). With 15 sheep per hectare, the total area covered by dung (radius = Rp), annually, is less than 5% unless there is an extended area of influence (radius = Rd) on pasture growth around the dung.

Results from the first experiment showed a very small contribution of S from sheep dung to the surrounding pasture within 5 cm of the dung. The maximum amount of plant S derived from the dung was about 2% for the pasture at the centre, where the labelled dung was deposited. This small amount, and the small increase of the total S uptake in the fertilized plot of the second experiment, are considered to have insignificant effects on increasing plant dry yield. Thus, based on these results (percent of plant S derived from dung, pasture yield, and S and P uptake), the extended radius of influence (Rd) of dung S on pasture growth is small being <5 cm. This suggested that approximately 3-11% of paddock may be influenced by dung return each year (Table 4.6)

4.5 CONCLUSION

Although about 5% of the paddock is covered by discrete sheep dung patches annually, in the short term the pasture dry matter yield in the area directly adjacent to the dung was insignificantly influenced by the nutrients P and S contained in the dung. Furthermore, the presence of the dung did not significantly influence the immobilization of soil or fertilizer derived phosphate and sulphate.

Very small amounts of plant P and S (about 2-5%) within 10 cm of the dung were derived from the dung. The total available nutrients in the dung are very small compared to those of readily available chemical fertilizers or that of available forms already existing in the soil. The majority of plant P and S were derived from soil sources, a large part of which will be derived from slowly mineralizing dung that has accumulated with time. The fertility of the soils at these experimental sites has been well maintained to ensure an optimum pasture production. Even when the dung at the normal rate of application (375 kg DM ha^{-1} $grazing^{-1}$) was evenly distributed over the whole pasture, there was no effect on pasture yield or nutrient content.

This study confirms Boswell's (1983) finding that the short term effect of dung on nutrient availability was small. Considerable amounts of S (approximately 34% of the S taken up from soil by pasture (Saggar *et al.*, 1990a, 1990b) are returned to the soil as dung. Therefore the role of dung is one of a slow S release component as it is decomposed by soil organisms and converted into soil humus which continues to release S. If the C : S ratio of the non-exchangeable S in the dung (Table 4.1) is approximately 200:1 and the soil microflora have a C : S ratio of 45:1 then it would take 2 generations of organisms with a growth yield of 0.4 (i.e. 60% of the carbon is evolved as CO_2 and all S is conserved at the site of decomposition) before a substantial amount of the dung S could be released as $SO_4^=$. The average generation time of microflora growing on the recalcitrant organic matter in dung may exceed 1 year (Dr, K. Tate, personal communication). Such simple calculations support the experimental evidence that dung S is a slow release S form.

In conducting future field trials designed to examine the short term fate of fertilizer S in well developed permanent pastures, it is not necessary to apply sheep dung to the experimental plots in order to simulate the effect of dung return because probably this will have little effect on the size of the 'bank' of the dung at various stages of decomposition in pasture soils. For long term (several years) experiments on permanent pasture or experiments on less well developed pasture where soil fertility is very low, animal dung may be needed to be deposited to maintain soil organic S reserves.

Many authors have accepted that the distribution of urine is similar to that of dung (Petersen *et al.*, 1956; Till, 1975; Taylor, 1980). Using data presented by Gillingham, (1978); Gillingham, (1980) and Gillingham *et al.,* (1980) it can be calculated that during a month, urine is returned to approximately 5% of the paddock if there is no overlap of urine spots.

It is well known that applications of sheep urine stimulate increased pasture growth (Sears and Goodall, 1948; Watkin, 1954; Kennedy and Till, 1981a; Boswell, 1983; Williams *et al.*, 1989; Morton and Baird, 1990; Sakadevan, 1991) mainly through the high concentrations of N, K and S that are returned to the soil through the urine. At the time of this study, other Ph.D. programs were investigating urine effects including S (Sakadevan, 1991) and it was considered unnecessary to duplicate these.

CHAPTER 5

EVALUATING FIELD EXPERIMENTATION TECHNIQUES USING ^{35}S LABELLED FERTILIZERS TO TRACE THE FATE OF SULPHATE AND ELEMENTAL S APPLIED TO PASTURE SOILS

5.1 INTRODUCTION

In many New Zealand soils the literature review indicates that sulphate-containing fertilizers such as gypsum, in superphosphate, may be prone to loss by leaching if applied in seasons when rainfall intensity and drainage are high. To some extent the amount of S loss may be reduced by spring rather than autumn application of superphosphate (Toxopeus and Gordon, 1971; Nguyen *et al.*, 1989a, 1989b). However, Saggar *et al.* (1990a, 1990b) estimated that even when applied during the spring and summer, the long term leaching losses of S from North Island hill country pasture ranged from 40-77% of the fertilizer (superphosphate) applied. Sakadevan (1991) showed that approximately 30% of this S loss was leached directly from the fertilizer application. Currently the use of S^o based fertilizers instead of sulphate-S fertilizers is recommended to reduce S leaching loss (Sinclair and Saunders, 1984). The mode by which it reduces leaching losses is either through slow release from S^o fertilizer maintaining lower soil sulphate concentrations during the period of intense leaching events or that relatively more S being incorporated into plants and soil organic matter from S^o than sulphate-based fertilizers. The relative importance of each process is unclear. Different forms of S fertilizers, labelled with ^{35}S, can be applied to pasture soils to test whether the fate of S^o and sulphate-S differ.

In studying the fate of ^{35}S labelled fertilizers in soil, two techniques are generally employed, direct labelling and inverse dilution (IAEA, 1976; Vose, 1980; Manzel and Smith, 1984).

In the direct labelling technique the fertilizer materials are labelled with ^{35}S radioisotope and added to soil systems. Then, after certain periods, plant and soil samples are harvested and determined for radioactivity in the samples. For example, Gregg and Goh (1978, 1979, 1982) and Goh and Gregg (1982a, 1982b) used ^{35}S labelled gypsum in the study of the fate of fertilizer S in pasture systems in South Island, New Zealand.

In the inverse dilution technique the soil system is labelled with carrier-free ^{35}S radioisotope. After equilibrium is obtained unlabelled fertilizers are applied to the soil and plant systems. The S released from the fertilizer material will dilute the specific activity of ^{35}S in the system.

Changes in the ratio of specific radioactivity (SR) of ^{35}S in soil or plant grown on fertilizer treated cores are measured. The specific activity ratio is calculated as follows:

$$SR = SAT / SAC$$

Where

SR = specific activity ratio
SAT = specific activity of ^{35}S in treated cores, Bq g^{-1} S
SAC = specific activity of ^{35}S in control cores, Bq g^{-1} S

When there is little release of S from fertilizer the ratio (**SR**) is about 1. Release of S from fertilizers will result in a reduction of this ratio (SAT is less than SAC). This technique has the advantage that comparison can be made of materials which may be difficult to label (e.g. rock phosphate, dung and urine). For example, Boswell (1983) employed this technique to study the fate of sheep dung and urine in pasture soil. However this technique has a drawback in that the nutrient released from fertilizer may be incorporated into non-active forms hence results may overestimate the plant availability of any nutrient released to soil (Freney *et al.*, 1971, 1975). Alternatively addition of fertilizer may stimulate root growth leading to higher isotope recovery.

5.2 OBJECTIVES

The general objective was to use ^{35}S as a tracer to study the fate of different forms of fertilizer S, namely sulphate and S^o, when applied to a pastoral soil. Two field trials were conducted to meet the general objective.

The first experiment investigated the redistribution of fertilizer-^{35}S between forms of soil S, plant S and sulphate leached from the top ten centimetres of pasture soil during a period of 150 days after a late spring fertilizer application. Two fertilizer S sources were used, namely, superphosphate (SSP) and elemental S (S^o); both were labelled with $^{35}SO_4^=$ and $^{35}S^o$, respectively.

The second short term experiment (60 days) investigated the use of an inverse isotopic dilution technique to determine the influence of applied fertilizer S on the size of the labile soil $^{35}SO_4^=$ pool. In the study a solution of carrier-free ^{35}S was used to pre-label soil S prior to fertilizer application.

5.3 MATERIALS AND METHODS

5.3.1 EXPERIMENT 1, ^{35}S LABELLED FERTILIZER

Superphosphate (SSP) and S^o were chosen for this experiment since these two fertilizers are common S fertilizers applied to New Zealand pasture soils. A microplot (Peverill and Douglas, 1976; Shedley *et al.*, 1979; Vose, 1980; Martin, 1985; Williams, 1988; Destain *et al.*, 1989) technique was employed.

5.3.1.1 Preparation of ^{35}S labelled S^o and SSP

An aqueous solution of $^{35}SO_4^=$ and a solution of $^{35}S^o$ dissolved in toluene, were obtained from the Radiochemical Centre, Amersham, U.K.

5.3.1.1.1 $^{35}S^o$ labelled S^o

About 462 MBq of carrier-free $^{35}S^o$ in toluene were mixed with 110 ml toluene containing 1.22 g of dissolved unlabelled S^o (or 53 mg per 5 ml). Five ml of the mixture were pipetted onto a 15 g layer of soil in a glass petri dish and dried overnight in a fume hood. This yielded 20.86 MBq per 53 mg of S with a ^{35}S specific activity of 394 KBq mg^{-1} S (Table 5.1). Later observations using a light microscope indicated that the S^o produced using this process was microfine with no particle exceeding 0.010 mm in diameter. Details were described in Section 3.1.3.

5.3.1.1.2 $^{35}SO_4^=$ labelled SSP

SSP labelled with $^{35}SO_4^=$ was prepared by adding carrier-free $^{35}SO_4^=$ to the sulphuric acid used to acidulate phosphate rock (Nunn and Dee 1952; Young *et al.*, 1985; Bolan *et al.*, 1987). Details were described in Section 3.1.4.

About 9 g of a mixture of 50:50 Christmas Island 'A' grade:Nauru phosphate rock was acidulated with 8.45 g of 65% H_2SO_4 containing 585 MBq of ^{35}S (15.8 mci) at an acid:rock ratio of 0.61 and then allowed to dry for 20 minutes. The product was dried overnight at 30 ^oC and then ground in a mortar and passed through a 100 mm sieve. The ground SSP was divided into 20 samples, 0.45 g each. Each sample contained 18.24 MBq of ^{35}S activity with ^{35}S specific activity of 342.07 KBq mg^{-1} S and 11.85% S (Table 5.1).

5.3.1.2 Trial method

The two sources of fertilizer-S, ^{35}S labelled SSP and S^o were applied to a 176.78 cm^2 area contained 15 cm diameter and 10 cm deep galvanized steel cores (microplot). Treatments were replicated four times and arranged in a randomized complete block allowing for five separate harvest dates, provided by 40 separate soil cores. The galvanized cylinders had been driven into the permanent pasture to isolate soil cores about 9 cm deep. On October 15, 1985, both fertilizer S forms were broadcast evenly over the soil cores at the rate of 30 kg S ha^{-1}. Monocalcium phosphate, giving an equivalent amount of P to the SSP treatment was also applied to cores fertilized with S^o. A basal application of muriate of potash (KCl) at the rate of 40 kg K ha^{-1} was also given to all cores.

5.3.1.3 Sampling and preparation of samples

At each harvest (i.e. 30, 60, 90, 120 and 150 days after fertilizer application) herbage from cores (microplots) was cut to within 2.5 cm of the soil surface and four replicate cores of each treatment were destructively sampled and transported to the laboratory. Schemes for sampling herbage and soil are shown in Table 5.2. After removing herbage stalks from the surface of each soil core, the core was divided into three equal sections of about 3 cm thickness; top (0-33 mm), middle (33-66 mm) and bottom (66-100 mm). The soil from each depth was mixed and approximately 5.00 g of the fresh wet soil was randomly sampled for analyses on fresh, field-moist soil. The remaining soil samples were put into plastic bags, frozen and then freeze-dried for 7 days. The freeze-dried soils were ground by a hammer mill. Both root and herbage samples were dried in an oven at 80 ^oC for 48 hours. A domestic coffee grinder was used for grinding herbage samples.

Table 5.1 Characteristics of ^{35}S labelled fertilizers used in this study.

^{35}S Labelled Fertilizers	Fertilizer applied mg core^{-1}	S applied mg core^{-1}	^{35}S Activity applied MBq core^{-1}	^{35}S Specific activity KBq mg^{-1} S
SSP	450	53.3	18.24	342.05
S^0	53	53.1	20.86	393.58

Table 5.2 Harvesting schedules of herbage from microplots at each harvest and number of soil cores destructively sampled.

	Days after fertilizer application				
Fertilizers	30	60	90	120	150
	Number of replications				
HERBAGE CUT					
SSP	20	16	12	8	4
S^0	20	16	12	8	4
SOIL CORES					
SSP	4	4	4	4	4
S^0	4	4	4	4	4

5.3.2 EXPERIMENT 2, INVERSE ISOTOPIC DILUTION EXPERIMENT

A field experiment using carrier-free ^{35}S was also carried out in conjunction with the previous experiment. The ^{35}S carrier-free solution was injected into the top layer of fertilized pasture soil enclosed in microplots. After injection and prior to fertilizer application, the ^{35}S isotope is expected to exchange with the labile soil sulphate pool. The main objective was to investigate the influence of fertilizers on the fate of the labile soil S pool.

5.3.2.1 Carrier-free ^{35}S preparation and injection technique

An aqueous solution of $^{35}SO_4^=$ was obtained from the Radiochemical Centre, Amersham, U.K.

A 702.08 MBq (18.975 mci) carrier-free $^{35}SO_4^=$ solution was diluted with 200 ml of deionized water and the solution containing 3.51 MBq ml^{-1} was used for labelling soil in microplots. A microplot injector (M.J. Hedley and R.W. Tillman, 1988, personal communication) as shown in Figure 5.1 was used to inject S carrier-free solution to soil cores.

5.3.2.2 Trial method

The microplot technique (as described in Section 3.2.2) was also used in this experiment. Twenty four galvanized steel cylinders were pressed into plots of the mowing plot trial (described in detail in Section 4.3.2) where plots having three fertilizer treatments (SSP, S^o and control) arranged in RCB design with four replications were laid out. Two cylinders were put in each plot. On October 15, 1985, about 6.8 ml of the carrier-free solution was injected into the top 1-6 cm soil layer in each microplot using the microplot injector (Figure 5.1). A week after the injection, granulated SSP and commercial grade S^o (100% <0.250 mm and 50%<0.150 mm particle size) were surface applied individually to these microplots at the rate of 30 kg S ha^{-1}. A basal application of monocalcium phosphate and muriate of potash was also applied in the same manner as described in Section 4.3.2.

5.3.2.3 Sampling and preparation of samples

At 30 and 60 days after the injection, all herbage from the microplots was cut to within 2.5 cm of the soil surface and the four replicate cores from each treatment were destructively sampled and transported to the laboratory. After removing herbage stalk, the whole soil cores and

herbage were dried in a forced-draught oven at 60 ^{o}C. The dried soil was then finely ground using a hammer mill. No attempt was made to subdivide soil cores into 3 sections as in the previous experiment. The herbage samples were dried and ground in the same manner as the previous experiment.

5.3.3 Experimental site

A permanent pasture site on the Tokomaru silt loam soil (yellow-grey earth, Fragiaqualf) was chosen for the study. It was located on the Keeble farm, 4 km south of the Massey University campus. The pasture consisted mainly of perennial ryegrass, white clover, subterranean clover together with other less dominant species of grass weeds. Before this experiment was started, the paddock had received S at the rate of approximately 20-30 kg S ha^{-1} $annum^{-1}$ as SSP. The average annual rainfall is about 1000 mm, distributed throughout the year but normally peaking in the months of December and June. Cattle and sheep had rotationally grazed the trial area. After the experiment had been established the experimental area was fenced to prevent access by stock. Before the fertilizer application, pasture on plots was mown to about 2.5 cm in height above ground level and discarded. This was done in conjunction with the small plot mowing trial described in Section 4.3.2. A general background of the soil used is given in Chapter 7, Table 7.1, and some chemical properties of the soil are shown in Table 5.3.

Table 5.3 Chemical properties[a] of S in three soil layers collected before the experiment.

Layer	Depth cm	Total S	Organic S	Ester sulphate S	Carbon bonded S	Extractable S	
						CaP-S	CaCl-S
				mg S kg^{-1} soil			
Top	0-3	540	525	184	343	15.1	9.0
Middle	3-6	380	382	193	189	7.0	1.2
Bottom	6-10	320	312	210	102	7.1	1.4

[a] *analytical methods were those of Landers et al., 1983*

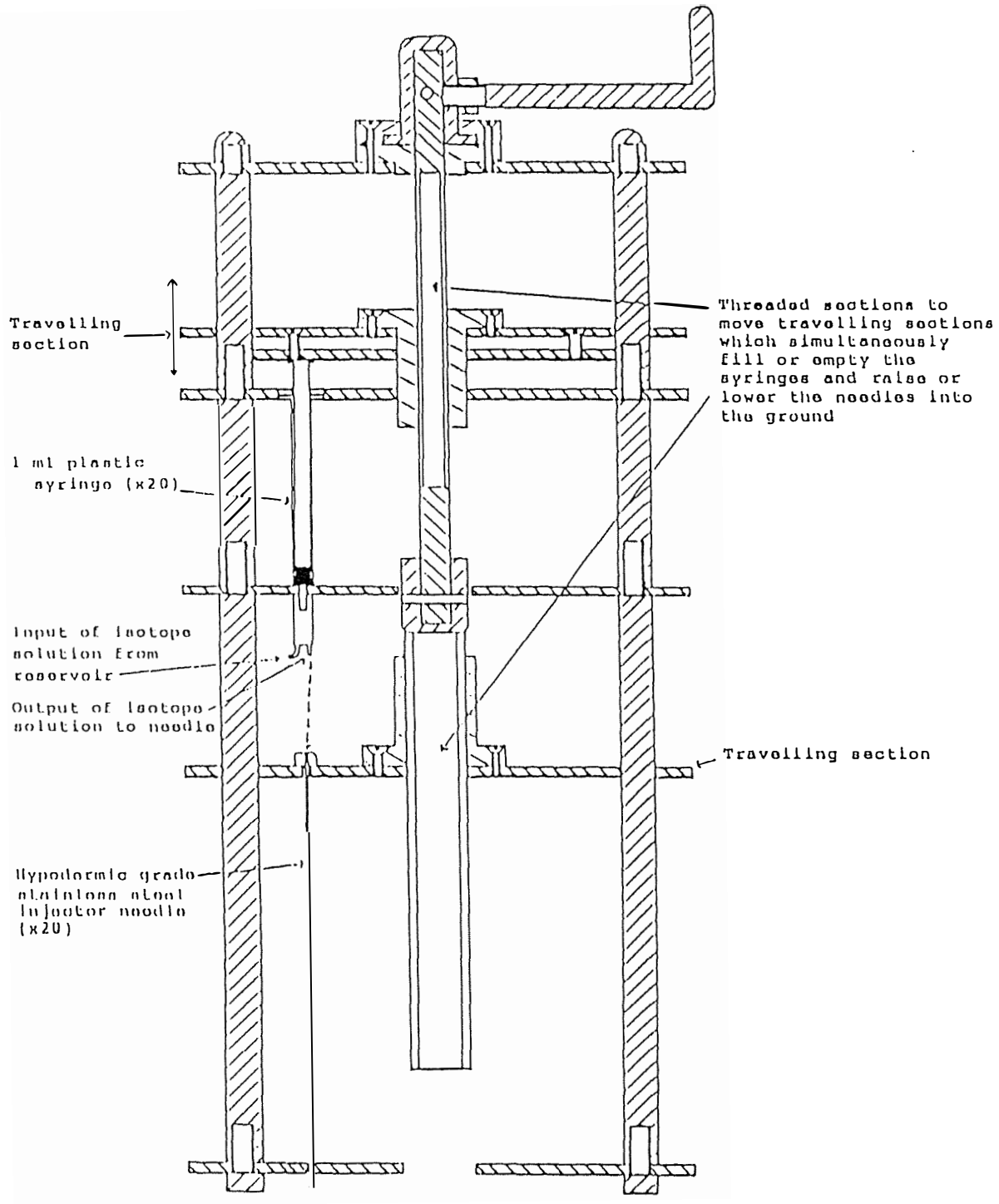


Figure 5.1 A cross sectional diagram of the injector system used to inject soil cores with radioisotope in the inverse isotopic dilution experiment (Hedley and Tillman, personal communication).

5.3.4 Chemical analyses

Chemical analyses of samples are listed in the following Table:

Table 5.4 Chemical analyses of soil, plant and fertilizer samples

Analyses	As described in Section	Chapter
Total S in soil sample	3.3.3	3
S^0 in soil sample	3.3.4	3
Extractable S^1	3.3.2	3
Soil organic S	3.3.5	3
Total S in herbage	3.3.1.1	3
Total S in fertilizers	3.3.7	3
HI-reducible S in extract	3.3.6	3
Radioassay of ^{35}S	3.3.8	3

[1] *extractable S (CaP-S) performed on freeze-dried soil*

5.3.5 Statistical analyses

To establish whether treatment effects were significant the measurements were subjected to variance analysis using the SAS package. Least significant differences (Lsd) at the 1 and 5% level of probability were used for comparison of means between treatments and result of the comparisons were presented in appropriate tables and appendices. The SAS (SAS Institute Inc., 1985) and Minitab (Minitab Inc., 1989) computer programmes were employed.

All ^{35}S activity data were normalized to the day when the ^{35}S fertilizers or injected solution were applied (October 15, 1985) using the relationship as described in Section 4.3.4. Some relevant herbage ^{35}S and soil ^{35}S calculations are presented as described in Section 4.3.4. Additional calculations are as follows:

a. Proportion of ^{35}S in different soil fractions in soil cores; amounts of ^{35}S (CaP-^{35}S, carbon-bonded ^{35}S, ester-$^{35}SO_4^=$, organic ^{35}S) expressed as a percentage of total ^{35}S remaining in soil cores.

$$P_t = T_a / T_r * 100 \quad \%$$

Where

P_t = proportion of ^{35}S in soil S fraction at time **t**
T_a = amount of ^{35}S activity appearing in that fraction at time **t**
T_r = total ^{35}S activity remaining in soil cores at time **t**

b. Percentage of applied ^{35}S cumulatively taken up by pastures

$$R_c = A_p / A_o * 100\ \%$$

Where

R_c = recovery percentage of ^{35}S in pasture at time **t**

A_p = amounts of ^{35}S activity cumulatively taken up by pasture at time **t**

A_o = total amount of ^{35}S activity in S labelled fertilizer applied at day **0**

A short term experiment was also designed to examine the recovery from soil of total ^{35}S activity from labelled fertilizers applied at day 0. Gypsum and microfine S^o containing ^{35}S activity of approximately 2.4 and 2.7 MBq g^{-1} S, respectively, were applied to slices (0-3 cm) of surface soils which were sampled from the field using the same size of galvanized steel cylinders as described in Section 5.3.1.2. There were three application rates for each fertilizer and the two treatments were replicated four times. After the labelled fertilizers were evenly applied to the soil surface, the whole soil cores were freeze-dried for 7 to 10 days. The dried samples were hammer milled before total ^{35}S determination.

Results are shown in Appendix 5.1. The recovery of ^{35}S activity was consistently low, 65-78% of that applied in fertilizer. There was no significant difference between application rates. The mean, 73%, calculated across all application rates, was used as a recovery factor to calculate the total fertilizer ^{35}S remaining in each soil core. The low recovery represents loss of fertilizer S during soil preparation for counting because spikes of $^{35}SO_4^=$ added to soil prior to digestion gave on average 100% recovery of spike activity.

5.4 RESULTS

5.4.1 Initial soil S properties and weather conditions during experiments

Initial soil S and other related properties are shown in Table 5.3 and Table 7.2 (Chapter 7). In general, the available soil S as measured by CaP-S at 10 ppm was considered to be optimum (Sinclair *et al.*, 1985) for pasture growth. This results from a history of regular annual top dressing with SSP at the rate of approximately 250 kg ha^{-1}. Sulphate retention was low hence some sulphate in soil solution is expected to leach down the soil profile. Other properties such as soil Olsen-P and pH were also near optimum for the study rate carried (Cornforth and Sinclair, 1984).

During the trial period, the experimental site received rainfall of approximately 554 mm and had total drainage water of approximately 176 mm (calculated according to Scotter *et al.*, 1979) as shown in Appendix 5.9. At the beginning of the trial about 5 mm of drainage water occurred. Larger amounts of drainage occurred during the second half of the trial period which may have influenced the leaching of sulphate.

5.4.2 EXPERIMENT 1, ^{35}S LABELLED FERTILIZERS

In this section the influence of different fertilizers on the redistribution of soil S and fertilizer S and ^{35}S are considered.

5.4.2.1 Accumulated herbage dry matter yield and total S uptake and comparisons between microplot and small plot experiments.

The accumulated dry matter yield and total S (soil S plus labelled fertilizer) uptake for five harvests in the microplot and small plot trials are presented in Table 5.5. The range of mean results of these key parameters are similar for both microplot and small plot trials. The microplot technique produced slightly more variable results (%C.V. for microplot range from 7 to 24% cf. small plot 5-31%). In experimenting with labelling fertilizers, the microplot technique is more preferable as it requires small amounts of costly isotope and the labelled fertilizer can be more easily contained to meet safety regulations. On average the results indicated that measurements made using the field microplot technique are applicable to and realistically simulate the results of the nearby small plot trial. This indicates that in both techniques, herbage S may be drawn from similar soil depths and volumes of soil. Thus, it appears probable that results from the microplot technique can be extrapolated to a larger field scale.

In general, total uptake of S by pasture from both fertilized treatments were higher than that of the unfertilized treatment (in the small plot experiment). Pasture growth, however, was not responsive to additional S fertilizer and increased S uptake did not generate higher herbage dry matter production on the S fertilized plots. Therefore, the results indicate that there was luxury consumption of the S by herbage in the fertilized plots.

During a five month period (spring-summer seasons), pasture production had a mean cumulative dry matter yield of about 9.0 t ha^{-1} on fertilized plots and a mean cumulative S uptake of about 41 kg S ha^{-1} (Table 5.5 and Figure 5.2). However, in the control plot about 34 kg S ha^{-1} of native soil sulphate S were taken up by pasture plants. Apparently, about 7 kg S ha^{-1} (17.5%) might be derived from fertilizers (fertilized plot - control plot) but from the ^{35}S recovery on the 0.177 m^2 cores the actual fertilizer uptake ranged between 5.3 and 4.3 kg ha^{-1} with SSP and S^o respectively (see later discussion).

Throughout the experiment, patterns of the cumulative plant S uptake on plots fertilized with SSP and S^o were similar.

Cumulative plant S uptake in both fertilized plots increased linearly with time (Figure 5.2 and Appendix 5.2) while the amount of soil total CaP-S (Figure 5.2 and Appendix 5.3) remained relatively unchanged after 60 days. The results indicated that during the trial period (October 14, 1985 to March 15, 1986) an average rate of S uptake from soil was about 0.45-0.48 mg S $core^{-1}$ day^{-1}, or approximately 0.25 kg S ha^{-1} day^{-1}. Without mineralization of organic S or atmospheric deposition, the total amount of CaP-S extracted from soil would be expected to decrease with time. As no significant decrease occurred after 60 days, then significant net mineralization of soil organic S must be invoked to explain the continued linear increase in herbage S, particularly during the period 60-150 days. This is the period after which much of the fertilizer S and original soil S (sulphate-S) had been rapidly immobilized by the soil organic cycle, or removed by leaching. The ^{35}S plant uptake and soil ^{35}S fractionation data presented in the following Sections should support or refute the mineralization hypothesis.

The contribution of S from atmospheric inputs within one month is considered to be minor (Smith 1979; Ledgard and Upsdell, 1991). During the trial period, there was about 554 mm rainfall and the S concentration in the rainfall would have been about 2 ppm (Smith, 1979; Heng, 1991). Therefore, on average, the contribution of S from rainfall was less than 2 mg $core^{-1}$ during the trial period. But the total S taken up by plant was about 70 mg $core^{-1}$.

Table 5.5 Cumulative dry matter yield and total pasture S uptake (soil plus fertilizer S) in microplots and small plot experiments during spring-summer seasons (October, 1985 - February, 1986).

	Days after fertilizer application				
Fertilizers	30	60	90	120	150
SMALL PLOTS					
Dry matter yield			.....g m^{-2}.....		
Superphosphate	183	385	590	670	884
Elemental S[1]	202	399	598	762	918
Control	229	428	628	725	908
Lsd 5%	ns	ns	ns	ns	ns
C.V. %	14.1	8.7	8.1	6.9	8.2
Cumulative S uptake			.....g S m^{-2}.....		
Superphosphate	0.84	1.96	2.88	3.41	4.12
Elemental S[1]	0.73	1.75	2.62	3.23	4.13
Control	0.75	1.56	2.28	2.76	3.38
lsd 5%	ns	0.28	0.31	0.28	0.31
C.V. %	12.60	10.20	7.40	31.10	5.20
MICROPLOTS					
Cumulative dry matter yield			.....g m^{-2}.....		
Superphosphate	181	385	572	702	860
Elemental S[1]	175	345	487	781	990
F-test	ns	ns	ns	ns	ns
C.V. %	24.0	9.9	7.3	12.2	19.5
Cumulative S uptake			.....g S m^{-2}.....		
Superphosphate	0.80	1.78	2.32	2.91	3.95
Elemental S[1]	0.78	1.65	2.01	3.18	4.18
F-test	ns	ns	ns	ns	ns
C.V. %	21.7	13.1	17.6	11.7	19.2
Sulphur concentration			.....%.....		
Superphosphate	0.44	0.47	0.39	0.47	0.41
Elemental S[1]	0.39	0.47	0.40	0.47	0.44
F-test	*	ns	ns	ns	ns
C.V. %	11.1	15.7	16.2	22.3	29.0

* = significant at 5% level; ns = not significant

1 particle size = <10µm

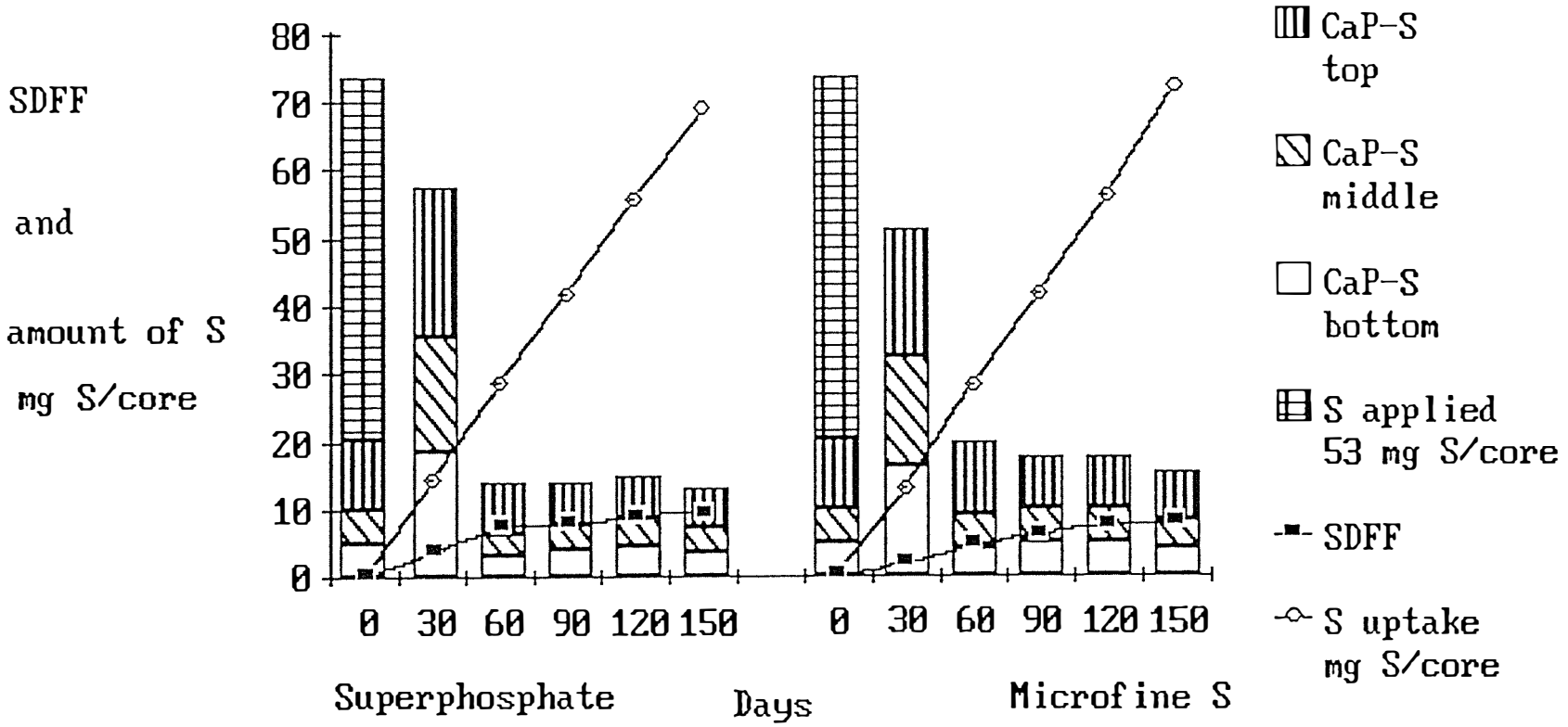


Figure 5.2 Cumulative plant S uptake (fertilizer plus soil S), amount of plant S cumulatively derived from ^{35}S labelled fertilizer (SDFF), extractable CaP-S at three soil depths and amount of S applied (microplots).

Of the total plant S uptake, the fractions of herbage S which were derived from labelled fertilizers (%SDFF) ranged from 19 to 29% at the 30 day harvest and decreased to 10-14% at the 150 day harvest (Appendix 5.2). In total this was equivalent to 7.6-9.3 mg S $core^{-1}$ (4.3-5.3 kg ha^{-1}) (application rate=53 mg S $core^{-1}$ = 30 kg S ha^{-1}) derived from fertilizer at the final harvest (Figure 5.2). This value is lower than the increase in S uptake caused by fertilizer application (approximately 7 kg S ha^{-1}, as mentioned above) and indicates that fertilizer application induced greater soil S uptake - commonly called 'priming effect'.

5.4.2.2 Total recovery of ^{35}S activity in soil and plants

Total recovery of ^{35}S in plant and soil samples are shown in Appendix 5.4 and Figure 5.3. In general, total recovery of ^{35}S was higher in the $^{35}S^{o}$ treated core. In the first 30 days considerable amounts of ^{35}S activity were lost and only 51% and 65% of the ^{35}S were recovered from total soil and plant analyses in the SSP and S^{o} cores, respectively. Thereafter the total ^{35}S recovered decreased further; however, the decrease in soil ^{35}S mainly occurred in the top 3 cm of soil in S^{o} fertilized core and in the top 6 cm in the SSP fertilized core and was mainly accounted for by plant uptake (Figure 5.3).

In soil, more residual ^{35}S from the fertilizer remained in the S^{o} treated core than that of SSP core. Irrespective of fertilizer treatment, the majority of total ^{35}S remained in the top 3 cm of the soil cores (Appendix 5.5 and Figure 5.3). On average the activity remaining in the S^{o} core was significantly larger (>2 fold) than in the SSP core, particularly in the top and middle soil layers (0-6 cm). This may be agronomically significant in terms of fertilizer S conservation since most pasture plant roots occur in the top 10 cm of soil (Williams, 1988).

At the conclusion of the experiment (150 days) about 60.8% and 44.2% of S from SSP and S^{o}, respectively, were not accounted for. The largest losses of ^{35}S occurred in the first 30 days of the experiment.

5.4.2.3 Extractable S in soil (CaP-S)

The total amount of CaP-S (soil CaP-S plus amount derived from labelled fertilizer S), the percentage of CaP-S derived from fertilizer (numbers in parentheses) and the amounts of extractable S derived from soil in each layer, and total amounts of CaP-S in the whole core are presented in Appendices 5.3A, 5.3B and 5.3C. The data presented In Appendix 5.3 are summarized in Figure 5.2.

The total amounts of CaP-S in the top 3 cm layer of both treatments were significantly higher than those in lower depths, partly reflecting the recent fertilizer application. At 30 days, the average CaP-S in the whole core fertilized with S^0 was lower than that of SSP core but thereafter (60-150 days) the amount of CaP-S in the S^0 treated core remained higher than in the SSP fertilized core. The differences are small and are not considered to be agronomically significant.

The amounts of CaP-S derived from the soil sulphate pool at each depth are presented in Appendix 5.3B. It was calculated as the difference between the total soil CaP-S and the amount of CaP-S derived from fertilizer S. The contribution from fertilizer was calculated by dividing the total CaP-^{35}S activity in the extract by the specific activity of ^{35}S in the applied fertilizers. For the first 30 days, the amount of CaP-S soil S in each of the three soil depths for both fertilizer treatments were similar and were not significantly different between treatments. Thereafter (30-60 days) the amounts decreased sharply from 15-16 ppm to 2-8 ppm but did not decrease further for the remainder of the experiment. From 0-30 days much of the decrease in the CaP-S could be accounted for in plant uptake, however at 60 days accumulated plant S uptake could not account for the decrease in CaP-S from 0-60 days (Figure 5.2). During this period 3 drainage periods occurred (Appendix 5.9) and some sulphate would have been leached. Further it is expected that the addition of fertilizer S which increased CaP-S level at 0-30 days stimulated S immobilization ('priming effect'). This effect lasted only 60 days.

The percentage contributions of fertilizer S to CaP-S (number in parenthesis in Appendix 5.3A) was initially larger (26-27%) but decreased markedly. Notably, by 60 days a substantial percentage of the fertilizer S remaining as sulphate had moved to the lower soil depth. This evidence supports the view that leaching of sulphate had occurred beyond the 10 cm depth of the intact soil cores.

5.4.2.4 Extractable ^{35}S in soil (CaP-^{35}S)

At time 0, all the SSP ^{35}S would have been in this fraction, by 30 days only 18% (Table 5.6 and Figure 5.4) of the applied ^{35}S remained in this fraction. These results confirm the large decreases in the CaP-S that occurred during this period (Section 5.4.2.3). Over the same time period (up to 30 days) increases in organic ^{35}S only accounted for 25.4% of the added ^{35}S (Table 5.6) and plant uptake, 6.4% (Appendix 5.4, Figure 5.4) giving a total ^{35}S recovery of 50.9%. The 49.1% not accounted for is presumed to have leached beyond 10 cm depth.

At time 0 in the S^o core, there is no ^{35}S in soil sulphate form or any other soil S fractions. The oxidation of the ^{35}S labelled microfine S^o was very rapid. Less than 2% of the ^{35}S label remained as S^o (acetone extractable) in the top layer of the soil cores at the first 30 days (Table 5.6). Thereafter less than 1% of the $^{35}S^o$ label remained as S^o. Negligible amounts of $^{35}S^o$ were detected in the lower layer of soil. A calculation using a model developed by Chatupote (1990) revealed that the oxidation of S^o having a particle size between 0.005-0.010 mm would be nearly complete within 25 days. The amounts of S^o were also determined in the acetone extracts and the results were consistent with the ^{35}S data (Table 5.6).

At 30 days, 48% of the added $^{35}S^o$ appeared as total organic soil ^{35}S, 3.6% in plant uptake, 9.7% as CaP-S and 1.8% remaining as S^o, giving a total recovery of 64.4%. At the end of the experiment 35.6% of the added S^o (Appendix 5.4) could not be accounted for and was presumed leached as $SO_4^=$-S.

The CaP-S fraction includes most of the total inorganic ^{35}S in the soil system. At 30 days the largest inorganic ^{35}S fractions were in the top soil layer (0-3 cm) (Table 5.7A). As with the CaP-S data shown in Figure 5.2, the ^{35}S activity in the CaP-S extracts decreased rapidly from 30-60 days. By 150 days, only 0.8% and 1.3% of S from the applied SSP and S^o respectively remained in this fraction in the top 0-3 cm layers.

When expressed as the percentage of total ^{35}S labelled fertilizers remaining in the soil core layers, the percent remaining as the CaP-S, generally increased with depth (Table 5.7B). This may suggest that transformations of inorganic S in the lower soil layers were slower than in the upper layers and this might be associated with more microorganism activity and more available carbon sources in the top layer. The higher amount of the CaP-^{35}S in the lower layers may be attributed to leaching of the $^{35}SO_4^=$ down the profile.

Data in Table 5.8 shows the relative proportions of ^{35}S fractions remaining in soil cores (0-10 cm). Initially, larger percentages, about 42% of ^{35}S labelled SSP remained as CaP-^{35}S. After 60 days, however, less than 10% of both fertilizers remained as CaP-S. Up to 60 days initial incorporation of ^{35}S labelled SSP into organic ^{35}S appeared slower than that of ^{35}S labelled S^o and might partly explain the larger cumulative ^{35}S uptake of the SSP fertilized pastures at the beginning of the trial.

In general, the amount of the added ^{35}S represented by the CaP-S fraction was much smaller than the ^{35}S organic form, as shown in Table 5.6.

5.4.2.5 Total organic ^{35}S

After only 30 days the organic fraction contained the majority of the ^{35}S other than that presumed leached. The percentage of applied ^{35}S accounted for by organic ^{35}S fractions is given in Table 5.6, Appendix 5.6A (as percentage of applied fertilizer), Appendix 5.6B (as percentage of ^{35}S remaining in the soil), Table 5.8 and Figure 5.5. Significantly more organic ^{35}S was formed from $^{35}S^{o}$ fertilizer (Table 5.6) but because more SSP-^{35}S was leached the proportions of ^{35}S remaining in the soil as organic ^{35}S were similar for both fertilizers (Table 5.8 and Appendix 5.6B). In both fertilized cores, larger amounts of labelled organic ^{35}S were formed in the top layers (0-3 cm) of the soils as shown in Appendix 5.6A and Figure 5.5. In the lower layers of the soil cores as shown in Appendix 5.6A, there were no significant differences between the amounts of organic S that were derived from microfine S^{o} or SSP-S. Significant differences occurred only in the top layer (0-3 cm).

The major transformation of both labelled fertilizers into organic forms was very fast and occurred during the first 30 days (Figure 5.5) and thereafter there was no measurable accumulation of ^{35}S into the organic fraction (Figure 5.5, Appendix 5.6A and Table 5.6). More precisely, the percentage of fertilizer derived ^{35}S in the organic fraction decreased from 60 to 150 days. During this period (90-150 days) mineralization of organic ^{35}S probably occurred because plant ^{35}S continued to accumulate slowly (Figure 5.3, Appendix 5.4) despite only small decreases in CaP-^{35}S (Table 5.6).

Expressing the recovery of ^{35}S in each fraction as a percentage of the total ^{35}S remaining in the three layers of each soil core, it is possible to show that initial conversion of S^{o} to organic S was more rapid than the S from SSP (Appendix 5.6B and Table 5.8). The more rapid conversion of S^{o} in this core may be associated with the additional microbial growth derived from oxidation of S^{o}. After the first 30 days, 58% of the total remaining ^{35}S in SSP and 81% in S^{o} fertilized soil core were present in organic forms (Table 5.8). After 60 days, there were only small differences among the proportions of ^{35}S remaining as organic ^{35}S throughout the soil layers with greater than 90% of the ^{35}S remaining in each layer having been incorporated into organic S.

5.4.2.6 Carbon-bonded and ester sulphate ^{35}S

By separating the organic S into carbon-bonded and ester-$SO_4^{=}$ fractions, it is evident that the majority of the organic S formed from fertilizer S during the first 30 days was carbon-bonded S (20% and 40% of the fertilizer ^{35}S added in the SSP and S^{o} cores respectively), Table 5.6 and

Figure 5.6, Appendices 5.7 and 5.8. During the same period only 6% and 9% of the ^{35}S of SSP and S^o respectively was transformed into ester-$SO_4^=$. In the S^o fertilized core three times as much carbon-bonded ^{35}S was formed compared to the SSP core. Between 60 and 150 days there was little effect of fertilizer form on the percentage of applied ^{35}S in the ester-$SO_4^=$ fraction.

Of the ^{35}S remaining in the soil cores (Table 5.8), carbon-bonded S remained the dominant fraction making up 55% and 77% of the soil ^{35}S in the SSP and S^o fertilized cores respectively. Ester-$SO_4^=$ made up a smaller percentage but was consistently higher in SSP fertilized core.

Results presented in both Tables 5.6 and 5.8 indicate that irrespective of fertilizer form, a greater percentage of fertilizer S was immobilized into soil carbon-bonded S than the ester-$SO_4^=$ fraction. Furthermore fertilizer applied as S^o tended to promote this process. Data presented in Figure 5.6B shows that the majority of the carbon-bonded S formed rapidly (first 30 days) in the top 3 cm of the soil cores. The greatest accumulation of ester-$SO_4^=$ occurred after 60 days and was again in the top 3 cm of soil. The percentage of ^{35}S in this top-soil ester-$SO_4^=$ fraction, however, declined gradually from 60-150 days, whereas the percentage in lower soil depths remained unchanged. The percentage of applied ^{35}S remaining as carbon-bonded S in the topsoil did not decline from 60-150 days.

If the data in Figure 5.6 is expressed as a percentage of ^{35}S remaining in each soil depth then some interesting trends with depth become evident (Figure 5.7). At all depths and for both fertilizers the percentage of ^{35}S remaining as CaP-^{35}S generally decreases with time (Table 5.7B and Figure 5.4). With decreasing soil depth the proportion of the ^{35}S present as ester-$SO_4^=$ increases but that present as carbon-bonded S decreases (Figure 5.7). The results probably reflect the relative input of carbon in each soil depth; ester-$SO_4^=$ being formed in areas of low carbon input. David and Mitchell (1987) found similar trends when they studied S transformations in a forest soil in New York state, USA, by adding ^{35}S sulphate to the forest floor. Recently Nguyen and Goh (1990) also found similar trends in New Zealand soils. Alternatively, the ester-$SO_4^=$ may remain more mobile than carbon-bonded S and is leached down the profile (Figure 5.7A and 5.7B).

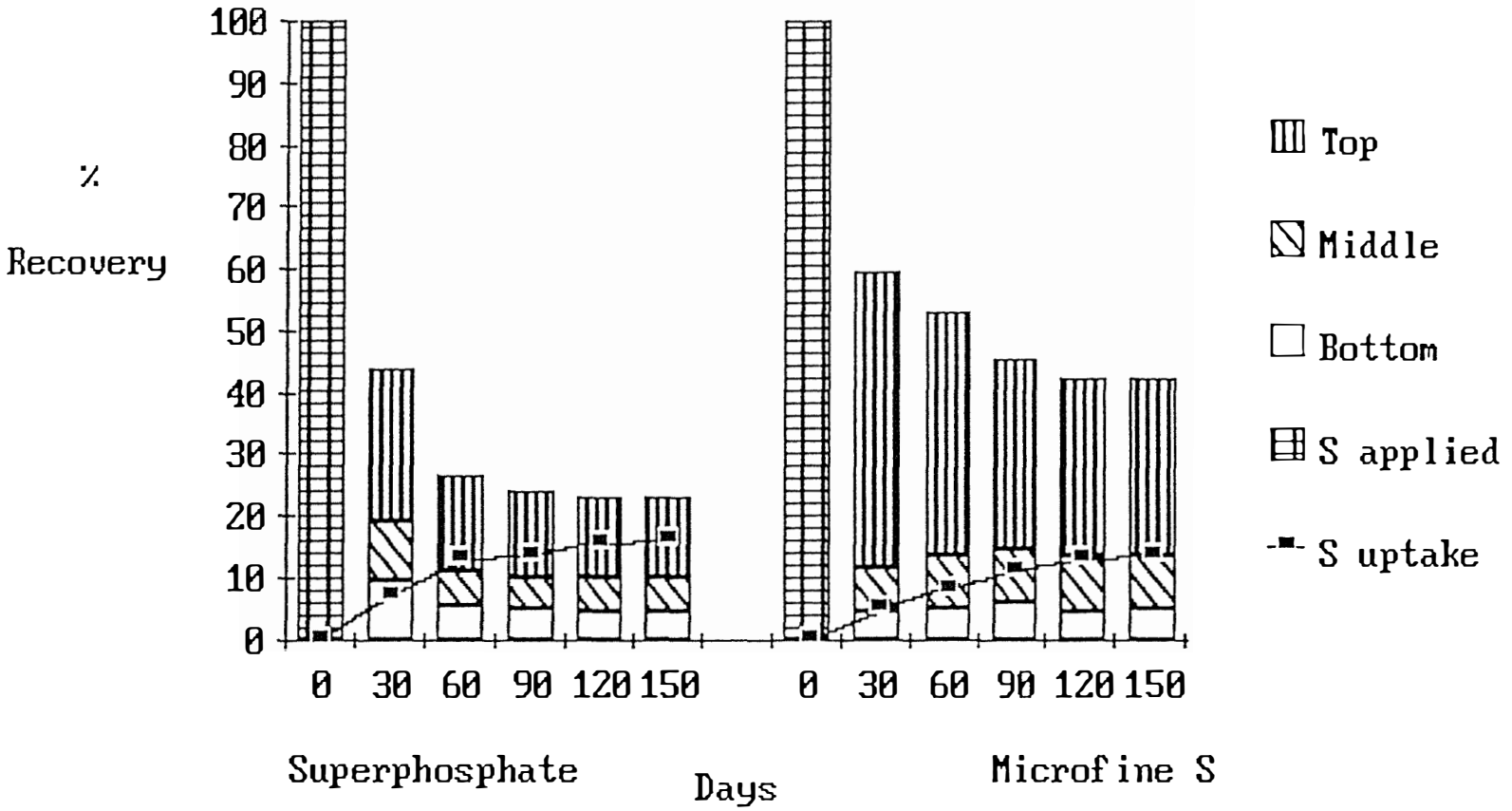


Figure 5.3 Percentage of applied ^{35}S labelled fertilizer recovered as total soil S in three soil layers and cumulative ^{35}S uptake by pasture.

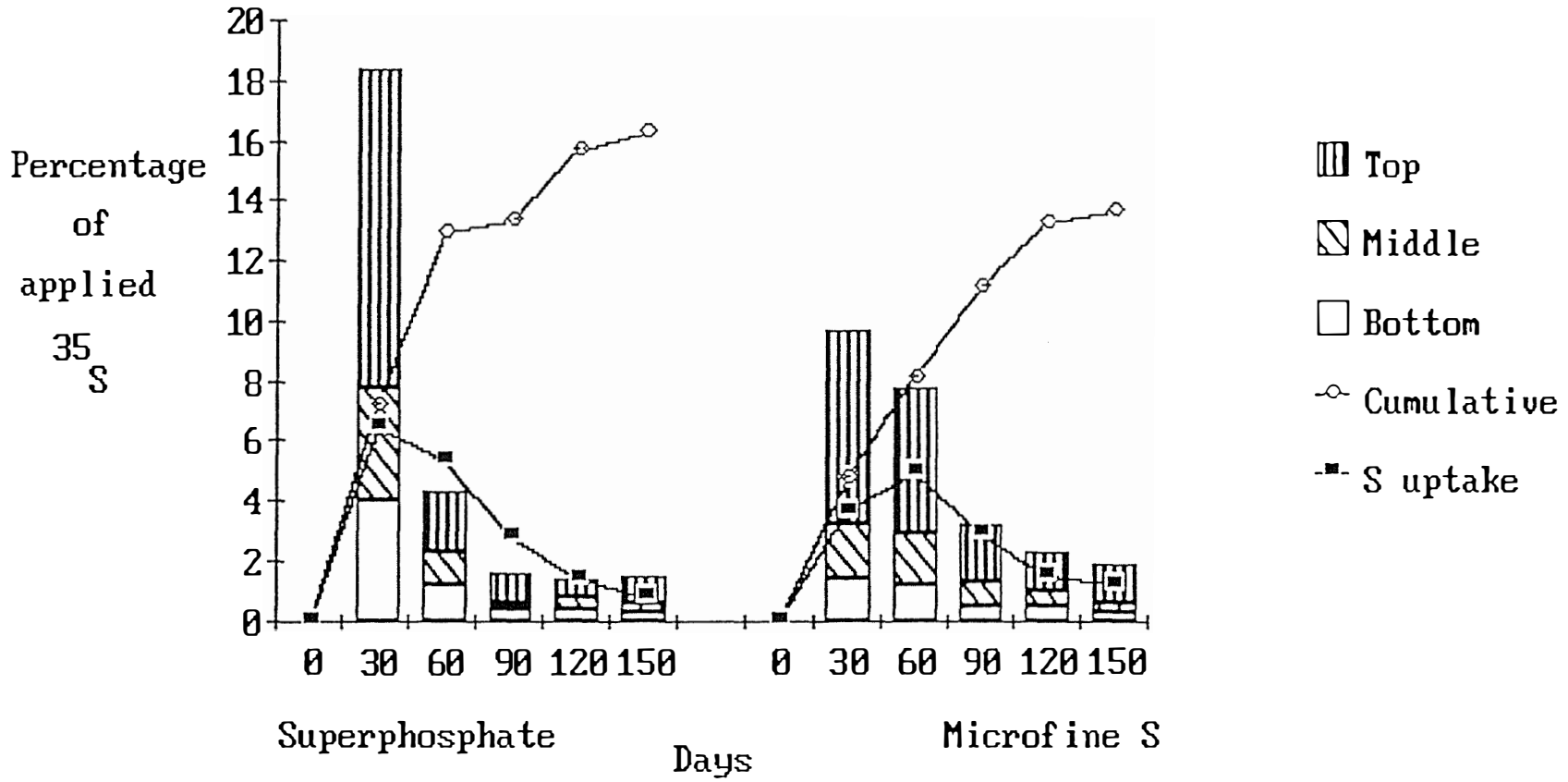


Figure 5.4 Percentage of applied ^{35}S present as extractable S (CaP-^{35}S) in three soil layers, cumulative plant ^{35}S uptake and ^{35}S taken up by pasture at each of five harvests.

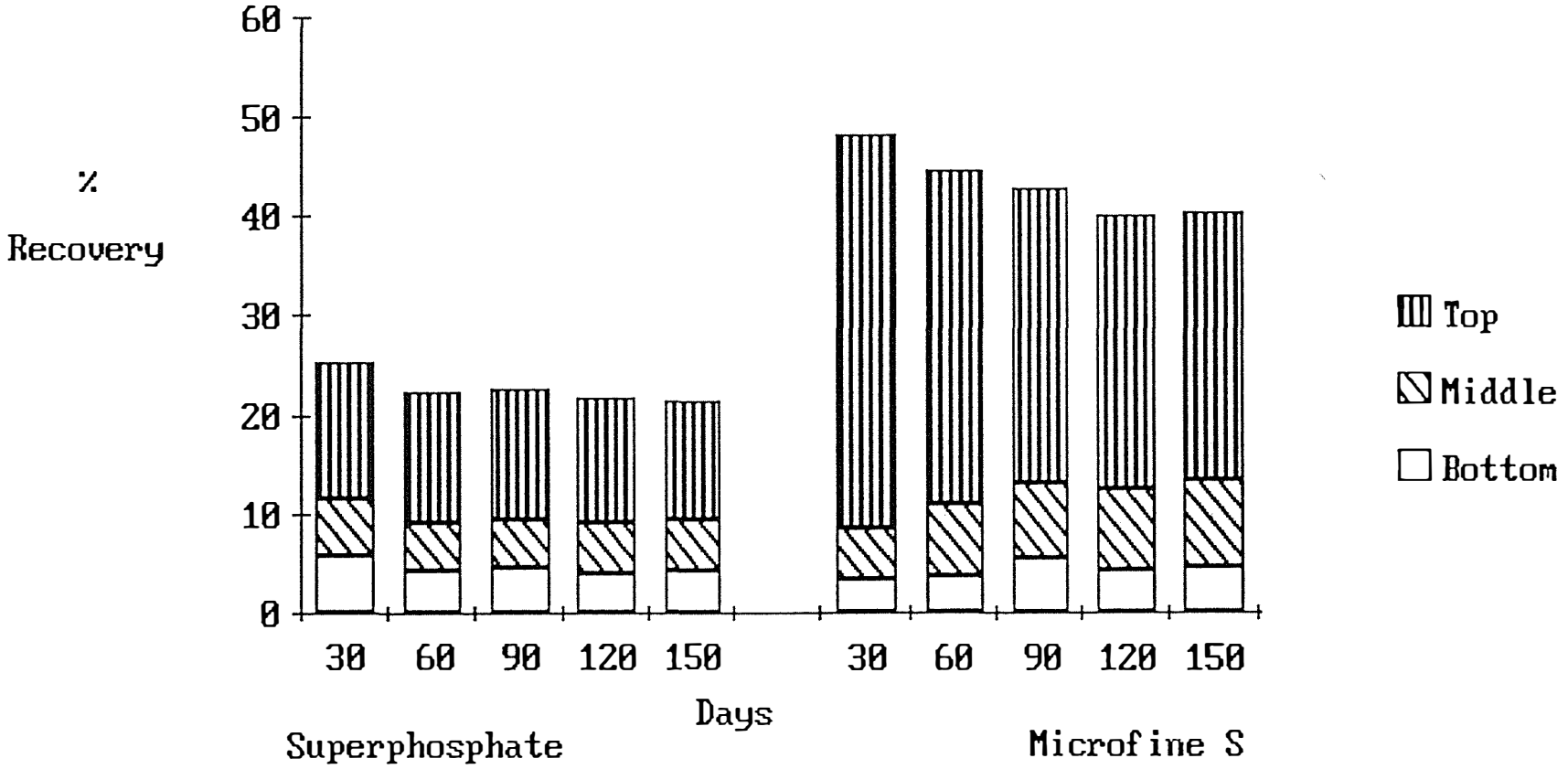


Figure 5.5 Percentage recovery of ^{35}S applied as fertilizer in organic S in three soil layers.

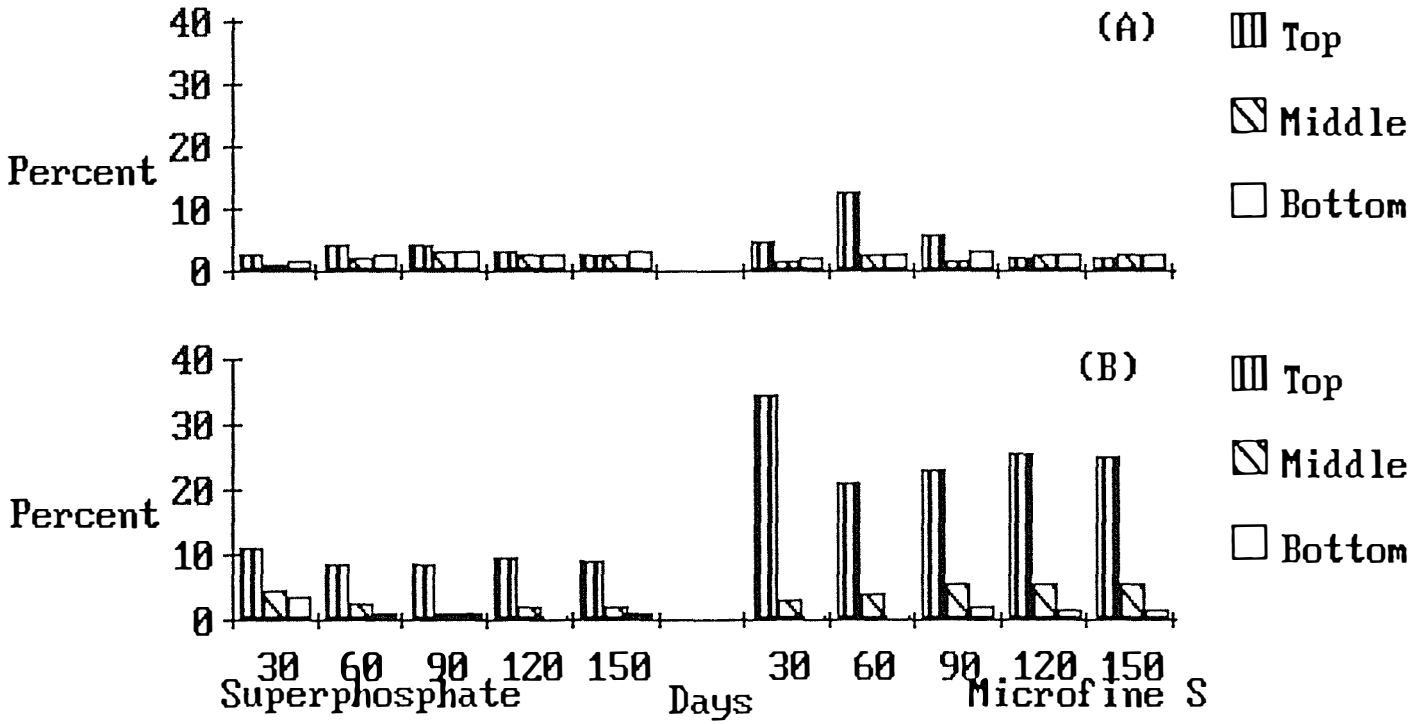


Figure 5.6 Distributions of ^{35}S ester sulphate (A) and carbon bonded ^{35}S (B) in three soil layers (value expressed as percentage of ^{35}S applied).

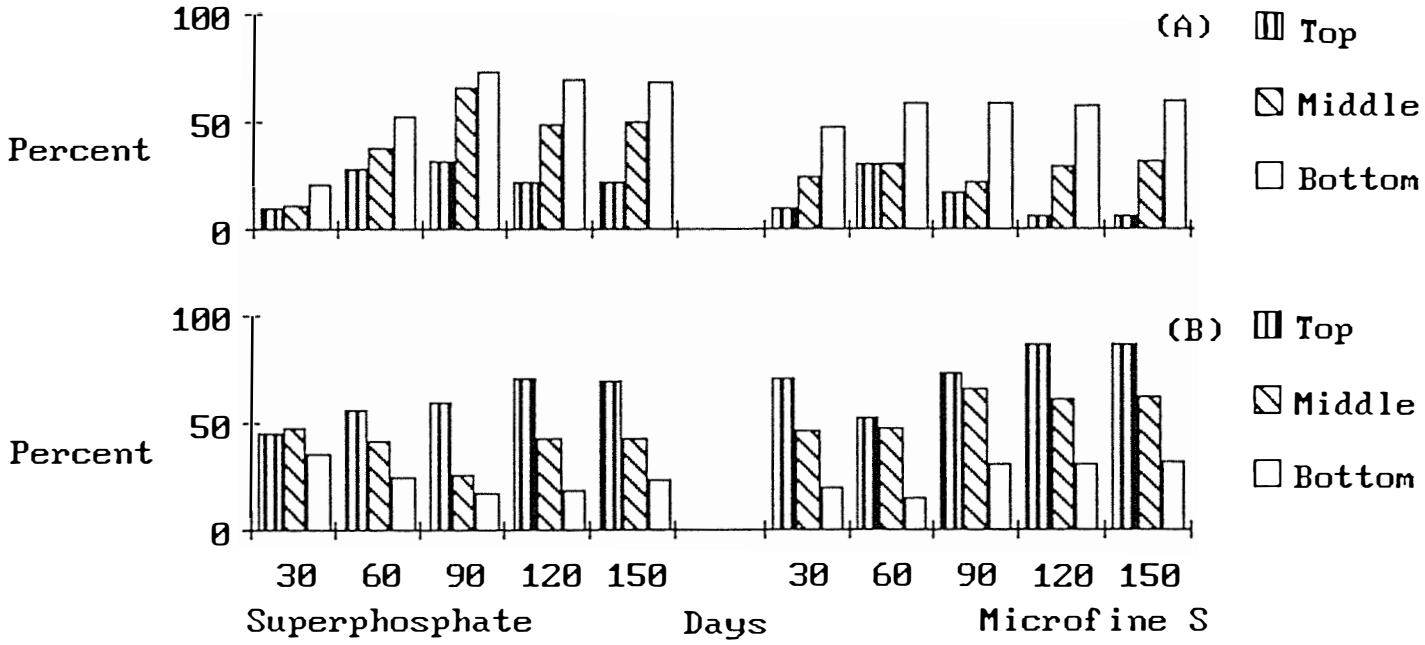


Figure 5.7 Distribution of ^{35}S ester sulphate (A) and carbon-bonded ^{35}S (B) in three soil layers (value expressed as percentage of total ^{35}S remaining in soil core, 0-10 cm).

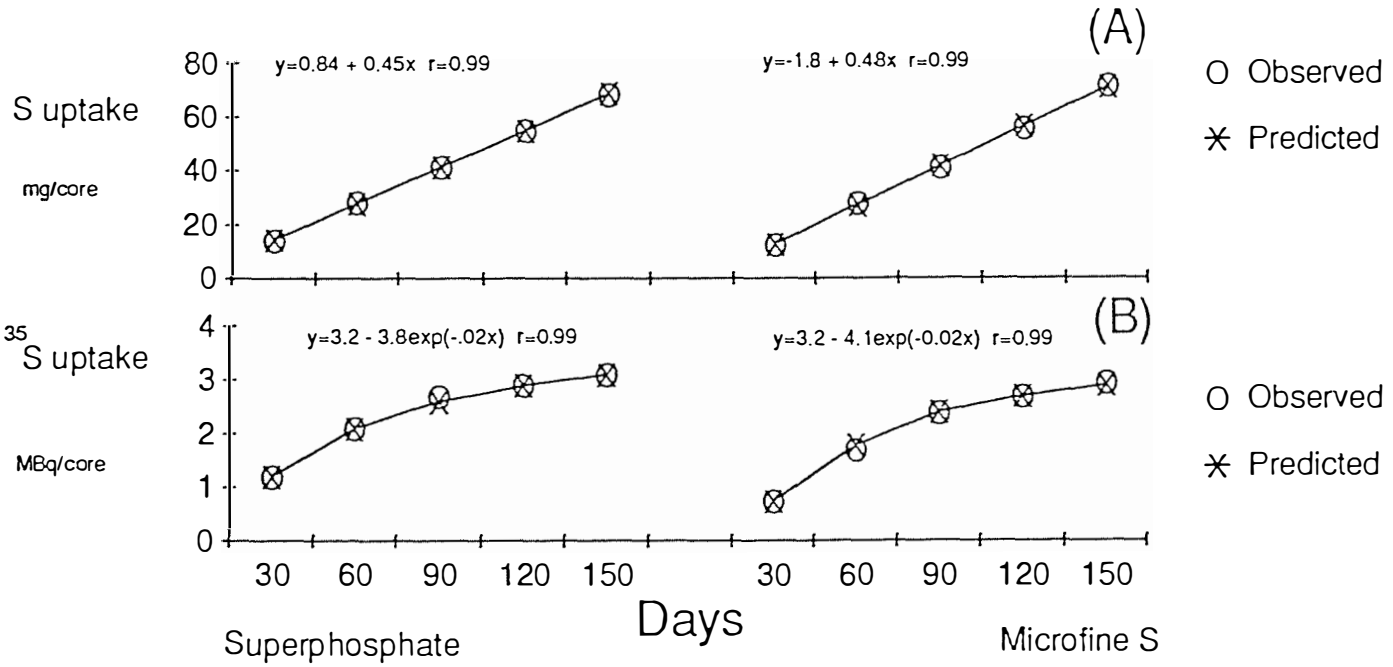


Figure 5.8 Cumulative S (A) and ^{35}S (B) taken up by plants at five harvesting times; observed vs. predicted.

Table 5.6 The percentage of total ^{35}S labelled fertilizers appearing in soil organic and inorganic ^{35}S fractions recovered from the 0-10 cm depth of undisturbed field soil cores at five harvesting times.

S forms	Fertilizers	Recovery percentage %				
Days after application		30	60	90	120	150
ORGANIC S						
Total						
	Superphosphate	25.4	22.4	22.5	21.9	21.5
	Elemental S[1]	48.1	44.8	41.9	40.1	40.3
	F-test	*	*	*	*	*
	C.V. %	14.4	9.7	13.0	4.9	13.4
Ester sulphate						
	Superphosphate	5.8	9.7	11.3	8.8	8.8
	Elemental S[1]	8.9	18.3	10.9	7.3	7.8
	F-test	*	*	ns	ns	ns
	C.V. %	15.3	23.6	24.5	37.9	37.0
Carbon-bonded S						
	Superphosphate	19.6	12.7	11.1	13.1	12.7
	Elemental S[1]	39.1	26.4	30.9	32.8	32.5
	F-test	*	*	*	*	*
	C.V. %	19.2	16.1	17.9	15.6	15.3
INORGANIC S (CaP-S)						
	Superphosphate	18.3	4.3	1.6	1.4	1.4
	Elemental S[1]	9.7	7.7	3.2	2.3	1.8
	F-test	*	*	*	*	*
	C.V. %	8.6	15.2	12.4	8.6	16.0
[a]Acetone extractable S^o						
	$^{35}S^o$	1.8	0.8	0.4	0.07	0.06
	S^o	3.7	1.8	1.0	0.80	0.60

** = significant at 5% level; ns = not significant*

[a] *Chatupote (1990);*

[1] *particle size = <10 μm*

Table 5.7 Average extractable ^{35}S (CaP-^{35}S) expressed as a percentage of total ^{35}S activity **applied** (PART A) and as a percentage of total ^{35}S **remaining** (PART B) in three soil layers at five harvests.

Fertilizers	Layers	Days after fertilizer application				
		30	60	90	120	150
PART A				.....% of applied.....		
Superphosphate						
	Top	10.6	2.0	0.9	0.6	0.8
	Middle	3.7	1.1	0.3	0.3	0.3
	Bottom	4.0	1.1	0.3	0.4	0.3
Elemental S[1]						
	Top	6.4	4.8	1.9	1.3	1.3
	Middle	1.9	1.7	0.8	0.6	0.3
	Bottom	1.4	1.2	0.5	0.4	0.3
Lsd 5% Within type[a]		1.8	1.0	0.2	0.2	0.2
C.V. %		25.9	34.9	17.9	20.7	20.6
PART B				.....% of remaining.....		
Superphosphate						
	Top	43.1	13.4	6.9	4.7	6.7
	Middle	39.4	18.8	5.4	6.2	5.7
	Bottom	42.0	21.3	7.4	9.6	6.0
Elemental S[1]						
	Top	13.7	12.0	6.4	4.4	4.8
	Middle	26.8	19.7	9.9	6.7	3.4
	Bottom	30.3	24.8	8.4	9.6	5.4
Lsd 5% Within type[a]		12.6	4.4	2.6	1.9	1.9
C.V. %		25.6	15.9	24.1	18.5	23.8

** = significant at 5% level;*

[a] *for comparison of means within each fertilizer type*

[1] *particle size = <10μm*

Table 5.8 Proportion of ^{35}S labelled fertilizers appearing in soil organic and inorganic fractions; amount expressed as percent of total ^{35}S recovered from the 0-10 cm depth of undisturbed soil cores at five harvesting times.

S forms	Fertilizers	Proportion % of remaining				
Days		30	60	90	120	150
Organic S						
	Superphosphate	57.5	83.8	93.4	94.0	93.6
	Elemental S[1]	80.4	83.9	91.8	94.4	95.3
	F-test	*	ns	ns	ns	ns
	C.V. %	6.5	2.7	1.5	0.5	1.1
Ester sulphate						
	Superphosphate	13.0	36.5	47.5	37.9	38.5
	Elemental S[1]	15.1	34.5	24.2	17.4	18.4
	F-test	ns	ns	*	*	*
	C.V. %	19.4	5.3	22.4	35.3	27.4
Carbon-bonded S						
	Superphosphate	44.8	47.3	45.8	56.1	55.1
	Elemental S[1]	65.3	49.5	65.7	77.0	76.9
	F-test	11.7	ns	18.3	11.3	16.4
	C.V. %	9.4	6.0	14.3	14.0	11.1
Extractable S (CaP-S)						
	Superphosphate	42.0	16.2	6.7	5.9	6.3
	Elemental S[1]	16.4	14.5	7.2	5.4	4.6
	F-test	*	ns	ns	ns	ns
	C.V. %	15.1	15.0	18.7	9.0	18.8
$^{35}S^{O}$ (acetone extractable)		3.1	1.4	0.9	0.2	0.2

** = significant at 5% level; ns = not significant*

[1] *particle size = <10μm*

5.4.2.7 Prediction of the extent of S transformation

The redistribution of ^{35}S isotope with time can be used to estimate the rate of redistribution of soil S between the available S pool, plants and soil organic S forms. Over a period of time **t,** with a time step Δt = 1 harvest interval (i.e. harvest n to harvest n+1), ^{35}S will be removed from the available S pool (**E**) by plant uptake (**P**), microbial immobilization (**I**) and leaching (**L**). After an initial period of immobilization some of the ^{35}S may also be added to this pool as previously immobilized ^{35}S is remineralized (**M**). The balance equation is as follows:

$$^{35}SE_{tn+1} = {}^{35}SE_{tn} - {}^{35}SP - {}^{35}SI - {}^{35}SL + {}^{35}SM \quad (1)$$

A similar equation can be written for the balance of ^{32}S

$$^{32}SE_{tn+1} = {}^{32}SE_{tn} - {}^{32}SP - {}^{32}SI - {}^{32}SL + {}^{32}SM \quad (2)$$

Three pieces of evidence suggest that the ^{35}SM is small. The amount of ^{35}S in the inorganic pool continues to decrease with time (Figure 5.4) not reaching equilibrium. Plant uptake of ^{35}S decreases rapidly with time (Figure 5.4) as the majority of soil ^{35}S appears confined to soil organic S in relatively constant proportions (Figure 5.5). Assuming then that ^{35}SM is negligible, the model in equation 1 and 2 can be used to estimate the net amount of SI formed during any period, if it is assumed that the CaP extract samples the ^{35}S specific activity of the available S pool, **E**. It is also assumed that the change in CaP-^{35}S specific activity between harvests is linear, and the average specific activity of pool **E** can be calculated as the arithmetic mean of specific activities at harvest n and n+1. The relationships derived to calculate the net immobilization and plant uptake of ^{32}S are given below:

$$\Delta SI_{tn...tn+1} = \frac{\Delta 35SI_{tn...tn+1}}{[(^{35}SE_{tn} + {}^{35}SE_{tn+1})/(SE_{tn} + SE_{tn+1})]} \quad (3)$$

Similarly the amount of S taken up by plant can be estimated as

$$\Delta SP_{tn...tn+1} = \frac{\Delta 35SP_{tn...tn+1}}{[(^{35}SE_{tn} + {}^{35}SE_{tn+1})/(SE_{tn} + SE_{tn+1})]} \quad (4)$$

This relationships only hold if ΔSI and ΔSP are approximately constant per unit time. For example, higher ΔSP for the first half of an uptake period, when the CaP-^{35}S specific activity was higher, would lead to an underestimation of total plant uptake. The Δ35SP between each

harvest was derived from the relationship between cumulative ^{35}S uptake by plants as shown in Figure 5.8. The equations shown were used to smooth the experimental data. By using the relationships in equation (4), the amount of S uptake in the superphosphate treated core during time periods of 30 days can be predicted are presented in Table 5.9. Predicted and observed plant uptake are very similar indicating that the specific activity of the CaP-S ($^{35}SE/SE$) is representative of the specific activity of the pool of S taken up by plants or immobilized into organic matter. Notable measure ΔSP was essentially linear for the study period (30-150 days).

Table 5.9 Observed plant S uptake and predicted values using relationships in equation 4 above.

	Plant S uptake (ΔSP)	
Days	Observed	Predicted
	....mg S $core^{-1}$....	
0-30	-	-
30-60	14.0	14.9
60-90	13.3	10.8
90-120	13.8	14.5
120-150	13.2	8.1
Total	54.3	48.3

The calculation could not be completed for $\Delta 32SI$ (S immobilized into soil organic matter) because apart from rapid immobilization of ^{35}S into the organic fraction in the first 30 days (Table 5.6) for the remainder of the growth period the net amount of organic ^{35}S changed little, decreasing slowly with time. Obviously a more detailed model including both mineralization and immobilization of S is required to predict actual immobilization rates. Such a model is discussed in Chapter 8. Similarly, ΔSL (leaching) occurs in intermittent events at varying intensities and requires a more complicated model which is capable of describing daily drainage events which leach labelled sulphate from an SE pool of known daily specific activity.

5.4.3 *EXPERIMENT 2, INVERSE ISOTOPIC DILUTION*

5.4.3.1 *Pasture dry matter yield and total S uptake*

Dry matters yields in both harvests and cumulative dry matter yields showed no significant differences between treatments (Table 5.10). Dry matter yields in this experiment were comparable to those of the previous experiment. As mentioned before, there was no yield response to fertilizer application because there was probably sufficient available S in this soil.

Application of fertilizers, however, significantly increased S uptake at both harvests. During the first 30 days, the uptake from the SSP treated cores was larger than from the S^o treated core. By the end of the experiment (60 days) both fertilized cores had similar total S uptake and were significantly higher than the control plot. This indicated a slow release of available S from the S^o which was of a coarser size fraction than the $^{35}S^o$ used in experiment 1.

Both fertilized cores produced larger S uptake than the control core and the results agreed with the small plot trial discussed in Section 5.4.2.1. Increase in S uptake indicated the luxury uptake of S by pasture plants which also occurred in the previous experiment.

5.4.3.2 *Total recovery of ^{35}S in soil and plant*

On average approximately 100% of the isotope injected to label the native soil sulphate pool could be accounted for by plant and soil analysis at 30 days (Table 5.11). The control core did have a lower recovery percentage than the fertilized cores, particularly the S^o core. This may result from experimental error because no logical reason for this exists. In contrast, at 60 days, the highest recovery was in the control core and lowest in the SSP treated core. The recovery in the S^o treated cores and control cores were similar. The unaccounted isotope is presumed to have leached.

Recovery of the ^{35}S radioactivity in herbage from both fertilized treatments were similar for both harvests, which suggested that fertilizer applications had no effect on the pasture uptake of the native $SO_4^=$ soil pool in both harvests. In other words, S application caused no 'priming effect'. This is contrary to the 'priming effect' observed In the direct labelling experiment

(Section 5.4.2.1) but agrees with the results of field studies with ^{35}S labelled gypsum reported by Gregg (1976) who found that a priming effect only occurred when there were positive or negative yield responses to fertilizer application. In this experiment there was no yield response.

It is assumed that the injected carrier-free sulphate ^{35}S solution and the soil sulphate pool at each depth were sampled by plant roots in a similar manner.

The specific activity of ^{35}S in pasture on the SSP treated core was lower than in pasture on the S^o and control cores at both harvests (as shown in Table 5.10). It is expected that the additional sulphate derived from SSP diluted the ^{35}S either in the soil or in the plants.

5.4.3.3 Recovery of injected ^{35}S in soil S fractions

In this experiment, it is assumed that the carrier-free ^{35}S injected into the soil was rapidly incorporated into native soil S pools and equilibrium was attained within less than one week. Any difference in the percentage of ^{35}S in different soil S forms between fertilized and the control cores will be the result of the fertilizer treatment applied to the soil core.

As anticipated, the injected ^{35}S sulphate solution was well mixed with the soil S pools as indicated by the amount of ^{35}S in the CaP-S fraction (Table 5.12). The CaP-^{35}S fraction was about 10% of the total soil ^{35}S (in the control core) which agreed with the proportion of native soil S present as CaP-S in the soil system before the trial.

5.4.3.4 Extractable ^{35}S (CaP-S)

Results are shown in Table 5.12. In the first 30 days, there was a larger amount of CaP-^{35}S activity remaining in the SSP treated core in the 0-10 cm soil layer than the S^o and control cores. The amount of CaP-^{35}S in the S^o treated core was slightly higher than that of the control core. By the end of 60 days, less than 7.2% of the applied ^{35}S activity was present in the extractable fraction of all cores. Larger amounts of CaP-^{35}S activity were present in both fertilized cores relative to the control core but, whereas the ^{35}S specific activities of the plant (discussed above) indicated isotope dilution by SSP addition, the CaP-^{35}S specific activities did not reflect this dilution.

These results may indicate that application of S fertilizer (SSP and S^0) resulted in an increase of the soil CaP-S pool further down the profile by leaching which transported ^{35}S to a zone where dilution by organic S mineralization was slower. The effect was more pronounced in the SSP treated core than the S^0 treated core and reflects an increase in the exchangeable sulphate, brought about by sulphate addition which may have led to more ^{35}S sulphate initially exchanging with this larger pool than the smaller sulphate pool in the control soil. This confirmed the result of the previous experiment (see Section 5.4.2). Goh and Tsuji (1979) also found an increase in native soil CaP-S after fertilizer S application. The specific activity of the CaP-^{35}S was also higher in the fertilizer treated cores. This may suggest that addition of fertilizer S has inhibited the mineralization of native soil S or that the rate of ^{35}S dilution and immobilization was slower with the increased sulphate pool size.

The total amount of CaP-S present in the soil cores (0-10 cm) at both harvests was higher than in the first experiment (labelled fertilizers experiment, Appendix 5.3).

This difference probably resulted from differences in soil drying methods (see Chapter 6). In the second experiment soil samples were dried in an oven at 65 ^oC rather than freeze-dried which might cause an increase in the CaP-S in the soil extracts. Many researchers have reported a large increase in CaP-S after the soil was dried at a high temperature (Barrow, 1961b; Peverill *et al.*, 1975; David *et al.*, 1982) which may be derived from microbial cell lysis (Sparling *et al.*, 1985).

Since the particle size of S^0 used in this experiment was a mixture of different particle sizes and larger (50%, <0.150 mm) than that of the previous experiment, oxidation was possibly slower. Thus, initially, the insignificant effects of the S^0 on both CaP-S and ^{35}S activity were not unexpected.

By the second harvest the amounts of CaP-^{35}S activity in the SSP treated core decreased by approximately 10% whereas the decreases in the S^0 and control cores were about 4% and 3% respectively (Table 5.12). The amount of decrease was considered to reflect the activity lost beyond 10 cm depth by leaching, rather than being taken up by plants or transformed to other soil S fractions because the ^{35}S activity taken up by herbage in the second harvest were similar in all treatments and concurrently there were larger decreases in total organic ^{35}S in both fertilized cores. These overall greater decreases in ^{35}S activity in fertilized cores imply that leaching losses were accelerated by both fertilizer applications.

5.4.3.5 Total organic ^{35}S

The percentage of ^{35}S recovered in organic form in the 0-10 cm soil layer, expressed as a percentage of the applied ^{35}S are shown in Table 5.13. As expected, the majority of carrier-free ^{35}S was incorporated into the organic ^{35}S fractions (ester-$^{35}SO_4^=$ plus carbon-bonded ^{35}S). By 30 days after the injection, the amount ranged from 67% in the control core to 92% in the S^o treated core. The amount of organic ^{35}S in the S^o treated core was higher than the other two cores. The control core had the smallest organic ^{35}S fraction at 30 days. This indicated greater immobilization which was caused by fertilizer S^o application. The higher organic ^{35}S fraction in the S^o fertilized core at the first 30 days harvest agrees with the results of the previous experiment (see Section 5.4.2).

By the end of the experiment (60 days), however, smaller amounts of total organic ^{35}S were detected in the fertilized cores (Table 5.13). This suggests mineralization and leaching of the recently immobilized ^{35}S. The amount of organic ^{35}S in the control core did not change.

As in experiment 1 the majority of organic ^{35}S formed in the first 30 days was carbon-bonded ^{35}S not ester-$^{35}SO_4^=$. The carbon-bonded ^{35}S fraction also appears to mineralize faster decreasing in size between 30 and 60 days whereas the percent of added ^{35}S in the ester-$SO_4^=$ fraction changed little. By the end of the experiment, approximately 90% of the ^{35}S remaining in the soil was in organic form being approximately two-thirds carbon-bonded S and one-third ester-$SO_4^=$. This balance in the top 10 cm of soil was more in favour of carbon-bonded S than the natural soil S distribution given in Table 5.3 and may indicate that carbon-bonded ^{35}S continues to mineralize. This supports the view of ^{35}S being immobilized by plant roots or growth of soil micro-organisms which senesced and slowly mineralized losing carbon and narrowing the C:S ratio which favoured ester-$SO_4^=$ formation (See Chapter 2, Sections 2.2 and 2.3)

Table 5.10 Dry matter yield (DM), total S uptake, cumulative dry matter yield and S uptake and specific activity of ^{35}S in herbage from two harvests.

	DM		Total S uptake		Cumulative		^{35}S specific activity	
					DM	S uptake		
Fertilizers	g core^{-1}		mg core^{-1}		g m^{-2}		KBq mg^{-1} S	
Days after injection	30	60	30	60	60	60	30	60
Superphosphate	3.5	3.8	17.2	20.0	416	2.1	137	70
Elemental S[1]	2.9	4.7	12.5	20.8	436	1.9	244	102
Control	2.8	4.6	10.6	13.6	419	1.4	236	129
Lsd 5%	ns	ns	5.1	6.2	ns	0.4	35.1	33.3
C.V. %	26.3	16.7	21.1	19.3	17.9	19.8	12.5	14.1

ns = not significant

[1] *particle size = 100% <250µm and 50% < 150µm*

Table 5.11 Mean recovery of ^{35}S from herbage and soils (0-10 cm depth) at two harvests, 30 and 60 days after injection of $^{35}SO_4^=$ into soil.

	Fertilizers	Recovery as % of radioactivity applied %	%
Days after injection		30	60
Total recovery (herbage plus soil)			
	Superphosphate	96.9	57.7
	Elemental S[1]	116.1	69.3
	Control	85.2	77.5
	Lsd 5% fertilizers	17.4	11.5
	C.V. %	9.0	9.1
Herbage			
	Superphosphate	9.2	6.0
	Elemental S[1]	12.6	8.7
	Control	10.4	7.4
	Lsd 5% fertilizer	ns	ns
	C.V. %	28.9	12.1
Soil			
	Superphosphate	87.2	51.7
	Elemental S[1]	103.5	60.6
	Control	74.8	70.2
	Lsd 5% Fertilizer	13.9	7.6
	C.V. %	9.8	7.8

ns = not significant

[1] *particle size = 100% <250μm and 50% < 150μm*

Table 5.12 Mean recovery of CaP-^{35}S and specific activity and total amount of CaP-S in 0-10 cm soil depth.

Fertilizers	Recovery % applied %		Recovery % remaining %		CaP-^{35}S specific activity KBq mg^{-1} S		Extractable S (CaP-S) mg S core^{-1}	
Days after injection	30	60	30	60	30	60	30	60
Superphosphate	16.6	6.0	18.9	11.8	61.9	31.2	63.2	45.9
Elemental S[1]	11.2	7.2	10.9	12.1	54.9	38.8	49.9	43.9
Control	7.8	4.8	10.6	6.8	42.9	30.7	44.5	37.2
Lsd 10%	5.8	1.5	6.5	3.8	5.5	4.6	13.0	4.5
C.V. %	35.9	19.3	35.1	30.6	31.1	32.2	18.1	7.7

[1] *particle size = 100% <250μm and 50% < 150μm*

Table 5.13 Mean recovery of soil ^{35}S organic S fractions from the 0-10 cm soil depth.

S forms	Fertilizers	Recovery % applied		Proportion % remaining	
Days after injection		30	60	30	60
Total organic ^{35}S					
	Superphosphate	71.2	45.7	81.0	88.2
	Elemental S[1]	92.3	53.4	89.1	87.9
	Control	66.9	65.4	89.4	93.1
	Lsd 5%	14.9	8.6	6.8	4.3
	C.V. %	12.1	9.8	4.9	3.0
Ester sulphate ^{35}S					
	Superphosphate	12.3	16.1	13.9	31.0
	Elemental S[1]	23.9	19.3	23.2	32.0
	Control	18.1	24.2	24.3	34.5
	Lsd 5%	5.1	3.5	6.1	ns
	C.V. %	20.5	13.1	21.8	9.6
Carbon bonded ^{35}S					
	Superphosphate	58.9	29.6	67.1	57.2
	Elemental S[1]	68.3	34.1	65.9	55.9
	Control	48.8	41.2	65.1	58.6
	Lsd 5%	12.2	6.9	ns	ns
	C.V. %	15.2	14.4	8.6	7.3

ns = not significant

[1] *particle size = 100% <250μm and 50% < 150μm*

5.5 DISCUSSION

5.5.1 Pasture yield and S uptake

Pasture growth and S uptake in both the small plot and microplot were comparable with no dry matter yield and S uptake responses to the applied fertilizer treatments. The applied fertilizer S was taken up at the expense of soil derived S. This was probably because the soil used in these studies had sufficient labile S for optimum pasture growth and because SSP has been applied annually to the paddock to maintain the optimum P fertility level. In this situation, S concentrations in mixed herbage throughout the trial period range from 0.39-0.47%, well above the critical level of 0.28-0.30% (Sinclair *et al.,* 1985). It has been suggested that for a paddock at this fertility level, applications of SSP can be withheld every two or three years without any depression in dry matter yield (Smith, 1976; Ledgard *et al.,* 1991). In this respect the majority of S taken up by plants was derived from the mineralization of organic soil S built up from previous fertilizer applications. Maintenance S fertilization is needed only to replace losses by animal transfer and leaching (Sinclair and Saunders, 1984) because pasture development has reached a stage where S immobilization into soil organic matter equals organic matter mineralization.

In terms of S uptake and dry matter yield, the performance of the microfine S^0 was comparable to SSP, but oxidation of this very fine particle size S^0 was very fast and probably complete within 25 days after application.

5.5.2 Short term fate of the fertilizer sulphur

In this experiment, interest was focused mainly on the immediate fate of fertilizers in the top 10 cm soil layer since most plant root activities impact on this layer. A previous study using ^{35}S in this soil revealed that about 90% S taken up by pasture plants was mostly derived from this layer (Horne *et al.,* 1992, unpublished data). Thus most fertilizer S moved beyond this layer may be considered a loss from the immediate (short term) cycling pool.

5.5.2.1 Uptake of ^{35}S labelled fertilizers

Labelled ^{35}S SSP was taken up by pasture mainly during the first 60 days and amounted to 6 to 7% of applied ^{35}S in each cut. Thereafter very small amounts were taken up. This pattern of isotope uptake is common for biologically active nutrients like S (Kennedy and Till, 1981b; Gregg and Goh, 1982; Goh and Gregg, 1982a) because microbially mediated immobilization and mineralization serve to remove ^{35}S from the available pool and dilute it with unlabelled S as time progresses. This is discussed more fully below. Plant uptake of ^{35}S during the earlier stages of the experiment (first 60 days) was greater in SSP fertilized cores but for the remainder of the experiment, ^{35}S uptake was larger (60-150 days) in the S^o cores. This probably reflects the slow release of ^{35}S by oxidation in the first 30 days and the greater amount of ^{35}S remaining in the S^o fertilized soil cores from 60-150 days.

5.5.2.2 Recovery of ^{35}S labelled fertilizer in the top 10 cm of soil

Losses of ^{35}S during the first 30 days of the direct labelling experiment were larger than expected and are unlikely to be completely explained by leaching loss because they represent S leaching losses from the SSP treatment exceeding 20 kg S ha^{-1} in the first 30 days even when the highest ^{35}S specific activity of the CaP-S pool is considered (i.e. ^{35}S added/ fertilizer plus initial CaP-S at day 0).

Although the large loss of ^{35}S remains largely unexplained by the measurement made in this experiment, the distribution of ^{35}S in soil S forms and in different soil depths provides useful information. A larger amount of ^{35}S from the labelled SSP could not be accounted for in the top 10 cm of soil (soil plus herbage) as compared to the labelled microfine S^o. By 30 days <3% of the S^o remaining in the soil was S^o. The greater retention of $^{35}S^o$ probably resulted from its faster rate of incorporation into soil organic S rather than the non-susceptibility of S^o to leaching. With coarser S materials (0.075-0.150 mm particle size) the non-susceptibility to leaching may be a more important mechanism in reducing S lost and is studied in Chapter 7.

5.5.2.3 Transformation of ^{35}S in top 10 cm of soil layer

As discussed above as both experiments proceeded the activity of CaP-^{35}S decreased in all soils and during the first 30 days there was a marked transformation of CaP-^{35}S to organic S. Larger amounts of ^{35}S labelled fertilizers remained as organic ^{35}S in the top 0-3 cm soil layer and this may be attributed to and/or associated with larger root and microbial activity taking

place in this surface horizon. Almost two-thirds of the ^{35}S remaining in the top soil was transformed into carbon-bonded S (as shown in Table 5.6). This trend in organic ^{35}S partitioning agrees with results of other investigators who observed that the rate of incorporation of radioactive ^{35}S into organic S was highest in the soil surface layer which contains larger amounts of organic residues (Swank and Fitzgerald, 1984; Schindler *et al.*, 1986; David and Mitchell, 1987) where larger amounts of carbon-bonded S were formed (Strickland *et al.*, 1987). In these pasture soils, pasture roots decrease logarithmically down the profile (Williams, 1988). Gregg (1976) also found that larger amounts of labelled ^{35}S gypsum were incorporated into organic ^{35}S in the improved pasture soils where larger amounts of organic matter had accumulated.

Transformation of ^{35}S from microfine S^o to organic forms was as fast as that of ^{35}S labelled gypsum in SSP, but the amounts transformed were twice those of the SSP treatments. It could not be explained why a larger amount of organic ^{35}S occurred in the S^o treated cores. The transformation may be associated with or occur concurrently with the microbial oxidation processes. During these processes autotrophs derived energy from S^o oxidation to fix carbon (Alexander, 1977). More detailed studies are required to determine the impact of S oxidizing microbes on the form of organic S formed. However, it is of interest to note that the capacity of soil to incorporate fertilizer S into organic forms differs with different fertilizer forms. In addition, it has been shown that the soil capacity for incorporation of S into organic forms varies with different soils (Autry and Fitzgerald, 1991). This was also indicated by the survey of pasture soil conducted by Jackman (1964a, 1964b) and relates to the climatically controlled biological productivity of the site and the ability of the soil to form organo-mineral complexes from plant and animal residues.

As a nutrient conservation point of view, it may be considered that S^o is more preferable than SSP, since larger amount of S were retained in organic forms which appeared to be slowly mineralized as the experiment proceeded.

Although larger amounts of organic ^{35}S occurred initially in the S^o treated cores, the proportions of ^{35}S remaining in the soil which were subsequently transformed into the organic fraction were about the same for both fertilizers (Table 5.8). More than 90% of the remaining ^{35}S was transformed into organic S. This indicates the importance of the transformation processes and suggests that it is possible that more ^{35}S from the SSP may be transformed into organic ^{35}S if losses by leaching can be reduced.

5.5.3 Comparison between labelled fertilizer and inverse dilution techniques

Theoretically, the inverse dilution technique is employed to detect changes in the labile pool or exchangeable pool of nutrients in a system when a treatment is applied after the labile pool or the exchangeable pool has been labelled and a maximum equilibrium has been attained. Change in the labile pool can be measured through plant uptake. Only the changes of the native soil pools can be quantified. But a quantification of changes of the applied treatment (e.g. fertilizers) cannot be achieved if the treatment is concurrently transformed into an inactive form (organic forms). Shedley (1982) employed this technique (inverse dilution) in a study of oxidation of S^o and found that a large amount of the oxidized S (sulphate form) was transformed into organic forms. The author considered that using changes of soil sulphate levels to estimate S oxidation is inaccurate and underestimated the oxidation rates.

In general, results of the inverse dilution technique were consistent with the labelling technique. Larger losses of ^{35}S occurred in the SSP fertilized cores and more ^{35}S was incorporated into organic S in the S^o fertilized cores. Additionally, the inverse dilution technique also revealed changes in the soil organic fractions, ester-$SO_4^=$ and carbon-bonded S. It appeared that carbon-bonded S was likely to be a greater source of mineralized S. This trend in organic S mineralization has been observed by Freney *et al.* (1975), McLaren and Swift (1977), McGill and Cole (1981), McLaren *et al.* (1988) and Ghani *et al.* (1991)

Isotope recovery and dilution data from both techniques remain difficult to interpret without models which are more descriptive of the factors accounting for daily removals of S from the exchangeable pool, in particular leaching losses.

5.5.4 The microplot technique (*undisturbed soil core*)

It is considered that the microplot technique employed in these studies gave results comparable to the small plot. The coefficient of variation (%C.V.) among treatments was small. Some investigators employed this technique to study the fate of fertilizer in soil systems, e.g. Peverill *et al.* (1977); Martin (1985); Destain *et al.*, (1989) and Williams *et al.* (1990a, 1990b). It was also considered that this method was less costly and presented few

practical difficulties, especially concerning the uniformity of fertilizer application. All of the applied nutrients can be conserved within the microplots and possible run-off of labelled fertilizers is prevented (Martin, 1985; Destain *et al.*, 1989).

5.6 CONCLUSIONS

1. Large unaccounted for losses of ^{35}S from the soil cores during the first 30 days of the experiment are unlikely to result solely from $SO_4^=$ leaching and therefore reduce confidence in calculated amounts of soil and fertilizer S transformed into other S fractions. However, clear trends in the relative rates of transformation of ^{35}S added as SSP or S^o are evident.

2. Incorporation of ^{35}S labelled fertilizer S into the soil organic fraction was the process that conserved the largest amounts of ^{35}S labelled fertilizers in the soil.

3. More of the microfine $^{35}S^o$ was transformed into the organic ^{35}S than ^{35}S from SSP indicating that more soil organic S reserves may be formed through the use of elemental S fertilizer than SSP based fertilizer. This may be a mechanism capable of reducing leaching loss in strongly leaching soil environments. Notably in all experiments the major organic S form labelled with ^{35}S was carbon-bonded S particularly in upper soil zones.

4. If it is assumed that unaccounted for loss of ^{35}S was due to leaching then leaching losses of S beyond the 10 cm soil layer were larger in the SSP treated cores and the majority of the loss occurred during the 30 days after application. Further studies, however, are required to confirm the nature of the loss because when expressed in terms of kg **S** leached per hactare the loss was unacceptably high.

5. The inverse dilution technique indicated that carbon-bonded S was likely to be the source of mineralized S. Both of the results from the inverse dilution and standard techniques were consistent.

5. In terms of plant S uptake, the microfine S^0 was initially slightly less effective than SSP. However, over a longer period both fertilizers showed a similar performance, reflecting the larger conservation of ^{35}S in the root zone of the S^0 treated cores. The particle sizes of S^0 used will have an important bearing on the rate of S^0 oxidation and these studies should be repeated with a range of S^0 particle sizes.

CHAPTER 6

THE MEASUREMENT OF AVAILABLE SOIL SULPHUR

6.1 INTRODUCTION

A large number of methods have been proposed to estimate the size of the immediately plant available soil sulphate pool (see Chapter 2; Section 2.3), among them extraction with 0.01 M $Ca(H_2PO_4)_2$. The amount of S extracted from air-dried soils (<2 mm) by 0.01 M $Ca(H_2PO_4)_2$ has been used by the New Zealand Ministry of Agriculture and Fisheries to modify the maintainance S recommendation for pastures (Sinclair and Saunders, 1984) into a short term fertilizer requirement. In this respect, the amount of S extracted from soil was considered to be an index of the soils ability to provide S to plants during the following growing season. A number of problems associated with this index are recognized. Different soil preparation techniques, in particular drying, storage, and grinding, influence the amount of S extracted (Peverill *et al.,* 1975; Searle and Sparling, 1987). Drainage events prior to sampling influence the soil test result (Ghani *et al.*, 1990) and the test does not reflect the varying amounts of plant available S that will be mineralized from the organic S present in the pasture soils (Nguyen and Goh, 1991). In general, the longer pasture has been influenced by fertilizer application and grazing, the greater the amount of organic S present in that soil (Jackman, 1964a, 1964b; Saggar *et al.*, 1990a, 1990b), the more S is mineralized from that soil (Sakadavan, 1991) and the less responsive dry matter yields are to the application of fertilizer S at a given soil S test (0.01 M $Ca(H_2PO_4)_2$) value (Sinclair *et al.*, 1985). Despite these problems most researchers adhere to the concept that 0.01 M $Ca(H_2PO_4)_2$ or 0.04 M $Ca(H_2PO_4)_2$ (Searle, 1979) extracts from soil are soluble and readily exchangeable (adsorbed) form of S, through which all S must pass before entering the plant root. Recently, Watkinson and Perrott (1990) and Watkinson *et al.* (1991) have demonstrated that up to 50% of total S in the extract can be present in the organic form. It is assumed that the organic S extracted is a labile form and easily hydrolysed for uptake by plants. Currently, research is in progress to improve the usefulness of soil S testing in determining fertilizer S requirements (Watkinson *et al.*, 1991).

The field experiment discussed in Chapter 5 provided a series of soil samples, taken at different times and from different soil depths under pasture fertilized with radioactively labelled superphosphate and elemental S (S^0). These samples provided the opportunity to evaluate soil preparation techniques and the ability of soil tests to determine the sources of the plant

available S pool in these soils by determining whether the specific activities of ^{35}S of particular extract S fractions are simply related to the ^{35}S specific activity in plants drawing their S supplies from these soils. Such information should be useful to those developing improved S soil tests.

If it is assumed that the extractable S (soluble S and adsorbed S) is the precursor of plant S, then a simple relationship should exist between the specific activity (SA) of herbage S and the specific activity (SA) of extractable S. If this relationship is examined for extractable S from increasing soil depths it may provide some indication of S uptake zones in soil and may indicate a soil depth which provides the best sample for analysis. For 600 days after fertilizing a paddock with labelled ^{35}S gypsum, Till and May (1971) found a significant relationship between the specific activity of extractable ^{35}S (0.01 M $Ca(H_2PO_4)_2$) sulphate from the 7.5 cm soil depth and herbage ^{35}S specific activity. Gregg (1976) also found close relationships between specific activity of plant ^{35}S and extractable ^{35}S (0.01 M $Ca(H_2PO_4)_2$) sulphate but the relationship varied among sites and from harvest to harvest. At most sites the uptake of ^{35}S was confined to the upper 15 to 30 cm.

With pasture soil, in New Zealand, it was reported that soil samples from the 0-7.5 cm depth gave satisfactory relationships between soil tests for available S and plant growth (Saunders and Cooper 1975; Saunders *et al.,* 1988; Nguyen *et al.*, 1989a, 1989b). However, Probert and Jones (1977) found that soil S extracted with 0.05 M calcium phosphate solution could be used to predict S response in establishing legumes in native pastures provided the whole profile (0-100 cm) was considered. When extractable S was below 10 ppm in the surface soil, using depth weighted means for extractable S in the profile enabled them to delineate responsive from the non-responsive sites. Soils with more than 15 ppm extractable S in the surface horizon could be categorized as non-responsive.

It would appear that relationships between extractable S and plant yield may well remain dependent upon soil characteristics, plant rooting depth and climate. Thus it is appropriate to investigate this relationship for permanent clover/ryegrass pasture on the Tokomaru silt loam studied in Chapter 5.

6.2 OBJECTIVES

The objective of the study reported in this Chapter was to compare the influence of soil sampling depth, soil preparation method and extractant type on the ability of soil tests to

identify the immediate source of plant available S in soil samples taken from the field study described in Chapter 5. Two soil preparation techniques were evaluated, freeze-drying and grinding compared to field-moist soil.

6.3 MATERIALS AND METHODS

The field trial and soil and herbage sampling techniques were those described in Chapter 5, Section 5.3. A brief discription of soil preparation and extraction techniques is given below.

6.3.1 Soil and herbage analyses

6.3.1.1 Extractable S

The soil samples from the field were stored overnight at 5 ^{o}C before being subdivided into three depths. Fresh moist soil was gently crumbled and then a known weight immediately extracted. A further sample of fresh soil was oven-dried at 105 ^{o}C for 12 hours to calculate the moisture content. The extractions were performed on samples of both fresh, field-moist soil and freeze-dried, finely ground soil using 0.01 M calcium chloride (CaCl-S) and 0.04 M calcium dihydrogen phosphate (CaP-S), pH 4, as the extractants. Five grams of wet soil or freeze-dried soil were extracted with 40 ml of either extracting solution (1:8, W/V) on an end-over-end shaker for 2 hours. The suspensions were centrifuged at 20,000 rpm, using a SS34 head on a Sorvall RC2B centrifuge, for 10 minutes and filtered with Whatman No. 42 paper. The filtrates were analysed for HI-reducible S as described in Chapter 3, Section 3.3.6. Aliquots of 1.0 ml of soil extract are used to determine ^{35}S activity as described in Section 3.3.8.

The specific activity (SS) of the HI-reducible ^{35}S in each extract was calculated as:

$$SS = A_S / S_S \quad \text{KBq mg}^{-1}\text{ S} \qquad (6.3.1)$$

where
- SS = specific activity of ^{35}S in the soil extracts
- A_S = amount of HI-reducible ^{35}S activity in the soil extract; KBq kg^{-1} soil
- S_S = amount of HI-reducible S in the soil extract; mg S kg^{-1} soil

The ^{35}S specific activity in the extract may have been slightly overestimated because ^{35}S associated with CaP-S soluble carbon bonded S would not be included in the HI-reducible S measurement. Comparisons of HI-reducible S and total S in the extracts from the Tokomaru soil suggest that carbon bonded S makes up less than 10% of the total S in the extract.

6.3.1.2 Herbage analyses

Total S and ^{35}S activity in herbage were analysed as described in Chapter 5, Section 3.3.1.1 and Section 3.3.8 and the specific activity of ^{35}S in herbage was calculated as:

$$SH = A_h / S_h \qquad \text{KBq mg}^{-1}\text{ S} \qquad (6.3.2)$$

where
- SH = specific activity of ^{35}S in herbage
- A_h = amount of ^{35}S activity in the herbage; KBq g^{-1} herbage
- S_h = amount of total S in the herbage; mg S g^{-1} herbage

6.3.2 Statistical analyses

Simple correlation and regression analyses were used to estimate the relationship between the specific activity of ^{35}S in herbage and soil sulphate S. The average specific activity of ^{35}S in the soil extracts of the first soil sampling and the second sampling were correlated and regressed against the specific activity of total ^{35}S in the herbage at the second harvest and so on. The SAS (SAS Institute Inc., 1985) and Minitab (Minitab Inc., 1989) computer programmes were employed. Specific activity data were paired as follows:

$$\frac{\sum {}^{35}A_s[H_{n-1} + H_n]}{\sum {}^{32}S_s[H_{n-1} + H_n]} \quad : \quad \frac{{}^{35}A_h[H_n]}{{}^{32}S_h[H_n]}$$

Where
- z = soil depth 1, 2 or 3 (top, middle and bottom)
- H = harvest time, n or n-1
- A_s, S_s, A_h and S_h = as described in Section 6.3.1

This treatment of data assumes that the rate of plant S uptake was constant over the period between the two harvests and that the decrease in the specific activity of the soil S extractable pool can be approximated by a linear relationship. Analysis of variance was also performed on data where correlations and regressions were significant.

6.4 RESULTS AND DISCUSSION

6.4.1 Effect of soil preparation and extractant

As described in Section 6.4.2 below, unlike the S^0 fertilized plots the specific activities of ^{35}S derived from the extraction of freeze-dried soil samples from the superphosphate treated plots were not influenced by residual S^0 slowly releasing sulphate into soil solution. Therefore, the effect of soil preparation and extractant type on the concentration of S and ^{35}S activity in the soil extracts are presented and discussed using the results from superphosphate fertilized plots only.

6.4.1.1 Effect of soil preparations

The statistical comparison between the extraction of S and ^{35}S from fresh, field-moist soil and freeze-dried soil samples were analysed separately for each extractant, namely CaCl-S and CaP-S. Results are presented in Table 6.1 and Table 6.2.

6.4.1.1.1 Effect of soil preparations on extractable S

The amounts of extractable soil S as affected by soil preparations are presented in Tables 6.1A (CaCl-S) and 6.1B (CaP-S). Both extractants extracted more (approximately twice the amount) S from the freeze-dried soils than the moist soils.

This increase, due to freeze-drying, may have resulted from sulphate being released from plant and microbial cells destroyed during the freeze-drying and grinding processes. At any one time in soils, soil microbial biomass may contain about 2-5% of the total soil S (Saggar *et al*., 1981a; Strick and Nakas, 1984; Chapman, 1987a, 1987b; Ghani *et al*., 1990). Pasture stolon and root materials were not removed from the freeze-dried soil samples prior to analysis and additional sulphate may also be released from these materials. The soil sampled from the fresh field-moist soil had less roots and underground plant materials because they tended to remain as a root mat and not as part of the 'crumbled' soil sample. After freeze-drying, however, the underground plant materials were very dry and brittle compared to those of the routine air drying method and they were easily ground and incorporated into the soil sample. The root, underground plant material and litter will contain some S in sulphate form. Horne *et al*. (1992) (unpublished data, M.J. Hedley personal communication) showed that up to 30% of the S in pasture roots was in the HI-reducible form. Lee *et al.* (1981, 1985), Watkinson and

Perrott (1990), Watkinson *et al.* (1991) and Nguyen and Goh (1991) have also shown that soil extracts using $Ca(H_2PO_4)_2$ as an extractant may contain about 30-50% of their S as soluble organic S (HI-reducible S) which may be of plant root and microbial origin.

Throughout the experimental period, the concentration of extractable S from freeze-dried soil in the top 3 cm was at least twice the concentration in the lower layers. Extractable S from moist soil did not show such marked differences with soil depth after the effect of recently added fertilizers (first 30 days) diminished (60-150 days). This is probably associated with the larger activity of plant roots and soil micro-organisms in this layer. In general, the results from the freeze-dried soil samples were less variable with time than those of the fresh field-moist soil. This is probably due to the more uniform soil sample produced by freeze-drying and grinding. The concentration of extractable S in the 30 day samples, in both moist and dry soils, were much higher than in later soil samples; this reflects the addition of superphosphate at day 0. In general, after 30 days the S concentration in all layers did not change much.

6.4.1.1.2 Effect of soil preparation on ^{35}S activity

The ^{35}S activity in the soil extracts as affected by soil preparations within each extractant are presented in Table 6.2A (CaCl-^{35}S) and PART B (CaP-^{35}S). In general, the average amount of extractable ^{35}S from the freeze-dried soil was similar to that from the fresh, field-moist soils. However, more ^{35}S was consistently extracted from the top layer of freeze-dried soil.

The effect of soil preparation on CaP-S extractable ^{35}S activity was similar to the effects on CaCl-S extractable ^{35}S described above. The greater extraction of ^{35}S after freeze-drying in the top layer probably reflects the release of ^{35}S incorporated into microbial cell and plant tissue. As discussed earlier in Chapter 5 more organic ^{35}S was synthesized in the top layer.

Throughout the experiment, extraction of freeze-dried soil produced more consistent results and showed consistent differences between the concentration of S and ^{35}S activities in top and lower soil layers. This was probably because of the more uniform mixing of soil samples during grinding which caused less sampling error.

In summary, the concentration of extractable S derived from the freeze-dried soils was much higher than that of fresh field moist soil whereas there were only slight differences in the concentration of the extractable ^{35}S, (i.e. the ^{35}S specific activity in the extracts from freeze-dried soil was less than that from moist soil, see discussion of Table 6.4 later). This inconsistency between the concentration of extractable S and ^{35}S activity suggests that

freeze-drying released HI-reducible S which had not exchanged with the isotope added. Such a source may be inactive soil microorganisms which are considered to make up 50% of the microbial population in soil (Sparling, 1985).

6.4.1.2 Effect of extractants

The effect of each extractant on the concentration of extractable S and ^{35}S activity in the soil extracts are presented in Table 6.3. A statistical comparison between the effect of each extractant was made on data from freeze-dried soil samples of the superphosphate treated plots.

6.4.1.2.1 Effect of extractant on extractable S

As shown in Table 6.3A, in general, the concentration of CaP-S was twice the concentration of CaCl-S (average CaCl-S : CaP-S ratios were 0.5-0.7). The larger amounts of S extracted by CaP-S are attributed to the ability of phosphate to displace sulphate from sorption sites (Fox *et al.*, 1964; Harward and Reisenauer, 1966). The chloride ion lacks this ability. As shown in Appendix 7.14, S sorption capacity in this soil was about 24-31%. As mentioned before, the CaP-S extract may also contain some soluble organic S (Lee *et al.*, 1981; Nguyen and Goh, 1991). In some soils organic S may contribute to 50% of the S in the CaP-S extract (Watkinson and Perrot, 1990; Watkinson *et al.*, 1991). Therefore the greater extraction of soil S by CaP-S than CaCl-S was attributed to extraction of native soil organic S (soluble organic S) and desorption of adsorbed sulphate S.

6.4.1.2.2 Effect of extractant on extractable ^{35}S activity

The comparison of the concentration of CaP-^{35}S and CaCl-^{35}S activities are presented in Table 6.3B. In general, amounts of ^{35}S activities from the CaP-S extraction were only slightly higher than ^{35}S activity from the CaCl-S the extraction (CaCl-^{35}S : CaP-^{35}S range from 0.7 to 1.0). It appears that much of the ^{35}S activity extracted is common to the CaCl-S and CaP-S extracts. Thus as discussed earlier the greater extraction of S by CaP-S than CaCl-S must be attributed to the extraction of a greater amount of non labelled (as described above) soil HI-S by CaP. This unlabelled S can originate from HI-reducible S released from inactive microorganisms by the freeze-drying process and from strongly sorbed sulphate on sorption sites within soil aggregates protected from exchange with added ^{35}S during the pot experiment in Chapter 5. Such aggregates may break down during extraction but the sorbed HI-reducible S would only be displaced by phosphate and not chloride.

Table 6.1 Concentrations of HI-reducible S in CaCl-S (Part A) and CaP-S (Part B) extracts from three soil layers using two soil preparations.

Methods		Layers	Days after fertilizer application: 30	60	90	120	150
PART A, EXTRACTABLE SULPHUR (CaCl-S)				..... mg kg^{-1} soil			
Field-moist soil		Top	10.3	1.2	0.9	2.3	0.6
		Middle	5.6	0.6	0.3	0.5	1.6
		Bottom	5.2	0.9	0.2	0.6	0.4
Ave.			7.0	0.9	0.5	1.4	0.8
Freeze-dried soil		Top	20.7	5.9	5.9	4.5	6.0
		Middle	14.9	4.3	1.9	2.9	2.6
		Bottom	17.6	2.8	2.1	2.7	2.4
Ave.			17.7	4.4	3.3	3.4	3.7
Lsd 5%	method		***	***	***	***	***
	Layer[2]		3.66	1.73	0.63	0.88	1.53
	Method*layer		ns	ns	ns	ns	ns
C.V. %			19.8	43.8	22.4	26.5	45.5
PART B, EXTRACTABLE SULPHUR (CaP-S)				..... mg kg^{-1} soil			
Field-moist soil		Top	15.6	1.2	4.3	3.8	3.3
		Middle	7.9	2.7	2.5	2.7	2.1
		Bottom	8.2	2.8	2.4	2.2	1.4
Ave.			10.6	2.2	3.1	2.9	2.3
Freeze-dried soil		Top	31.9	10.6	8.6	8.4	8.3
		Middle	24.2	4.2	5.4	6.2	4.8
		Bottom	26.3	5.1	5.8	6.4	5.7
Ave.			27.5	6.6	6.6	7.0	6.2
Lsd 5%	Method		***	***	***	***	***
	Layer[2]		5.50	1.70	2.31	1.51	1.62
	Method*layer		ns	ns	ns	ns	ns
C.V. %			19.4	28.0	32.3	20.5	25.7

**** = significant at 0.1% level; ns = not significant;* [2] *lsd for comparison between layers in each soil preparation*

Remark, data from labelled superphosphate treated plots

Table 6.2 Concentration of HI-reducible ^{35}S in CaCl-S (Part A) and CaP-S (Part B) extracts from three soil layers using two soil preparation techniques.

Methods	Layers	Days after fertilizer application: 30	60	90	120	150
PART A, EXTRACTABLE ^{35}S (CaCl-S)		..... KBq g^{-1} soil				
Field-moist soil	Top	3.09	0.26	0.18	0.08	0.04
	Middle	0.94	0.19	0.13	0.06	0.02
	Bottom	0.91	0.39	0.09	0.07	0.02
Ave.		1.65	0.27	0.13	0.07	0.02
Freeze-dried soil	Top	2.38	0.38	0.26	0.13	0.21
	Middle	0.73	0.33	0.08	0.07	0.06
	Bottom	0.69	0.21	0.07	0.07	0.05
Ave.		1.27	0.31	0.13	0.09	0.11
Lsd 5%	Method	ns	ns	ns	ns	*
	Layer[2]	0.93	0.12	0.09	0.03	0.04
	Method*Layer	ns	ns	ns	ns	ns
C.V. %		42.8	27.6	46.0	23.8	38.7
PART B, EXTRACTABLE ^{35}S (CaP-S)		..... KBq g^{-1} soil				
Field-moist soil	Top	2.39	0.39	0.12	0.09	0.06
	Middle	1.00	0.53	0.51	0.07	0.04
	Bottom	1.03	0.51	0.35	0.07	0.04
Ave.		1.65	0.47	0.32	0.08	0.04
Freeze-dried soil	Top	2.76	0.53	0.25	0.17	0.22
	Middle	0.97	0.29	0.07	0.09	0.08
	Bottom	1.04	0.29	0.09	0.11	0.07
Ave.		1.59	0.37	0.14	0.12	0.13
Lsd 5%	Method	ns	ns	ns	ns	*
	Layer[2]	1.22	0.11	0.13	0.03	0.03
	Method*layer	ns	ns	ns	ns	ns
C.V. %		20.8	17.4	38.9	24.1	23.1

** = significant at 5% level; ns = not significant; [2] lsd for comparison between layers in each soil preparation*
Remark, data from labelled superphosphate treated plots

Table 6.3 Concentration of HI-reducible S (Part A) and ^{35}S activity (Part B) in CaCl-S and CaP-S extracts from freeze-dried soils taken from three soil layers.

Extractants		Layers	Days after fertilizer application 30	60	90	120	150
PART A, EXTRACTABLE SULPHUR							
			..... mg kg^{-1} soil				
CaCl-S		Top	20.7	5.9	5.8	4.5	6.1
		Middle	14.9	4.3	1.9	2.9	2.6
		Bottom	17.7	2.8	2.1	2.7	2.4
	Ave.		17.8	4.3	3.3	3.3	3.7
CaCl-S/CaP-S			0.7	0.7	0.5	0.5	0.6
CaP-S		Top	31.9	10.5	8.5	8.4	8.3
		Middle	24.2	4.2	5.4	6.2	4.8
		Bottom	26.3	5.1	5.8	6.4	5.7
	Ave.		27.5	6.6	6.6	7.0	6.2
Lsd 5%	Extractant		*	*	**	**	**
	layer[2]		4.1	2.2	0.9	1.1	1.9
	Extr*layer		ns	ns	ns	ns	ns
C.V. %			12.3	28.0	12.3	14.3	25.9
PART B, EXTRACTABLE ^{35}S							
			..... KBq g^{-1} soil				
CaCl-S		Top	2.38	0.38	0.26	0.13	0.21
		Middle	0.73	0.33	0.08	0.07	0.06
		Bottom	0.68	0.21	0.07	0.07	0.05
	Ave.		1.27	0.31	0.14	0.08	0.11
CaCl-^{35}S/CaP-^{35}S			0.8	0.9	1.0	0.7	0.9
CaP-S		Top	2.77	0.53	0.25	0.17	0.22
		Middle	0.97	0.29	0.07	0.09	0.08
		Bottom	1.05	0.29	0.08	0.11	0.07
	Ave.		1.59	0.37	0.14	0.12	0.13
Lsd 5%	Extractant[1]		*	*	ns	*	ns
	Layer[2]		0.46	0.10	0.10	0.03	0.04
	Extr*layer		ns	ns	ns	ns	ns
C.V. %			22.0	20.1	29.6	21.2	25.7

** and ** = significant at 5 and 1% level; ns = not significant; [2] lsd for comparison between layers in each soil preparation*

Remark, data from labelled superphosphate treated plots

6.4.2 Specific activity of ^{35}S in soil extracts and herbage

Average total S and ^{35}S activity in herbage from superphosphate and microfine S^0 treated cores are presented in Appendix 6.1. (these results have already been discussed in Chapter 5). The average specific activity of ^{35}S in soil extracts in each soil depth and herbage are shown in Table 6.4.

In general, the specific activity of ^{35}S from the moist soil samples was higher than that of freeze-dried soils. On average, the specific activity of ^{35}S from the CaCl-S extraction was higher than that from the CaP-S extraction. Possible reasons for this are discussed above.

6.4.3 Relationships between the specific activity of ^{35}S in herbage and in soil extractable ^{35}S

The specific activities of extractable ^{35}S in soils harvested at the first 30 and 60 days were averaged (see methods in Section 6.3.2) and correlated with the specific activity of ^{35}S in herbage harvested at day 60 and so on for the subsequent growth period. The averaged specific activities of ^{35}S in soil extracts and herbage are shown in Figure 6.1. Decreasing ^{35}S specific activity in the soil extracts and plants with time is consistent with the slow mineralization of non-radioactively labelled soil organic S. Simple linear relationships between specific activity of ^{35}S in herbage and specific activity of ^{35}S in soil extracts (Table 6.4) are presented in Table 6.5, Figures 6.2. 6.3, and 6.4.

In general, better relationships were obtained from the correlation between the specific activity of ^{35}S from the extraction of freeze-dried soil and the specific activity of herbage. Extractions of fresh field-moist soil yielded a poorer relationship and underestimated the specific activity of ^{35}S taken up by the herbage, as shown in Table 6.5. In addition the variability in sampling moist soils caused apparent increases in specific activity of ^{35}S extracted from the top soil layer with time. In a moist soil system it is impossible for the specific activity of the plant available S pool in the top soil layer to increase with time after sulphate fertilizer application (S^0 addition may cause this, as S^0 is oxidized and also at lower soil depths this may occur as a single drainage event which may move labelled sulphate S into a lower depth). Thus the procedure of moist soil samplings would appear to produce non-uniform soil samples some containing 'hot spots' of applied ^{35}S labelled fertilizer.

Regardless of the number of soil depths considered, the specific activity of HI-reducible ^{35}S from the extraction of freeze-dried soil using either calcium chloride (0.01 M) or calcium dihydrogen phosphate (0.04 M) most closely correlated with the herbage ^{35}S specific activity for the superphosphate fertilizer plots.

The 1:1 relationship was approximated better by considering the top and middle layers and using CaP-S as the extractant (Figure 6.3). Other authors (Till and May, 1971; Gregg, 1976) have suggested that soil extract : plant specific activity ratios of close to 1 indicate a form of extractable soil S available to plants. In the present study such an interpretation suggests that CaP-S is a better extractant of the plant available pool than CaCl-S. These authors suggestion, however, is flawed because no direct association can be made between CaP-S extractable S and S taken up by the plant. The results within the present study simply indicated that the pool of S taken up by the plant has a lower specific activity than CaCl-S extracts and is similar to the specific activity of CaP-S extracts. Notably by increasing the soil sampling depth the specific activity of CaCl-S extracts could be made to approach that of the plant (compare Figure 6.2A with Figure 6.4A). The same improvement, but to a greater extent, was achieved with CaP-S extracts (compare Figure 6.2B with Figure 6.4B).

The lower specific activity of plant S than CaCl-S (soluble S) results from the plants deriving a certain amount of their S from an unlabelled source, such as, the continued mineralization of native soil organic matter and/or unlabelled HI-reducible S from greater soil depths. The ability of CaP-S extracts to predict plant specific activity will depend upon the proportional contribution to plant S from either source. If most of the unlabelled S is derived from HI-reducible S from greater soil depths then the relationship in Figure 6.3B is a causal one; i.e. plants are deriving their ^{35}S from a pool of soil sulphate defined by CaP-S extractable soil S. If mineralized soil organic S is the major plant source as indicated by the data discussed in Chapter 5 and the continued decline in CaP-S specific activity from the top soil depth (Table 6.4 and Figure 6.1) with time, then the pool size available for plant uptake will depend upon the mineralization rate. It is unlikely that this can be predicted from a single soil test taken at 1 point.

Freeze-drying caused a decrease in specific activity probably due to the release of unlabelled, but labile organic S or aggregate protected S. Thus, the better soil extract : plant specific activity relationships obtained using freeze-dried soils may suggest the importance of organic S in the soil extracts as a contribution to plant available S in a soil S test. Higher amounts of extractable S in freeze-dried soils, therefore resulted in lower specific activities of ^{35}S in the soil extracts and close relationships with the herbage ^{35}S specific activities. This result further emphasizes the need to investigate the inclusion of the labile organic S fraction in soil S testing procedures to improve the estimation of soil S status (Swift *et al*., 1988; Nguyen and Goh, 1991).

Of the three soil layers, the ^{35}S specific activity of the CaP-S extract from the top 0-6 cm of soil had the best relationship with herbage specific activity. This also suggests that pasture S, in this superphosphate treated plot, was mostly taken up from this soil layer.

Table 6.4 The specific activity of ^{35}S in CaCl-S and CaP-S extracts of field-moist and freeze-dried soils and plants.

Fertilizers	Layers	Days after fertilizer application 30	60	90	120	150
FIELD-MOIST SOIL EXTRACTION						
		..... KBq mg^{-1} S				
CaCl-S						
Superphosphate	Top	312	203	246	41	77
	Middle	176	390	552	108	16
	Bottom	207	461	753	143	83
Elemental S	Top	161	182	316	64	70
	Middle	344	153	122	92	37
	Bottom	509	289	77	104	72
CaP-S						
Superphosphate	Top	174	472	37	25	20
	Middle	130	201	206	29	18
	Bottom	129	197	172	35	36
Elemental S	Top	131	163	106	49	39
	Middle	125	137	212	43	43
	Bottom	102	140	213	38	27
FREEZE-DRIED SOIL EXTRACTION						
CaCl-S						
Superphosphate	Top	116	69	43	29	36
	Middle	50	80	43	25	27
	Bottom	40	76	35	25	23
Elemental S	Top	68	91	55	42	41
	Middle	39	91	47	28	28
	Bottom	26	84	40	31	23
CaP-S						
Superphosphate	Top	86	50	29	20	28
	Middle	40	75	13	14	18
	Bottom	40	65	15	17	13
Elemental S	Top	73	95	52	35	40
	Middle	24	78	24	15	15
	Bottom	18	61	20	19	12
PLANT ^{35}S SPECIFIC ACTIVITY						
Superphosphate		83	67	38	18	12
Elemental S		60	66	45	22	15

Table 6.5 Simple linear relationships, y = bx, between specific activity of total ^{35}S in plants (y) and specific activity of ^{35}S in extracts from different soil layers (x).

		Soil Preparation			
		Field-moist soil		Freeze-dried soil	
		Relationships	r	Relationships	r
SOIL LAYERS; TOP[1]					
CaCl-S	Superphosphate	$0.13X^{***}$	ns	$0.63X^{***}$	0.96^{***}
	Elemental S	$0.10X^{***}$	ns	$0.67X^{***}$	0.68^{***}
CaP-S	Superphosphate	$0.09X^{**}$	ns	$0.86X^{***}$	0.96^{***}
	Elemental S	$0.34X^{***}$	0.66^{**}	$0.61X^{***}$	0.68^{***}
SOIL LAYERS; TOP+MIDDLE[2]					
CaCl-S	Superphosphate	$0.12X^{***}$	ns	$0.72X^{***}$	0.97^{***}
	Elemental S	$0.26X^{**}$	0.55^{*}	$0.66X^{***}$	0.57^{*}
CaP-S	Superphosphate	$0.21X^{***}$	0.55^{*}	$1.01X^{***}$	0.99^{***}
	Elemental S	$0.29X^{***}$	ns	$0.70X^{***}$	0.55^{*}
SOIL LAYERS; TOP+MIDDLE+BOTTOM[3]					
CaCl-S	Superphosphate	$0.11X^{***}$	ns	$0.78X^{***}$	0.95^{***}
	Elemental S	$0.28X^{***}$	0.91^{***}	$0.68X^{***}$	ns
CaP-S	Superphosphate	$0.23X^{***}$	0.55^{*}	$1.09X^{***}$	0.98^{***}
	Elemental S	$0.29X^{***}$	ns	$0.77X^{***}$	ns

, ** and * = significant at 5, 1 and 0.1% level, respectively; ns = not significant*

[1] Specific activity of extractable ^{35}S (**SS**) was calculated as:

$$SS = A_{s1} / S_{s1}$$

where

$$A_{s1} = \Sigma^{35}S_{H(n-1)+Hn}[top] \quad \text{KBq core}^{-1}$$

$$S_{s1} = \Sigma S_{H(n-1)+Hn}[top] \quad \text{mg core}^{-1}$$

[2] Specific activity of extractable ^{35}S (**SS**) was calculated as:

$$SS = A_{s12} / S_{s12}$$

where

$$A_{s12} = \Sigma^{35}S_{H(n-1)+Hn}[top + middle] \quad \text{KBq core}^{-1}$$

$$S_{s12} = \Sigma S_{H(n-1)+Hn}[top + middle] \quad \text{mg core}^{-1}$$

[3] Specific activity of extractable ^{35}S (**SS**) was calculated as:

$$SS = A_{s123} / S_{s123}$$

where

$$A_{s123} = \Sigma^{35}S_{H(n-1)+Hn}[top + middle + bottom] \quad \text{KBq core}^{-1}$$

$$S_{s123} = \Sigma S_{H(n-1)+Hn}[top + middle + bottom] \quad \text{mg core}^{-1}$$

where

H = harvesting time, n; **SS**, A_s and S_s as described in section 6.3.1.1.

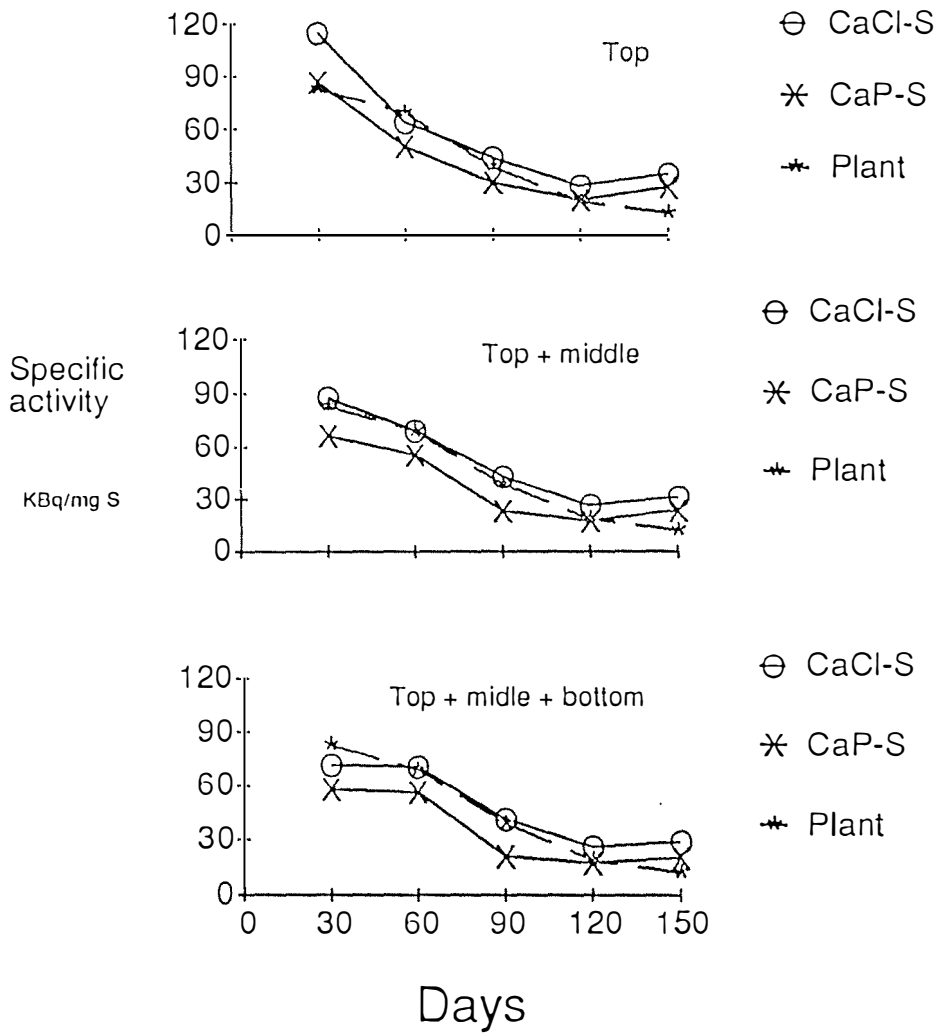


Figure 6.1 Specific activity of ^{35}S in plants and soil extracts (CaCl-S and CaP-S) from superphosphate treated cores at five harvests for three soil depths.

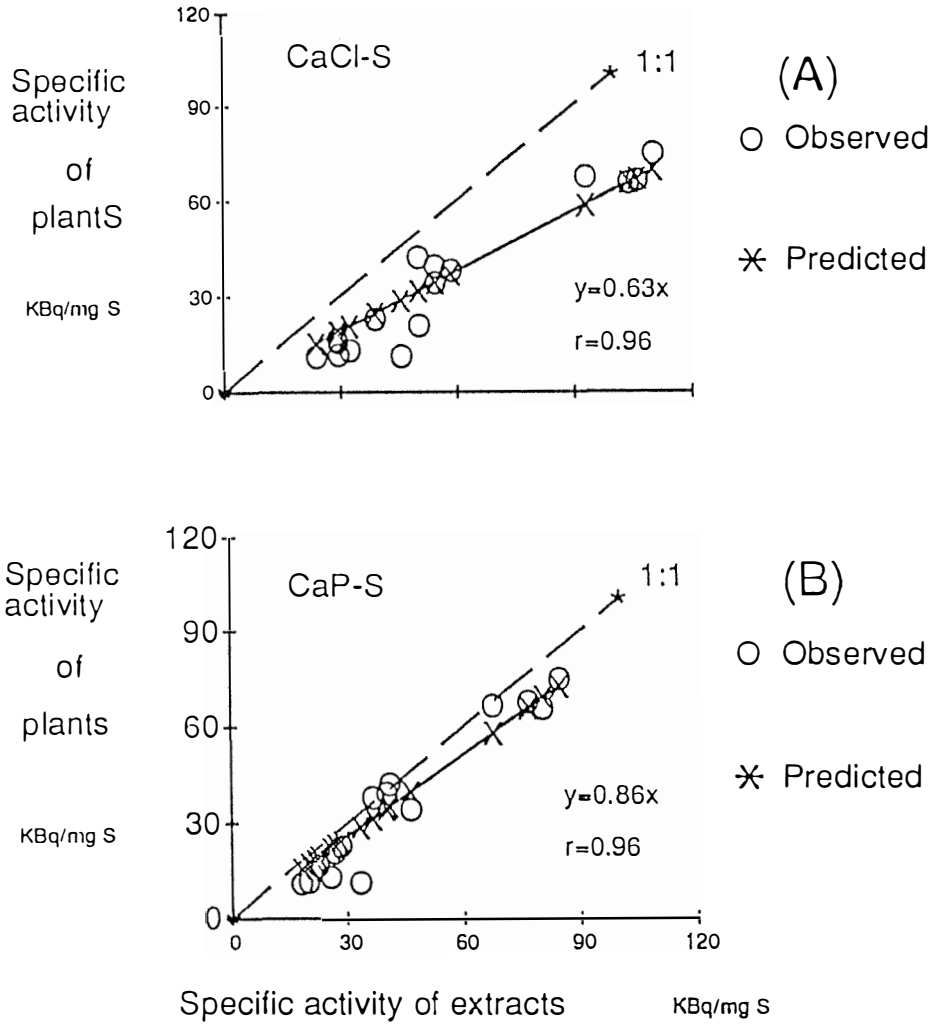


Figure 6.2 Relationships between specific activity of ^{35}S in plants and HI-reducible ^{35}S in soil extracts from the top layer; (A) = CaCl-S and (B) = CaP-S.

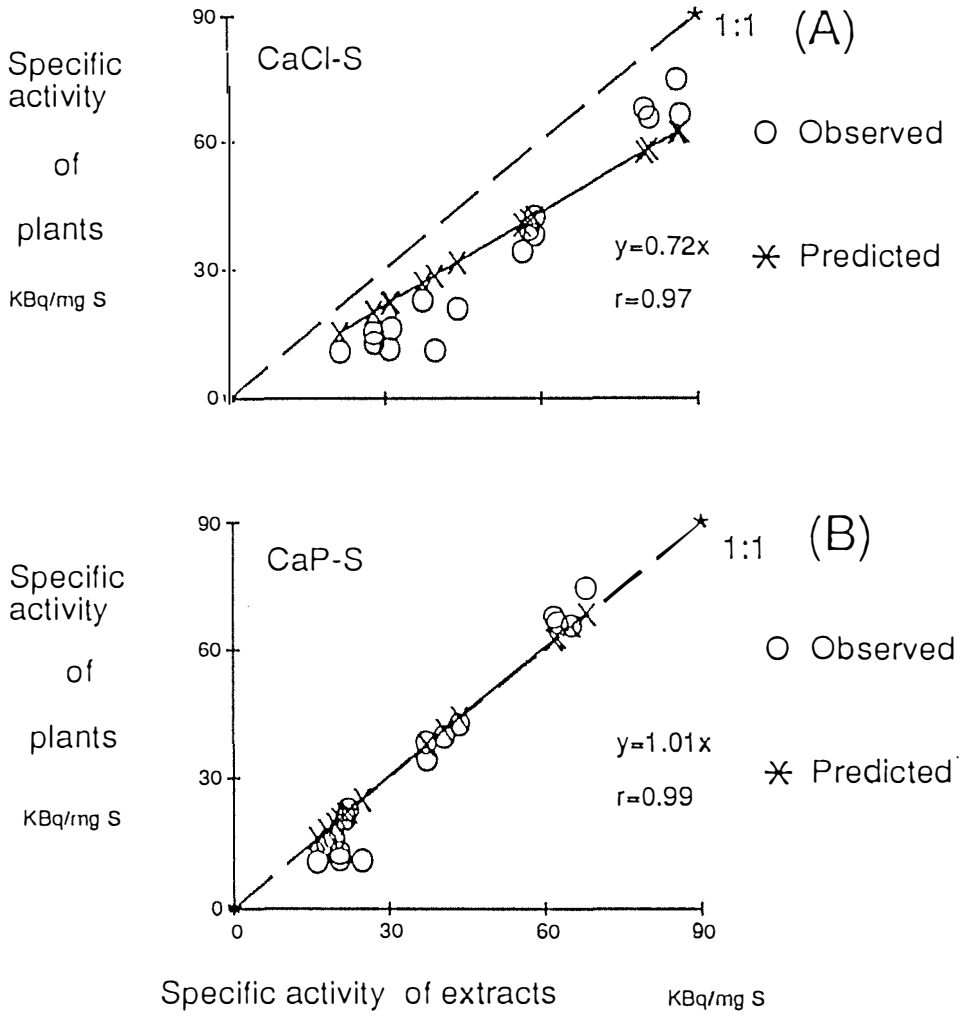


Figure 6.3 Relationships between specific activity of ^{35}S in plants and HI-reducible ^{35}S in soil extracts from the top plus middle layers; (A) = CaCl-S and (B) = CaP-S.

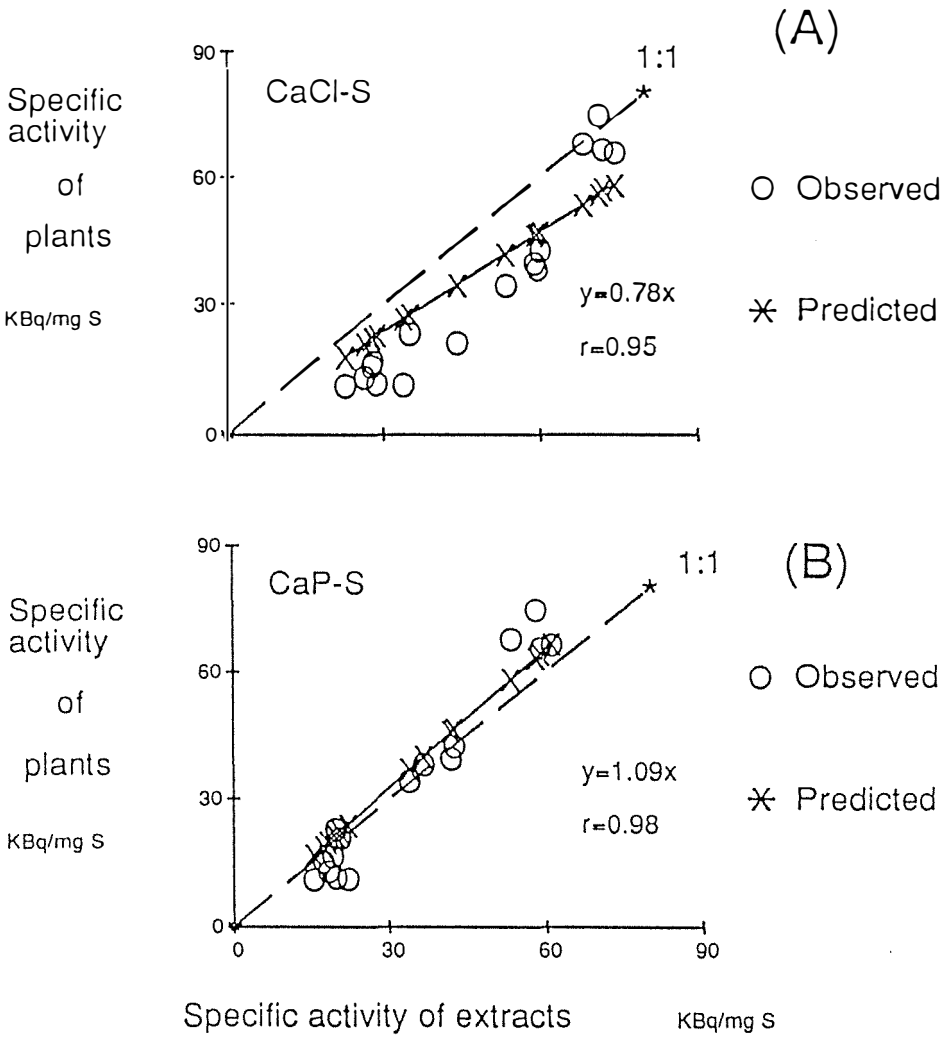


Figure 6.4 Relationships between specific activity of ^{35}S in plants and HI-reducible ^{35}S in soil extracts from the top plus middle and bottom layers; (A) = CaCl-S and (B) = CaP-S.

6.5 CONCLUSIONS

1. Of the two soil preparation techniques (extraction of moist-crumbled soil or freeze-dried and ground soil), freeze-drying and grinding causes significantly more S, but not more ^{35}S, to be extracted from soil samples. Therefore, freeze-drying exposes a form of soil S to extraction that was not freely exchangeable with added sulphate-^{35}S even during 150 days of plant growth.

2. The average ^{35}S specific activity in a CaP-S extract from a freeze-dried sample of the top 6 cm of pasture soil was the extract specific activity most closely related to that of S taken up by plants over several periods of plant growth. Calcium chloride (CaCl-S) extracts from freeze-dried soil and CaCl-S and CaP-S extracts from moist soils had ^{35}S specific activities that were generally higher than that of S taken up by plants.

3. Results indicated that plant roots have access to forms of soil S that could not be accessed by soil water and exchangeable sulphate ions (i.e. extracted by $CaCl_2$ from moist soil) during 150 days of plant growth. Such forms are likely to be: (a) organic S that is mineralized during plant growth or (b) aggregate-protected, adsorbed sulphate, ($SO_4^=$), which becomes accessible to the penetrating roots or root hairs during plant growth but not to $CaCl_2$ extraction of moist soil.

4. The specific activities of CaP-S extractable S from samples of freeze-dried and ground soil (0-6 cm) were similar to that of S taken up by plants indicating that this extract from this soil depth better represents the plant available S pool than the other extraction methods, however, this result does not confirm that the S in the extract is the precursor of S taken up by plants. Confirmation that CaP-S extracts plant available S requires a quantitative assessment of plant S uptake relative to depletion of CaP-S. The CaP-S pool is dynamic and requires a consideration of all factors influencing its magnitude. This is attempted in Chapter 8.

CHAPTER 7

THE INFLUENCE OF FERTILIZER FORM ON THE FATE OF SULPHUR IN SOILS

7.1 INTRODUCTION

Extensive field trials in New Zealand (Ludecke, 1965; Sinclair and Enright, 1983; Sinclair *et al.*, 1985; Boswell and Swanney, 1986, 1988, 1991; Lee and Boswell, 1988; Boswell *et al.*, 1988a, 1988b; Swanney *et al.*, 1988) have demonstrated that finer particle sizes of elemental S (S^0) oxidize more rapidly than coarser particles and create high initial plant S uptake rates. Leaching losses of S were not measured in these experiments but it may be predicted that more rapid S oxidation may lead to greater S leaching as observed in Chapter 5 when microfine S^0 was used. Thus for S conservation in a grazed pasture system, a compromise must be reached between oxidation rate, plant uptake and leaching loss. This compromise may best be achieved by varying the particle size distribution of the S^0 fertilizer for specific situation. Before such fertilizer products can be designed the effect of different S^0 particle sizes on the fate of S in soils must be determined.

Most S^0 applied to pasture in New Zealand is combined with P fertilizer such as S^0/RPR mixtures and sulphurized SSP in granule forms. The presence of phosphate fertilizer may influence S^0 oxidation (Kittams and Attoe, 1965; Attoe and Olsen, 1966; Bloomfield, 1967; Lee *et al.*, 1987) and the fate of fertilizer S in the soil/plant system (During, 1984; Boswell, 1983; Bolan *et al.*, 1986, 1987). In this series of experiments the effect of phosphate fertilizer on the fate of S from both sulphate-S and S^0 was examined as was the effect of elemental S particle size.

In Chapter 5, it was found that accumulation of organic S in soil was larger, and estimated leaching losses of S from field soils under pastures were smaller, when S^0 was applied rather than single superphosphate (SSP). Apparent sulphate leaching losses of applied fertilizer S beyond 10 cm depth in this field experiment were large, 61% for SSP and 44% for S^0 treated soil cores. These losses were uncontrollable due to the unpredictable nature of seasonal rainfall. The large leaching loss of S from S^0 was probably due to the fact that the S^0 used was microfine and was rapidly oxidized to sulphate. In the light of these results it was necessary to repeat supplement this earlier experiment with particle size ranges of S^0 which reflect the size ranges normally applied in fertilizer material.

7.2 OBJECTIVES

1. To examine the influence of S^0 particle size on the fate of S^0 in pasture soils
2. To examine the effect of phosphate fertilizer on the fate of S from S^0 and sulphate-S fertilizers
3. To examine the effect of granulation on the fate of S from S^0 and S^0/phosphate rock fertilizers

7.3 MATERIALS AND METHODS

A second experiment using undisturbed soil cores, isolated in galvanized steel cylinders similar to those used in Chapter 5, was designed and conducted on two soil types; a Tokomaru silt loam (yellow-grey earth), a pasture soil of lower fertility than that studied earlier and Ramiha silt loam, a yellow-brown earth/yellow-brown loam intergrade, sited in an area with higher rainfall and altitude. At each site only 10% of the soil cores prepared were left in situ for field studies, the remaining undisturbed soil cores were transferred to the glasshouse where leaching events could be controlled and the leachates collected for analysis

7.3.1 Soils

The experiments were conducted both under field and glasshouse conditions at Massey University using undisturbed field soil cores of Tokomaru and Ramiha silt loam soils contrasting in organic matter contents, S contents and P retention, vegetated by predominantly ryegrass/clover pasture. General soil properties of these two soils are shown in Table 7.1 and Table 7.2. These results are derived form a series of small soil cores (15 cores, 2.5 cm diameter by 10 cm depth) sampled from each site prior to trial establishment. Soil cores were bulked, air-dried and sieved through 2 mm prior to analysis.

Tokomaru soil is genetically moderately leached and moderately acidic, classified as a New Zealand yellow-grey-earth (Typic fragiaqualf). It was formed on thick deposits of loess of fine sandy loam texture. Drainage is impeded during the wet season which results in a common occurrence of a pale-coloured horizon bearing iron/manganese concretions lying beneath the top and sub soils (Cowie, 1978). The field site was located at the Massey No. 4 Dairy farm 5 km from Palmerston North. The pasture composition on Tokomaru site were predominantly

clover *(Trifolium repens)* and ryegrass *(Lolium perenne)* and had not been fertilized for at least 13 years. The lack of recent fertilization is reflected in the low Olsen P and calcium phosphate extractable S (CaP-S) soil test values (Sinclair and Saunders, 1984).

Ramiha soil is derived from similar parent material to Tokomaru soil, but formed under high rainfall and has been more strongly leached. The higher P retention is due to the weathering of volcanic ash present in the loess which produces amorphous type clay which are absent in Tokomaru silt loam soil. Naturally, this soil is low in exchangeable bases and is acid in reaction. The site used for the undisturbed soil core study and collecting undisturbed soil cores for glasshouse studies was a hill soil (intergrade between a New Zealand yellow-brown-earth/yellow-brown-loam) at Tuapaka farm at an altitude of 300 meters above the sea level, 10 km east of Palmerston North. The climate is cool-temperate with an evenly distributed rainfall throughout the year, normally peaking in June and December (Pollok and Mclaughlin, 1986). On the Ramiha site there was a significant amount of browntop *(Agrostis capillaris)*, together with ryegrass and white clover. The Ramiha pasture had received superphosphate at the rate of 200 kg ha^{-1} (1983-1984) and 300 kg ha^{-1} (1984-1985). In 1985-1986, it received 200 kg ha^{-1} of Hyphos-S+Se (6% S) and no fertilizer was applied during the 1986-1987 trial period. Although the site had a low Olsen-P value, CaP-S levels were medium (Sinclair and Saunders, 1984). Both sites were under established pastures grazed by sheep.

7.3.2 Preparation of soil cores

Galvanized steel cylinders (1 mm wall thickness, 15 cm diameter and 10 cm length) were driven into pasture of uniform sward content at both field sites. The soil cores were then removed and the bottoms were sealed with nylon mesh (1.00 mm opening). The cores were placed in a glasshouse and maintained at a moisture content of approximately 90% of field capacity by weighing and watering every 3 days. This provided a simulated pasture situation, with soil microflora and fauna, top-soil profiles and plants virtually undisturbed.

7.3.3 Design of the experiments

After surface application to the undisturbed soil cores the fate of S^o of three particle sizes, was studied for a pasture growth period of 180 days. An application rate of 30 kg S ha^{-1} was chosen for the study. Treatments consisted of three particle size ranges of ^{35}S labelled S^o namely, <0.150 mm (**SS**), 0.150-0.250 mm (**MM**), and 0.250-0.500 mm (**LL**) and two granulated $^{35}S^o$ labelled, S^o materials made from the <0.150 mm $^{35}S^o$ labelled S^o (granulated S^o plus fine ground North Calorina phosphate rock and granulated without

phosphate rock, **SS/PR** and **SS/gr**). Two additional check soil cores, granulated phosphate rock (**PR**) and control (**Ctrl**) were also included in the study. These treatments were applied to the surface of pre-trimmed undisturbed soil cores on the 29 November, 1987. Herbage and soil samples in both glasshouse and field trials were taken at various harvest intervals during the 180 days of growth. Treatments and replications are presented in Tables 7.3, 7.4, 7.5 and 7.6.

In the glasshouse trial, two additional treatments with four replicates each were also applied to both soils along with the above study. These treatments were ^{35}S labelled gypsum (**GP**) and superphosphate (**SSP**) and were carried out for 90 days.

Table 7.1 General properties of the soils used in the studies.

Description	Soils	
	Ramiha	Tokomaru
GENERAL PROPERTIES		
Location	10 km east of Palmerston North	5 km south of Palmerston North
Land form	Tararua range, rolling	High river, flat
Rainfall (mm)	1270-1520	890-1140
Soil type	Ramiha silt loam	Tokomaru silt loam
NZ soil group	Yellow-brown earth/ yellow-brown loam	Yellow-grey earth
Soil taxonomic classification	Andic Dystrochrept	Typic Fragiaqualf
Parent materials	Siliceous loess over graywacke[a]	Siliceous loess[b]
Clay minerals	Vermiculite, some illite, allophane[b]	Mica/illite some vermiculite[a]
Bulk density (0-5 cm) ($kg\ m^{-3}$)	963	1035
Soil pH, in water (1:2)	5.5	5.7
Organic carbon (%)[c]	7.6	4.5
P retention (%)[d]	88	20
Extractable P[e] ($mg\ kg^{-1}$)	11	8

[a] *Pollok (1975);* [b] *McLaughlin (1983);* [c] *Bremner and Tabatabai, 1971;* [d] *Saunders, 1965;* [e] *Olsen et al. (1954).*

Table 7.2 The forms and distribution of soil S[a] in three soil layers collected from field sites before the experiment.

Layer	Depth	Total S	Organic S	Ester sulphate S	Carbon bonded S	Extractable S[b]	
	cm					CaP-S	CaCl-S
				mg S kg^{-1} soil			
RAMIHA							
Top	0-3	540	524	201	323	15.2	10.0
Middle	3-6	434	425	198	327	8.2	4.2
Bottom	6-10	445	437	211	226	7.7	3.0
TOKOMARU							
Top	0-3	395	384	125	259	9.0	6.4
Middle	3-6	365	358	131	227	7.3	4.2
Bottom	6-10	320	313	138	175	7.1	3.1

[a] *method of Landers et al., (1983);* [b] *contains some soluble organic S (HI-reducible S) (Watkinson et al., 1991)*

7.3.4 Labelling fertilizer S

7.3.4.1 Labelling S^o containing fertilizers

Three different particle sizes, <0.150 (SS), 0.150-0.250 (MM) and 0.250-0.500 mm (LL), of S^o labelled with $^{35}S^o$ were made for this study. The manufacturing methods were described in Section 3.1.3.

Radioactively labelled $^{35}S^o$ was manufactured in two Lots: **Lot I**, low activity, destined for soil cores removed form the field and glasshouse during the early stages (0-60 days) of the experiment and **Lot II**, high activity, destined for soil cores sampled late in the experiment (90-180 days)

Lot I.

A 0.4 ml aliquot of 651.2 MBq ml^{-1} of carrier-free ^{35}S in toluene was added into 4.509 g of finely ground elemental S. This yielded 3.207 MBq per 53 g of S (standard deviation=1.35) or a ^{35}S specific activity of 60.51 MBq g^{-1} S. Soil cores that received this labelled material were destructively sampled at 15, 30, 45 and 60 days.

Lot II

A 2.1 ml aliquot of 651.2 MBq ml^{-1} of carrier-free plus residual $^{35}S^{o}$ from lot I were added into 7.306 g of finely ground elemental S. This yielded elemental ^{35}S with 94.295 MBq per 53 g S (standard deviation=2.89) or a ^{35}S specific activity of 1779 MBq g^{-1} S. The soil cores that were destructively sampled at 90, 120 and 180 days received this labelled material.

The particle size separates (<0.150 mm (**SS**), 0.150-0.250 mm (**MM**), and 0.250-0.500 mm (**LL**)) of ^{35}S labelled elemental S from both Lots, Lot I and Lot II, were prepared in the same manner by crushing and sieving through nylon cloth in a plastic bag in a fume hood. . The finer size (**SS**) had approximately 30% of the particles less than 0.050 mm. During crushing and sieving approximately 2-4% of the S^{o} was not recovered from either mortar or sieve. The characteristics of the labelled fertilizers are shown in Table 7.3.

7.3.4.2 Labelling sulphate containing fertilizers

Finely ground ^{35}S labelled superphosphate (**SSP**) and gypsum (**GP**) were also prepared as described in Section 3.1.4. For ^{35}S labelled SSP 2.46 g of 65% H_2SO_4 containing 92.5 MBq of $^{35}SO_4^{=}$ was added into 2.62 g of a 1:1 mixture of Christmas Island A : Nauru phosphate rocks. This produced SSP containing 14.748 MBq g^{-1} (SD=0.104) or ^{35}S specific activity of 147.48 MBq g^{-1} S (10.00% S sd=0.02). The product was dried at room temperature and finely ground.

About 1.65 g of calcium carbonate (99%) were mixed with 3.2 g of H_2SO_4 (50% w/w) containing 92.5 MBq of $^{35}SO_4^{=}$ and dried in forced drought oven at 30 ^{o}C. The GP produced had a specific activity of 148.02 MBq g^{-1} S (18.5% S sd=.015) or 27.383 MBq g^{-1} of gypsum (sd=0.105). The product was also finely ground after drying. Results of the ^{35}S labelling of GP and SSP are shown in Table 7.4

7.3.4.3 Granulation of S^{o} and S^{o}/phosphate rock

Granules of $^{35}S^{o}$ with and without finely ground phosphate rock were prepared as described by Chatupote (1990). Fine $^{35}S^{o}$ (<0.150 mm) and a mixture of fine $^{35}S^{o}$ (<0.150 mm) and finely ground North Carolina reactive phosphate rock (NCRPR, 13.2% P; 100% <0.150 mm, 80% <0.075 mm) were granulated (0.5-1 mm granules size) with 1% agar and saturated KCL solution and yielded fertilizer granules containing 9% S^{o}, Table 7.3, (Chatupote, 1990).

Table 7.3 Characteristics of $^{35}S^{o}$ labelled fertilizer used in this study.

Lot No.	Fertilizers and sizes (mm)	Abbrev.	S content %	S Applied[a] mg S core^{-1}	^{35}S Activity applied MBq core^{-1}	^{35}S Specific activity MBq g^{-1} S
Lot I						
	<0.150	SS	100	53	3.207	60.51
	0.150-0.250	LL	100	53	3.207	60.51
	0.250-0.500	MM	100	53	3.207	60.51
	SS/PR*	SS/PR	8.9	53	3.041	57.38
	SS*	SS/gr	8.6	53	3.207	60.51
Lot II						
	<0.150	SS	100	53	9.429	177.91
	0.150-0.250	LL	100	53	9.429	177.91
	0.250-0.500	MM	100	53	9.429	177.91
	SS/PR*	SS/PR	8.9	53	8.797	165.98
	SS*	SS/gr	8.7	53	9.429	177.91

** Elemental S of particle size of <0.150 mm was used and were granulated with/without finely ground phosphate rock and the total P content in the SS/PR granules was 10.6%.*

[a] *equivalent to a rate of 30 kg S ha^{-1}*

Table 7.4 Characteristic of $^{35}SO_4^{=}$ labelled superphosphate and gypsum used in this study.

Fertilizers	Amount applied mg core^{-1}	S applied mg core^{-1}	S content %	^{35}S Activity applied MBq core^{-1}	^{35}S Specific activity MBq g^{-1} S
GP	285	52.7	18.5	7.81	148.38
SSP	485	48.5	10.0	7.15	147.37

7.3.5 Experimental conduct

7.3.5.1 General

Before fertilizer application, herbage on soil cores from the glasshouse and field were cut to about 2.5 cm height and the herbage discarded. The fertilizers were surface applied on the undisturbed soil cores at the rate of 53 mg S $core^{-1}$ (30 kg S ha^{-1}). Treatments, replications, and harvesting and sampling schedules for the field trials are shown in Table 7.5 and those for the glasshouse trials are shown in Tables 7.6 and 7.7.

A hand sprayer was used to simulate small amounts of rainfall in the glasshouse trials. Distilled water was sprayed onto the surface of the undisturbed soil cores twice a week. Additional watering was conducted by adding water to the plastic container in which the pot stood to maintain the core at 90% field capacity. Under the glasshouse conditions, a minus N, P, and S nutrient solution (Middleton and Toxopeus, 1973) was applied regularly to supply other nutrient to soil cores. At the field site the soil cores were fenced to prevent access by stock.

7.3.5.2 Leaching events

In addition to regular watering the undisturbed soil cores were leached on five occasions during the first few weeks with a total volume of water equivalent to 50 mm of rainfall (10 mm in each event) to simulate early winter rain events. The leachates were collected in plastic bags, weighed and analysed for HI-reducible S and ^{35}S activity.

Three leachings (at days 7, 14 and 44) were made when the soil in the undisturbed soil core was close to maximum water holding capacity and two leachings (at day 21 and 56) were made when the cores were close to wilting point.

7.3.5.3 Soil and herbage sampling

Field herbage harvesting and soil sampling schemes and the number of treatment replications sampled at each harvest are shown in Tables 7.5, 7.6 and 7.7. Herbages were cut at 2.5 cm above the soil surface and analysed as described in Table 7.8. Soil samples were sectioned into top, middle and bottom layers (0-33, 3-66, and 6-100 mm, respectively). The soil samples from the radioactive treatments were frozen and freeze-dried to stop microbial activity before being ground to <1 mm particle size using a large hammer mill. The non-radioactive treatment soil samples were air-dried at room temperature (20 ^{o}C)

Table 7.5 Treatments, soil sampling and herbage harvesting schedule for treatments with different particle sizes of elemental S in the glasshouse trial.

			Days after fertilizer application							
Treatments	Abbrev.	Lot	15	30	45	60	90	120	150	180
Herbage Samplings			*Number of replications*							
< 0.150 mm	SS	I	1	3	1	3				
< 0.150 mm	SS	II	10	1	10	1	10	7	7	7
0.150-0.250 mm	MM	I	1	3	1	3				
0.150-0.250 mm	MM	II	1	10	1	10	10	7	7	7
0.250-0.500 mm	LL	I	1	3	1	3				
0.250-0.500 mm	LL	II	1	10	1	10	10	7	7	7
SS + PR*	SS/PR	I	1	3	1	3				
SS + PR*	SS/PR	II	1	10	1	10	10	7	7	7
SS*	SS/gr	I	1	3	1	3				
SS*	SS/gr	II	1	10	1	10	10	7	7	7
PR[1]	PR	nl[2]	1	13	1	13	10	7	7	7
Control	Ctrl	nl[2]	1	13	1	13	10	7	7	7
Soil Samplings			*Number of replications*							
< 0.150 mm	SS	II	1	3	1	3	3	-	-	7
0.150-0.250 mm	MM	II	1	3	1	3	3	-	-	7
0.250-0.500 mm	LL	II	1	3	1	3	3	-	-	7
SS + PR*	SS/PR	II	1	3	1	3	3	-	-	7
SS*	SS/gr	II	1	3	1	3	3	-	-	7
PR[1]	PR	nl[2]	1	3	1	3	3	-	-	7
Control	Ctrl	nl[2]	1	3	1	3	3	-	-	7

* S^0 *of particle size of < 0.150 mm was used and granulated with/without finely ground phosphate rock*

1 *Finely ground and granulated phosphate rock was used*

2 *nl = not labelled*

Table 7.6 Treatments soil sampling and herbage harvesting schedule for treatments with different particle sizes, S^0 in the field trials.

Treatments	Abbrev.	Lot	Days after fertilizers application: 15	30	45	60	90	120	150	180
Herbage Samplings						*Number of replications*				
< 0.150 mm	SS	II	-	3	-	3	3	3	3	3
0.150-0.250 mm	MM	II	-	3	-	3	3	3	3	3
0.250-0.500 mm	LL	II	-	3	-	3	3	3	3	3
SS + PR*	SS/PR	II	-	3	-	3	3	3	3	3
SS*	SS/gr	II	-	3	-	3	3	3	3	3
PR[1]	PR	nl[2]	-	3	-	3	3	3	3	3
Control	Ctrl	nl[2]	-	3	-	3	3	3	3	3
Soil Samplings						*Number of replications*				
< 0.150 mm	SS	II	-	-	-	-	-	-	-	3
0.150-0.250 mm	MM	II	-	-	-	-	-	-	-	3
0.250-0.500 mm	LL	II	-	-	-	-	-	-	-	3
SS + PR*	SS/PR	II	-	-	-	-	-	-	-	3
SS*	SS/gr	II	-	-	-	-	-	-	-	3
PR[1]	PR	nl[2]	-	-	-	-	-	-	-	3
Control	Ctrl	nl[2]	-	-	-	-	-	-	-	3

* *S^0 of particle size of <0.150 mm was used and granulated with/without finely ground phosphate rock*

1 *Finely ground and granulated phosphate rock;*

2 *nl = not labelled*

Table 7.7 Treatments and soil sampling and herbage harvesting schedule for treatments with ^{35}S labelled gypsum and superphosphate fertilizers in the glasshouse trial.

Treatments	Abbrev.	Days after fertilizer application: 30	60	90
		Number of replications		
Herbage sampling				
Gypsum	GP	4	4	4
Superphosphate	SSP	4	4	4
Control	Ctrl	4	4	4
Soil sampling				
Gypsum	GP	-	-	4
Superphosphate	SSP	-	-	4
Control	Ctrl	-	-	4

7.3.6 Chemical analyses

7.3.6.1 Soil, plant and fertilizer samples

Analyses for S and related ^{35}S activities in soil, plant and fertilizer materials are listed in Table 7.8:

Table 7.8 Analyses for S and related ^{35}S activities in soil, plant and fertilizer materials.

Analyses	Described in Section	Chapter
Soil samples		
Total S	3.3.3	3
Total S^o (acetone extract)	3.3.4	3
Extractable S (CaP-S)	3.3.2	3
Organic S	3.3.5	3
Plant samples		
Total S	3.3.1.1	3
Fertilizer materials		
Sulphate S	3.3.7.1	3
S^o	3.3.7.2	3
HI-reducible S in soil extract and digested samples	3.3.6	3

7.3.6.2 Sulphate retention

Sulphate retention capacities of Tokomaru and Ramiha soils from each soil depth (Appendix 7.14) were determined as described by Gregg (1976). Five grams of soil were shaken for 16 hours in an end-over-end shaker with 25 ml of 0.01 M $CaCl_2$ containing different concentration of S (0, 50, 100, 150, 200, 250, 500, ppm S as Na_2SO_4). To suppress microbial activity, a few drops of chloroform were added before shaking. The soil suspension were filtered through Whatman No 42 filter papers. Then the extracts were diluted to a concentration range between 5 to 10 mg S l^{-1} and the S concentration measured using an automated, reduction-distillation technique for measuring HI-reducible S (as described Section 3.3.6).

The amount of sulphate retained by soil at each level of sulphate addition was calculated as followed:

$$\%S \text{ retention} = [(A + X) - Y]/A*100$$

where

A = amount of S contained in the 0.01 M $CaCl_2$ solution

X = amount of soil sulphate released into 0.01 M $CaCl_2$ (no S added) solution in a separated extraction

Y = amount of sulphate left in solution after shaking the soil with 0.01 M $CaCl_2$ containing different concentrations of $SO_4^=$

Results are presented in Appendix 7.14

7.3.6.3 Recovery of added fertilizer $^{35}S^o$ activity from soil cores at day 0

A short term experiment was also designed to examine efficiency of the recovery of total ^{35}S activity from soil cores fertilized with $^{35}S^o$ labelled fertilizers at day 0. Elemental S was labelled with small amount of $^{35}S^o$ activity, about 3.3 MBq per g S^o (as described in Section 3.1.3.2) and crushed into two particle sizes; <0.150 mm and 0.250-0.500 mm. Each particle size was divided into three application rates, namely 12.5, 25.0 and 50.0 mg S^o $core^{-1}$ (15 cm diameter). After the labelled fertilizers were evenly applied onto the soil surface (0-3 cm layers), the whole upper soil layer was freeze-dried for 7 to 10 days. The dried samples were then hammer milled before total ^{35}S determination; total ^{35}S ($NaHCO_3$ + Ag_2O; Landers *et al.*, 1983), total S^o and $^{35}S^o$ activity (Chatupote, 1990), as described in Section 3.3.7.1

Results are presented in Appendix 7.15. There was no significant difference between treatment means. Recoveries range from 70-100% and grand means of the percentage recoveries of added fertilizer ^{35}S were used as correction factors to correct measured amounts of S^o to achieve amounts of ^{35}S present in soil cores.

7.3.7 Statistical analyses

Single replicated soil data collected at 15 days and 45 days used for monitoring rate of S^o oxidation, were not subjected to the statistical analysis and data were not shown. Other replicated data on herbage and soil according to Table 7.5 were subjected to analysis of variance to determine the significance of treatment effects. Data from both soils and herbage harvest were statistically analysed on a single harvest date basis. The Duncan Multiple Range

Test (DMRT)and the Least Significant Difference (Lsd) were employed in comparisons of treatment means where appropriate and result of the comparisons are presented in appropriate Tables and Appendices. The SAS (SAS Institute Inc., 1985) and Minitab (Minitab Inc., 1989) computer programmes were employed.

Data on ^{35}S activities were normalized to the day when the $^{35}S^{o}$ labelled fertilizers were applied (November 29, 1987). Some related ^{35}S calculations have been presented in previous Chapters (Section 4.3.4 and Section 5.3.5). Additional relevant calculations are presented here:

a. Percentage of applied ^{35}S cumulatively taken up by pasture

$$H_t = C_t/A_{to}*100$$

Where

H_t = percentage of applied ^{35}S cumulatively taken up by pasture at time **t**

C_t = amount of ^{35}S activity cumulatively taken up by pasture at time **t**

A_{to} = total amount of $^{35}S^{o}$ or $^{35}So_4^{-2}$ activity in labelled fertilizer applied to soil core at time **0**

b. Percentage of applied $^{35}S^{o}$ in soil core

$$S_t = A_t/A_{to}*100$$

where

A_t = amount of $^{35}S^{o}$ activity recovered in acetone extract of soil cores at time **t** (corrected using recovery factors determined in Section 7.3.8.3

A_{to} = total amount of $^{35}S^{o}$ activity in labelled fertilizer applied to soil core at time **0**

c. Percentage of oxidized $^{35}S^{o}$ cumulatively taken up by pasture

$$P_t = H_t/(100 - S_t)*100$$

where

P_t = percentage of oxidized $^{35}S^{o}$ cumulatively taken up by pasture at time **t**

H_t = percentage of applied ^{35}S cumulatively taken up by pastures at time **t**

S_t = percentage of applied $^{35}S^{o}$ in soil core at time **t**

7.3.8 Calculation of specific rates (K) of S^{o} oxidation.

The acetone extraction of S^{o} from soil samples in this Chapter was conducted by W.Chatupote as a part of his Ph.D. thesis (Chatupote, 1990). An iterative computer programme developed in the thesis was used to calculate the **K** values (specific oxidation rate $\mu g\ S\ cm^{-2}\ day^{-1}$). The

programme assumes that S^o particles are spheres and that specific oxidation rate is constant per unit surface area of S^o exposed. A description of these relationships was given by (Watkinson, 1988, 1989)

7.4 RESULTS AND DISCUSSION

Weekly amounts of rainfall, and drainage water, and average weekly maximum and minimum temperatures which represented the climate conditions in the Palmerston North district and of the field sites during these experiments were presented in Appendix 7.16 (Taken from DSIR Grasslands, P.N. Meteorological records). Drainage water percolating beyond 10 cm, was calculated as described by Scotter *et al.* (1979). Drainage occurred in the field soils at both sites before the trials were laid down and thereafter no drainage occurred. Rainfall during the trial period was approximately 450 mm. However, there were some dry periods which produced soil water deficits that probably limited pasture growth during weeks 14-20 and 26. On average, maximum and minimum temperature were about 20 and 10 ^oC which was considered to be adequate for S^o oxidation in soils (Janzen and Bettany, 1987b).

In general soil pH and available P (Table 7.1) for both soils were slightly below optimum for pasture growth (Cornforth, 1981; Cornforth and Sinclair, 1984). Extractable S (CaP-S, Table 7.2), in the Ramiha soil was optimum, but was considered to be low for the Tokomaru soil (critical level = 10 ppm S, according to Sinclair *et al.*, 1985). The majority (>95%) of S in both soils was present as organic S (Table 7.2). The S retention (adsorption) of both soils was considered to be low (see $SO_4^=$ adsorption isotherm results in Appendix 7.14) despite the fact that the Ramiha soil had a high phosphate retention capacity. The S retention capacity in the top 0-3 cm soil layer of Ramiha soil was lower than that of Tokomaru soil, probably due to larger amounts of organic S and adsorbed P (as indicated by Olsen-P in the Ramiha soil relative to the Tokomaru soil). Searle (1982), Johnson *et al.* (1979), Johnson and Todd (1983) have shown the negative influence of organic matter on sulphate adsorption.

In general, fertility status of the Ramiha soil was considered to be higher than that of Tokomaru soil partly as a result of its better fertilizer history.

7.4.1 The percentage recovery of $^{35}S^o$ and %SDFF in pasture as influenced by fertilizer specific activity

Data on percentage recovery of applied ^{35}S in pasture and the percent of accumulated plant S derived from fertilizer (%SDFF) at 30 and 60 days after fertilizer application were used to compare results obtained using $^{35}S^o$ of different specific activity (**Lot I** and **Lot II**, Table 7.3).

Only data from the SS treatments (<0.150 mm) could be used for this comparison during early stages of the experiment because the amount of $^{35}S^{o}$ released from the coarser particle sizes MM and LL was low and subject to variation. Results are presented in Table 7.9. Sample count rate had no significant effect on the apparent recovery of fertilizer S in the plants and therefore such results obtained from the two different sources of labelled $^{35}S^{o}$ could be compared directly when expressed as a percentage of the original isotope added.

Table 7.9 Effect of fertilizer specific activities on ^{35}S recovery in pastures and percent of plant S derived from fertilizers (%SDFF).

		30 days[1]		60 days[2]	
Soils	Lot No.	Recovery	%SDFF	Recovery	%SDFF
		%	%	%	%
Ramiha					
	Lot I	2.5	24.3	4.8	67.8
	Lot II	3.1	23.5	4.4	58.5
F-test		ns	ns	ns	ns
C.V. %		25.4	17.9	23.5	23.8
Tokomaru					
	Lot I	1.3	17.4	2.3	36.6
	Lot II	1.5	13.7	1.5	35.2
F-test		ns	ns	ns	ns
C.V. %		23.3	19.4	35.7	22.7

ns = not significant; [1] average of seven replications; [2] average of three replications

7.4.2 THE INFLUENCE OF PARTICLE SIZE AND FERTILIZER FORM ON THE FATE OF S^o

In this Section, discussion will be focused on the fate of $^{35}S^o$ as influenced by $^{35}S^o$ particle size (SS, MM and LL), the effect of granulation of fine S^o (SS/gr) and granulation of fine S^o with phosphate rock (SS/PR).

7.4.2.1 Recovery of radioisotope in soils and plants

The cumulative uptake of ^{35}S radioactivity by plants and that remaining in the top 10 cm of the glasshouse and field soil are shown in Table 7.10 for the Ramiha soil and Table 7.11 for the Tokomaru soil.

By 180 days cumulative plant uptake under glasshouse conditions accounted for less than 20% of the isotope and the majority (75-90%) remained in the soil. The amount of isotope leached beyond the 10 cm soil in glasshouse cores was negligible (see later, Table 7.15). In many cases the total (soil+plant) recovery of added ^{35}S was apparently greater than 100%. This error is attributable to the difficulty of obtaining a uniform distribution of the particulate S^o in each soil sample taken for analysis for total $^{35}S^o$ (acetone extraction) and total ^{35}S ($NaCO_3$+Ag_2O digestion), and was a particular problem with the coarse S^o particle size (LL). This problem has also been noted by Watkinson *et al.,* (1987) and Barrow (1968). These errors could be reduced by increasing the number of undisturbed cores in each replicate but this would have produced an unmanageable workload. Notably, there is a closer spread of recoveries at 180 days when seven replicate cores were harvested.

The recovery of ^{35}S applied to Ramiha field cores was lower than glasshouse cores. There may have been greater losses of sulphate S due to leaching at the higher rainfall Ramiha site. Recoveries of ^{35}S activity lower than glasshouse cores were not recorded for the Tokomaru field cores.

Table 7.10 Recovery of labelled ^{35}S fertilizers in pasture, soil and total recovery in Ramiha soil cores in glasshouse and field trials (average of three replications from individual microplots).

Treatments		Days after fertilizer aplication 30	60	90	180[1]	Field 180
				..... % recovered		
CUMULATIVE PASTURE UPTAKE						
	SS	2.5[a]	7.5[a]	12.5[a]	20.5[a]	9.8[a]
	LL	0.4[c]	1.4[c]	1.9[c]	4.2[c]	1.7[b]
	SS/PR	1.5[b]	4.0[b]	7.7[b]	14.7[b]	8.1[a]
	SS/gr	1.7[b]	6.1[b]	6.2[b]	13.7[b]	8.3[a]
	F-test	**	**	**	**	***
	C.V. %	39.9	24.7	36.4	24.8	34.2
SOIL						
	SS	114.9	91.6	91.3	75.4	74.5
	LL	131.9	119.8	109.9	97.8	87.7
	SS/PR	104.4	101.9	98.4	99.2	81.3
	SS/gr	98.6	88.3	87.0	80.3	78.2
	F-test	ns	ns	ns	ns	ns
	C.V. %	14.3	15.8	4.9	11.2	20.7
TOTAL						
	SS	117.4	99.1	102.8	95.9	84.3
	LL	132.4	121.2	111.6	101.9	89.4
	SS/PR	106.9	105.9	105.3	113.8	89.4
	SS/gr	99.8	94.4	90.8	93.4	86.5
	F-test	ns	ns	ns	ns	ns
	C.V. %	13.9	15.4	4.2	8.8	13.4

, ** and * = significant at 5, 1 and 0.1% level, respectively; ns = not significant; mean separation by DMRT at 5% level denoted by letters*

[1] *average of seven replications*

Table 7.11 Recovery of labelled ^{35}S fertilizers in pasture, soil and total recovery in Tokomaru soil cores in glasshouse and field trials (average of three replications from individual microplots).

Treatments		Days after application 30	60	90	180[1]	Field 180
				..... % recovered		
CUMULATIVE PASTURE UPTAKE						
	SS	1.2[a]	3.5[a]	6.3[a]	13.9[a]	4.2[a]
	MM	0.4[b]	1.9[b]	1.4[c]	3.5[c]	1.1[b]
	LL	0.2[c]	0.9[c]	0.8[c]	1.9[c]	0.6[b]
	SS/PR	0.5[b]	2.1[b]	4.7[b]	12.6[b]	5.8[a]
	SS/gr	0.4[c]	1.3[b]	4.0[b]	10.4[b]	3.9[a]
	F-test	**	**	**	**	***
	C.V. %	35.5	55.9	34.7	28.7	34.2
SOIL						
	SS	115.1	100.9	88.4	92.9	94.9
	MM	136.9	132.1	113.1	101.8	100.4
	LL	126.7	122.6	141.3	104.4	105.5
	SS/PR	116.3	111.1	109.4	99.1	101.4
	SS/gr	120.6	107.5	86.2	87.3	91.5
	F-test	ns	ns	ns	ns	ns
	C.V. %	13.7	15.2	12.7	13.7	11.4
TOTAL						
	SS	116.3	104.5	92.8	106.9	99.2
	MM	137.2	133.9	115.3	105.4	101.5
	LL	125.8	123.5	142.3	106.3	106.1
	SS/PR	116.7	112.6	113.9	122.5	107.1
	SS/gr	120.9	108.8	90.9	97.7	95.4
	F-test	ns	ns	ns	ns	ns
	C.V. %	13.7	14.9	12.5	12.6	20.0

, ** and * = significant at 5, 1 and 0.1% level, respectively; ns = not significant; mean separation by DMRT at 5% level denoted by letters*

[1], *average of seven replications*

7.4.2.2 Plant uptake of S and ^{35}S and percentage of plant S derived from fertilizer (%SDFF)

Cumulative S uptake (soil S plus fertilizer S) by the herbage in glasshouse and field trial are shown in Figures 7.1A and 7.2A and Appendices 7.1A and 7.2B, respectively. By the end of the trial (180 days), only the smaller particle size (SS) produced increased S uptake significantly higher than unfertilized control (Ctrl) in both soils in the glasshouse trial which is partly due to the higher oxidation rate of finer S^0 (Watkinson *et al.*, 1987). Throughout the trial period the <0.150 mm S^0 (SS) treatment on Tokomaru soil which initially had a lower sulphate status than the Ramiha soil (Table 7.2), had significantly higher S uptake than the other two treatments (MM and LL) which produced more plant S than the unfertilized control (Ctrl). These significant differences did not occur on the higher S status Ramiha soil. In general, the S uptake from soil plus fertilizer was slightly higher on the Ramiha soil in both glasshouse and field trials. This soil had high initial sulphate status (Table 7.2). Fertilizer application caused no increase in plant S uptake in the field trials which suggested that other factors (e.g. P, N availability, soil moisture) might have limited S uptake which was 3-5 times lower than that occurring under glasshouse conditions where nutrients solution (K, Mg, Ca and some micro-nutrients) was also provided.

In general, both SS/PR and SS/gr treatments yielded less cumulative S uptake (soil plus fertilizer S uptake at 180 days) than that of SS treatments in glasshouse trials (Figure 7.2, Appendix 7.1A), particularly on the Tokomaru soil. In the field trials there was no effect of granulation (SS/gr) and phosphate rock addition (SS/PR) on S uptake (Appendix 7.2A).

Lower cumulative plant S uptake on the Tokomaru soil than on Ramiha soil cores in the glasshouse trials may be attributed to lower phosphate extractable S and total S in the intact soil cores of Tokomaru soil (Table 7.2).

Accumulated dry matter yields in the glasshouse and field trials are shown in Figures 7.3, 7.4 and Appendices 7.1B, 7.2B, respectively. Applications of S^0 and/or phosphate rock did not significantly increase plant dry matter yields in both glasshouse and field trials. Dry matter yield of herbage grown on the glasshouse undisturbed soil cores were consistently higher than on the field cores. This is due to the more favourable growth conditions (temperature and moisture) in the glasshouse than in the field. The dry matter yields on both soils in the glasshouse trials were similar but were slightly higher for the Ramiha soil in the field trials.

The smaller particle size (SS) consistently, but not always significantly, Increased herbage S concentrations at the last five harvests under glasshouse conditions and at the last field

harvest (Apppendix 7.3A and 7.3B). At the first sampling, under the glasshouse conditions S concentrations of pastures were above the critical level of 0.30% (McNaught and Christoffels, 1961; Cornforth *et al.*, 1983; Sinclair *et al.*, 1985). Thereafter, the S concentration of pastures fell below the critical level, except at the last sampling time. The increases in the plant S concentration at the last sampling might be attributed to increased mineralization of organic S as indicated by increase in phosphate extractable S (CaP-S) in the soil cores at the last sampling (Table 7.13B).

Although, there were some significant differences in the S concentration of pasture at the last harvest of the field trials (Appendix 7.3B), the S concentrations were below the optimum level (0.30%). The low plant S concentrations in the field trial may be partly attributed to periods of low soil moisture content during the trials which would limit mineralization. Also higher evapotranspiration under glasshouse conditions would contribute to more plant S uptake than in the field, i.e. greater water use and therefore more S uptake.

Results also showed no effect of granulation and phosphate rock on pasture S concentration. This indicated that surface applied granules (SS/PR and SS/gr) did breakdown and released S in the same manner as the powder form (non-granulated, SS). However, the increases in plant S uptake did not generate increased dry matter yield in either the glasshouse or field trials.

There was larger cumulative recovery of ^{35}S labelled fertilizer in pastures under glasshouse than field conditions (Figure 7.5 and Appendix 7.4). The glasshouse grown swards accumulated approximately twice the activity of ^{35}S accumulated by the field pasture. The smallest particle size (SS) produced the highest recovery percentage throughout the whole trial period irrespective of whether it was granulated in either the presence or absence of phosphate rock. The greater amount of pasture grown on the Ramiha soil took up more ^{35}S from labelled fertilizer than that grown on the Tokomaru soil. In general, in both soils pasture fertilized with the small particle size (SS) took up 4-5 times more ^{35}S than the larger particle sizes (MM and LL) and this can be attributed to faster S oxidation rates of the finer materials (see Tables 7.12 and 7.13). On Tokomaru soil, in both glasshouse and field trials, the recovery of ^{35}S by pastures grown using MM S^o was greater (but not significantly) than the recovery of ^{35}S from LL fertilized soil.

The trend in cumulative ^{35}S uptake (Figure 7.6) revealed the effect of granulating SS with (SS/PR) and without (SS/gr) phosphate rock better than cumulative S uptake (Figure 7.2). In the glasshouse trial, throughout the period of pasture growth, granulated S^o alone (SS/gr) and

S^0 granulated with phosphate rock (SS/PR) produced significantly less cumulative ^{35}S uptake than the non-granulated S^0 (SS) (Figure 7.6 and Appendix 7.4A). But the negative effect of granulation with or without phosphate rock was less pronounced in the field trials and addition of phosphate rock in the granulation mixture slightly increased the cumulative ^{35}S uptake at the final samplings in the field trial on Tokomaru soil (Figure 7.8B, Appendix 7.4B).

Percentages of plant S cumulatively derived from fertilizer (%SDFF) are presented in Figure 7.7 and Appendix 7.5 and show similar trends to data on recovery of ^{35}S in pasture, as mentioned above. These results demonstrated that S^0 of finer particle size (SS) provided substantially more S to plants than the medium (MM) and coarser S^0 fractions (LL).

It was interesting to note that both glasshouse and field trials produced similar %SDFF values and this was in contrast to the results of cumulative ^{35}S recovery and cumulative S uptake of pastures. The same %SDFF in both glasshouse and field trials might indicate that the volume of soil occupied by the fertilizer ^{35}S were the same in both situations. The %SDFF tended to increase during the trial period, this indicates that the ^{35}S labelled S^0 continued to oxidize and release plant available ^{35}S into the plant available pool during the experiment. This was confirmed by the recovery of residual S^0 from soil (see later discussion, Tables 7.12A and 7.13B).

Similar %SDFF's in the glasshouse and field trial indicate similar efficiencies of S^0 use. But as discussed above, there were large differences in the amount of S and ^{35}S uptake in both systems as a result of differences in the plant growth environments. This suggests that as climatic conditions suitable for the uptake of soil S improved (moving from field to glasshouse) so the rates of S^0 oxidation must have also increased such that the amount of ^{35}S made available to the plant per unit of soil S remained essentially constant.

Notably granulation of the SS particle size alone generally decreased the %SDFF, but not always significantly (Figure 7.8 and Appendix 7.5). Initially, up to 90 to 120 days significantly smaller %SDFF's occurred in treatments with SS/gr and SS/PR in both glasshouse and field trials. In the glasshouse trial on Ramiha soil and in the field trial on Tokomaru soil, the negative effect of granulation (SS/gr) on %SDFF prevailed throughout the trial period. However, by the end of the trial the differences between the %SDFF for SS and SS/gr were less pronounced. Addition of ground phosphate rock (SS/PR) significantly alleviated the negative effect of granulation on the %SDFF of pasture in field trials on Tokomaru soil. Overall, in both glasshouse and field trials addition of phosphate rock (SS/PR) slightly mitigated the negative effect of granulation on %SDFF. This could have resulted from improved granule dispersion or through the positive influence of phosphate rock (PR) on S^0 oxidation (Chatupote, 1990).

In all soils fertilized with <0.150 mm S^0 (SS), 27-43% of plant S (%SDFF) was derived from

the fertilizer, however, several of these treatments did not generate increased dry matter yield or increased plant S (soil plus fertilizer S) uptake. This indicated that fertilizer S was taken up at the expense of native soil S (Rennenberg, 1984).

When the results were expressed as the percentage of oxidized $^{35}S^{o}$ cumulatively taken up by pasture, it was evident that at most harvests in the glasshouse experiment and at the final harvest in the field trial, plants recovered oxidized ^{35}S more efficiently from S of smaller particle size (Figure 7.9 and Appendix 7.6). The higher percentage of oxidized $^{35}S^{o}$ taken up by pasture fertilized with finer S^{o} particles (SS) was probably because at the same rate of S^{o} application the finer particles (SS) fertilized a greater soil volume and thereby created a greater chance for root interception of the sulphate released from S^{o}. At the final glasshouse harvest, however, plants grown on Ramiha soil fertilized with fine (SS) and coarse (LL) S^{o} recovered similar percentages of oxidized S^{o}. It is unclear why this occurred because in the Tokomaru soil there were consistent reductions in the percent of oxidized $^{35}S^{o}$ taken up by plant as the S^{o} became coarser.

Notably, the percentage recoveries of oxidized S^{o} by plants were lower in field cores than glasshouse cores again probably reflecting the greater water use by and S demand of the glasshouse plants.

Granulation of the SS particle size with (SS/PR) and without (SS/gr) phosphate rock initially reduced amounts of oxidized $^{35}S^{o}$ taken up by plants (Figure 7.10, Appendix 7.6) but as the experiment continued inclusion of phosphate rock on average produced higher percentage recoveries of oxidized $^{35}S^{o}$.

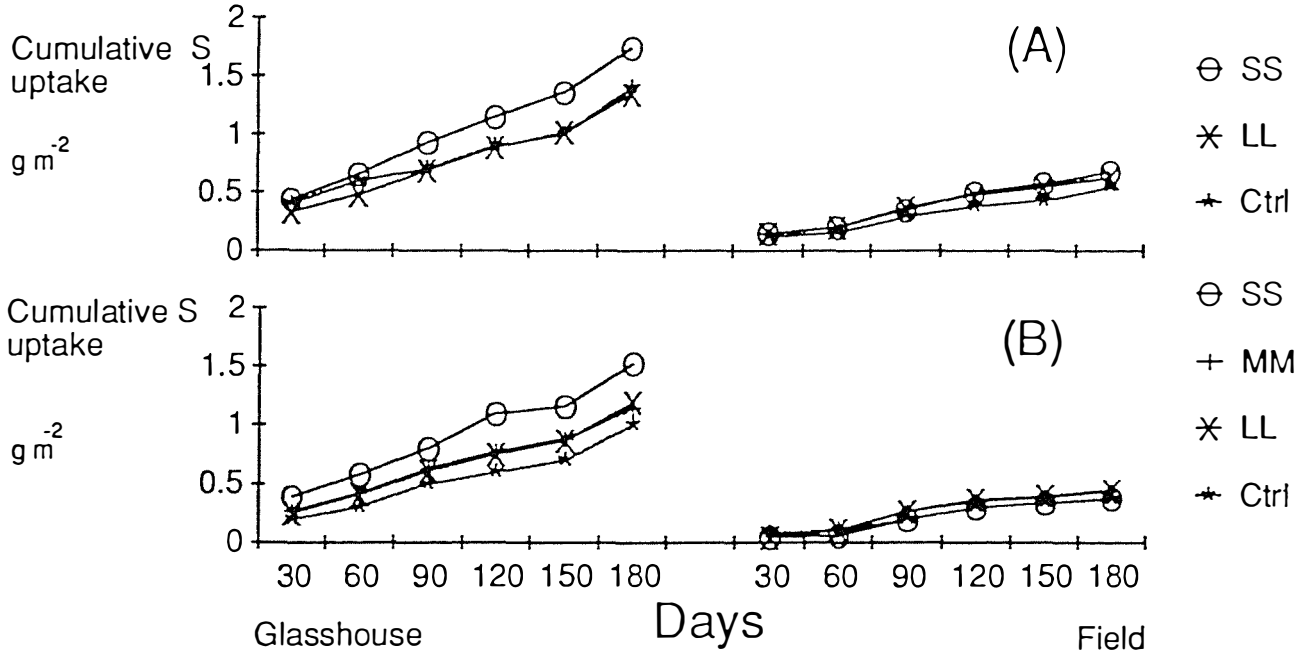


Figure 7.1 The effect of S^0 particle size on the cumulative S taken up by pasture grown on Ramiha (A) and Tokomaru (B) soils.

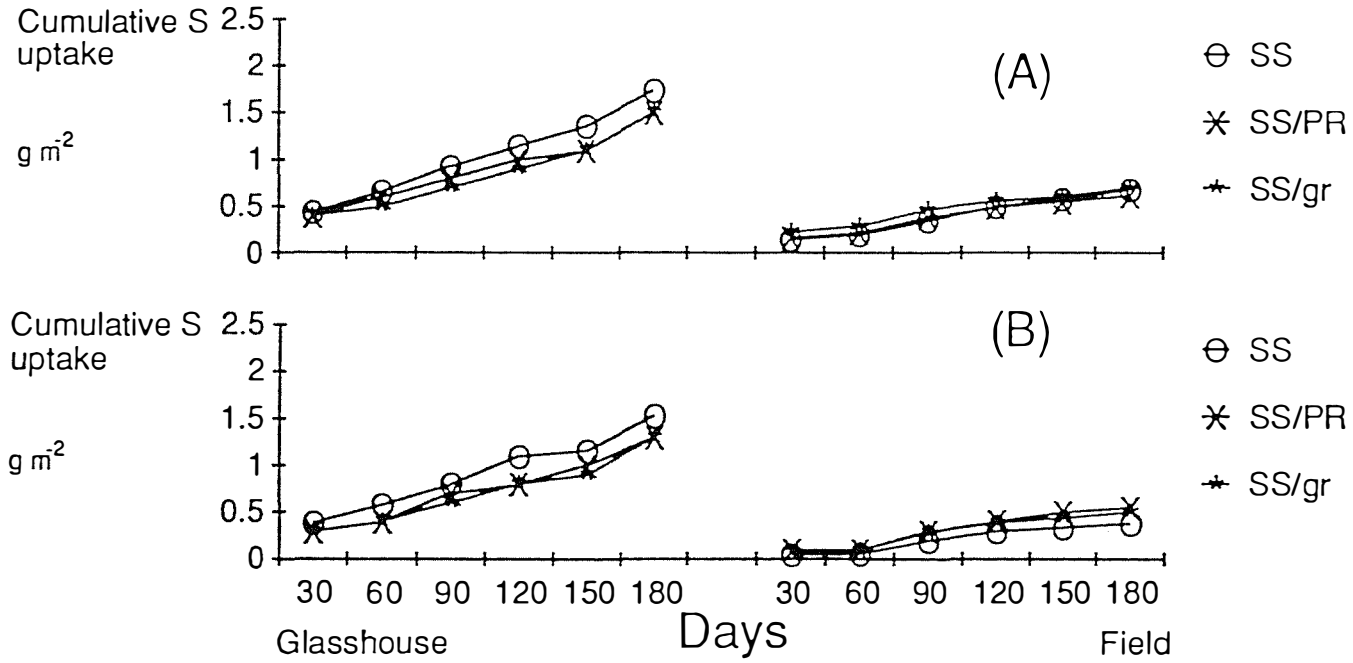


Figure 7.2 The effect of granulation of S^0 with or without phosphate rock (SS/PR and SS/gr compared with SS) on the cumulative S taken up by pasture on Ramiha (A) and Tokomaru (B) soils.

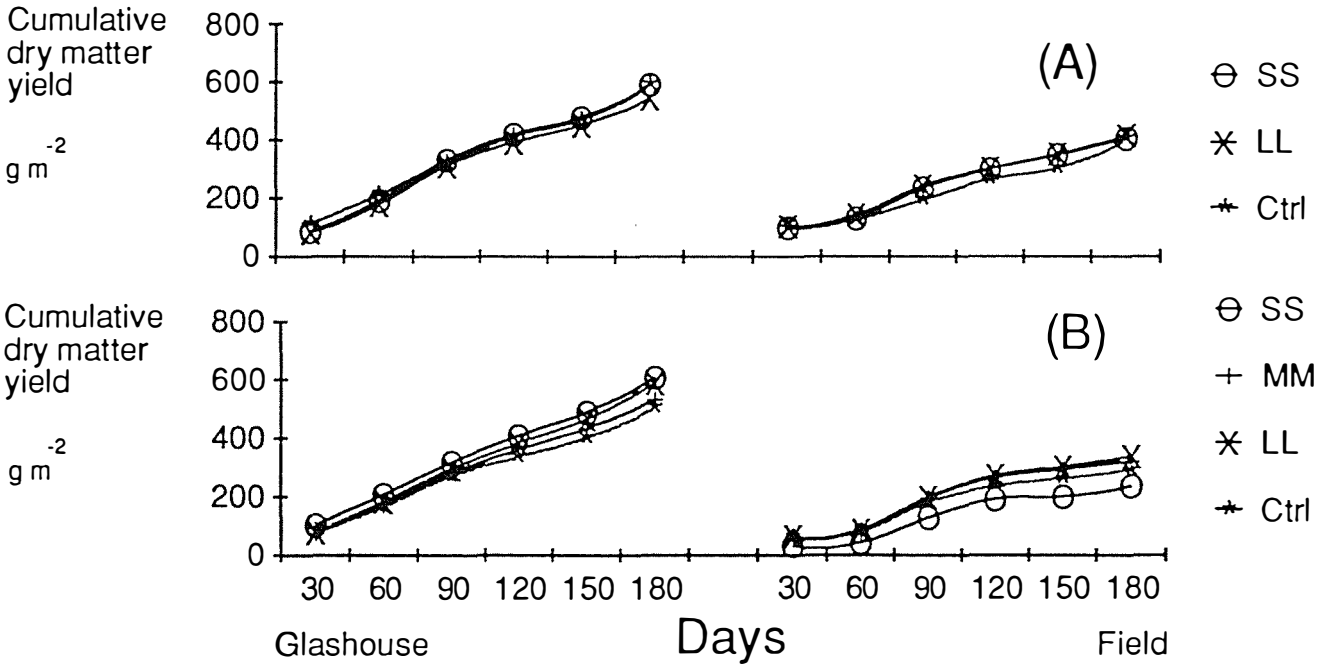


Figure 7.3 The effect of S^0 particle size on the cumulative dry matter yield of pasture on Ramiha (A) and Tokomaru (B) soils.

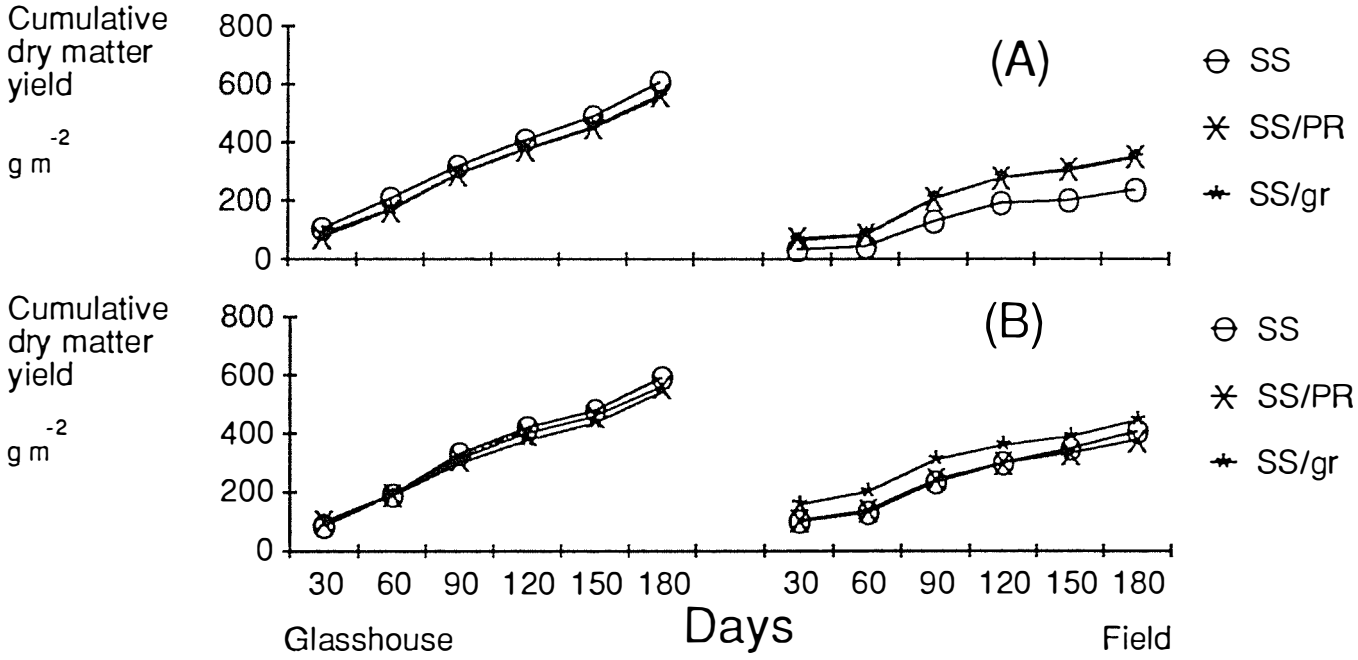


Figure 7.4 The effect of granulation of S^0 with or without phosphate rock (SS/PR and SS/gr compared with SS) on the cumulative dry matter yield of pasture on Raimiha (A) and Tokomaru (B) soils.

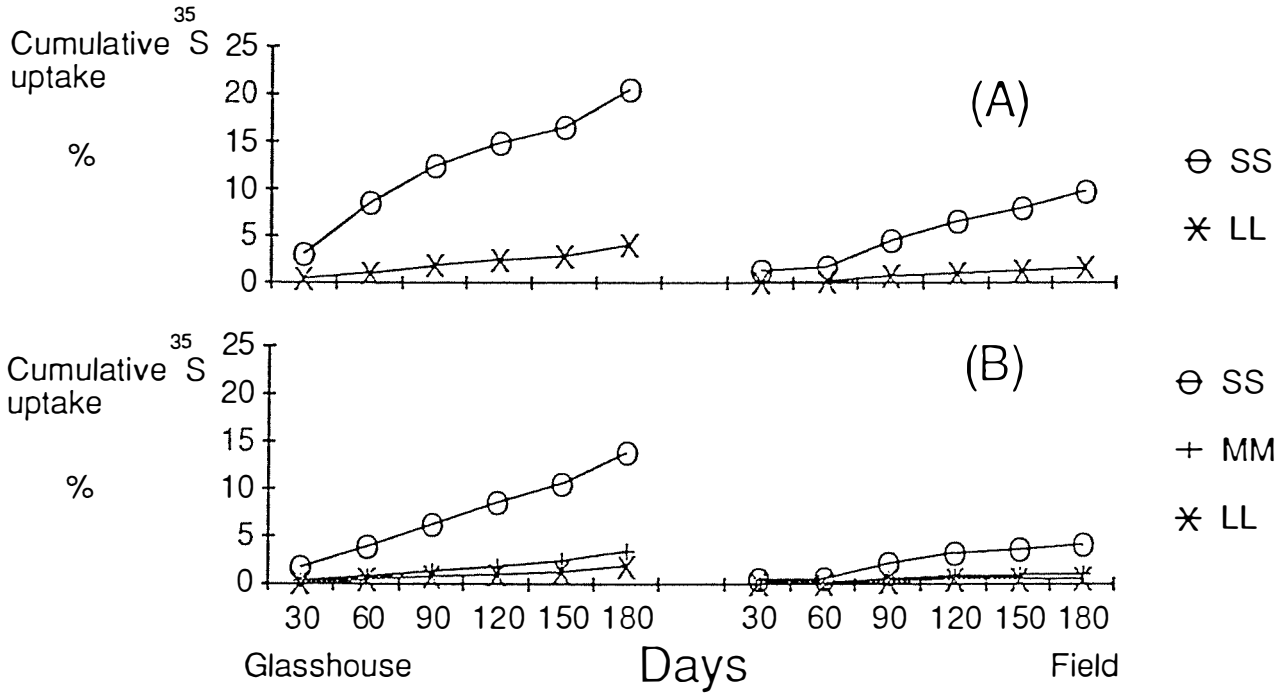


Figure 7.5 The effect of S^0 particle size on the cumulative percentage ^{35}S uptake by pasture on Ramiha (A) and Tokomaru (B) soils.

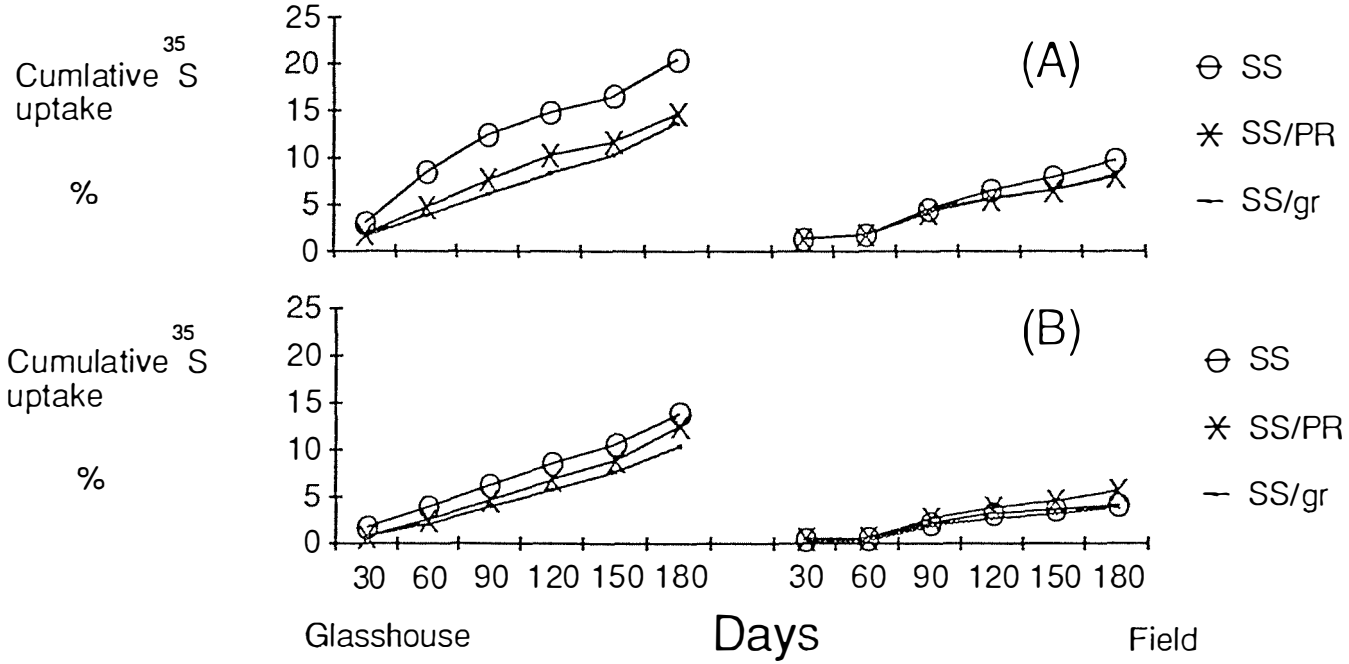


Figure 7.6 The effect of granulation of S^0 with or without phosphate rock (SS/PR and SS/gr compared with SS) on the cumulative ^{35}S taken up by pasture on Ramiha (A) and Tokomaru (B) soils.

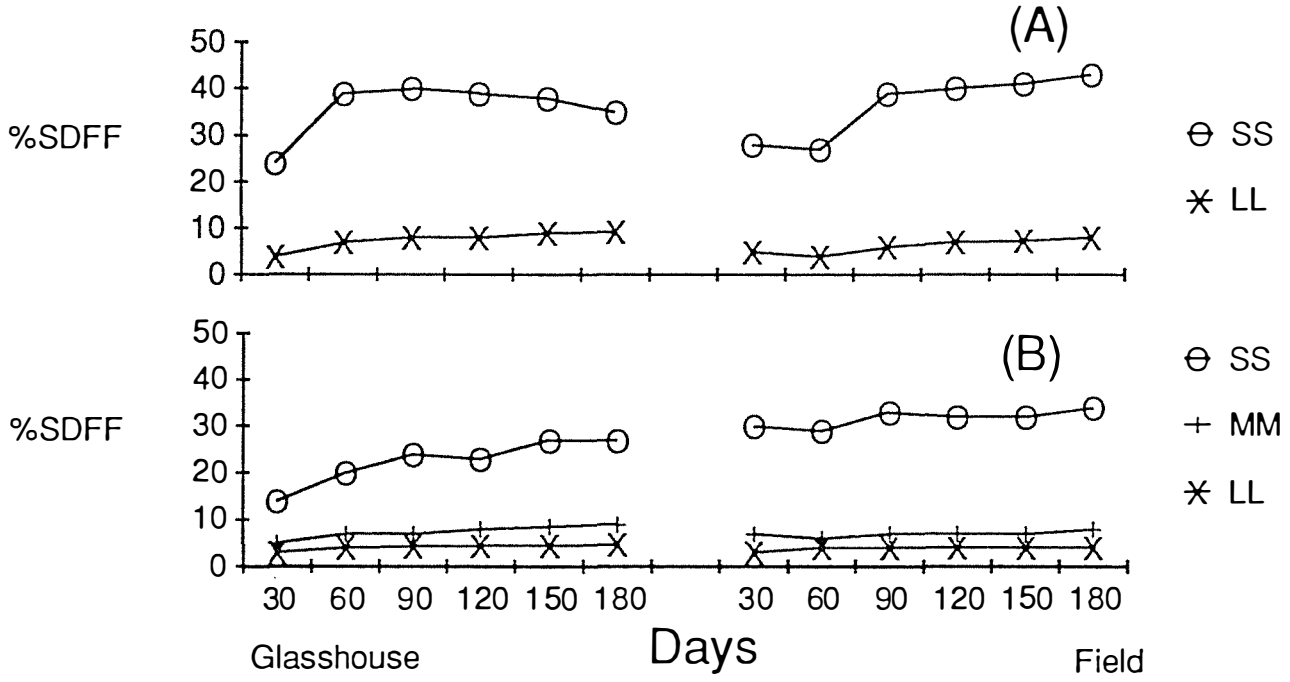


Figure 7.7 The effect of S^0 particle size on the percentage of cumulative plant S derived from fertilizer (%SDFF) on Ramiha (A) and Tokomaru (B) soils.

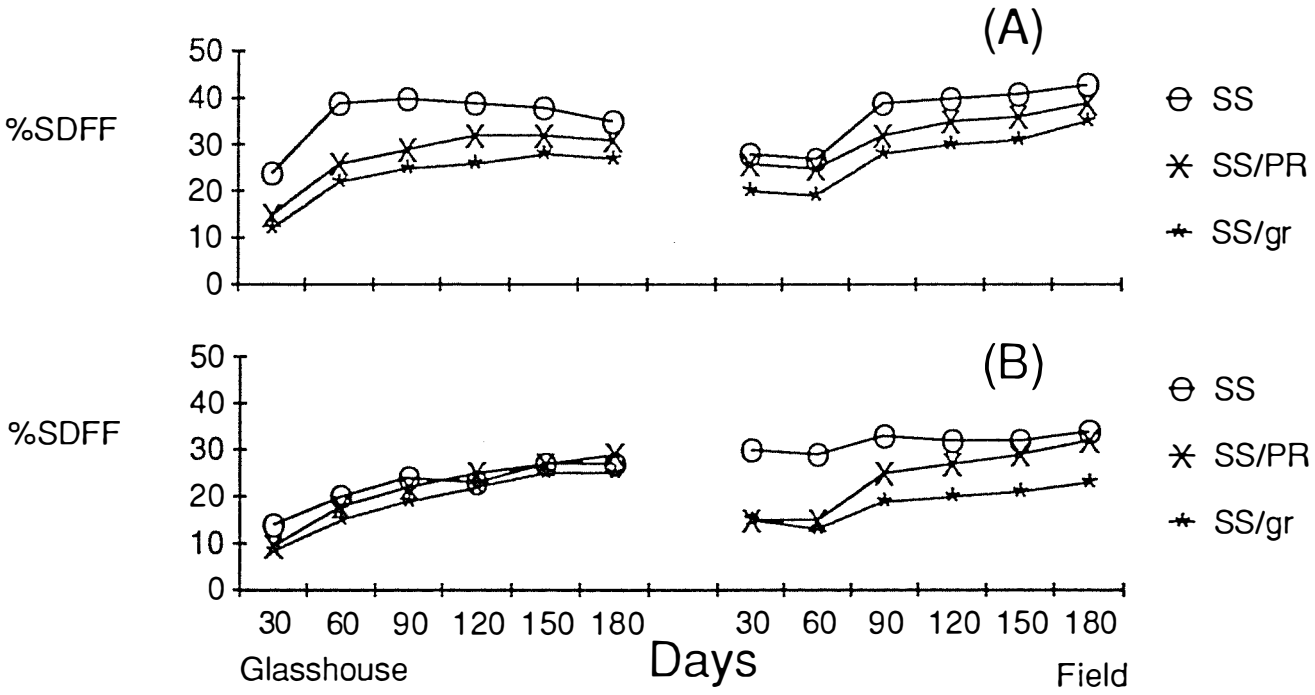


Figure 7.8 The effect of granulation of S^0 with or without phosphate rock on the percentage of cumulative plant S derived from fertilizer (%SDFF) on Ramiha (A) and Tokomaru (B) soils.

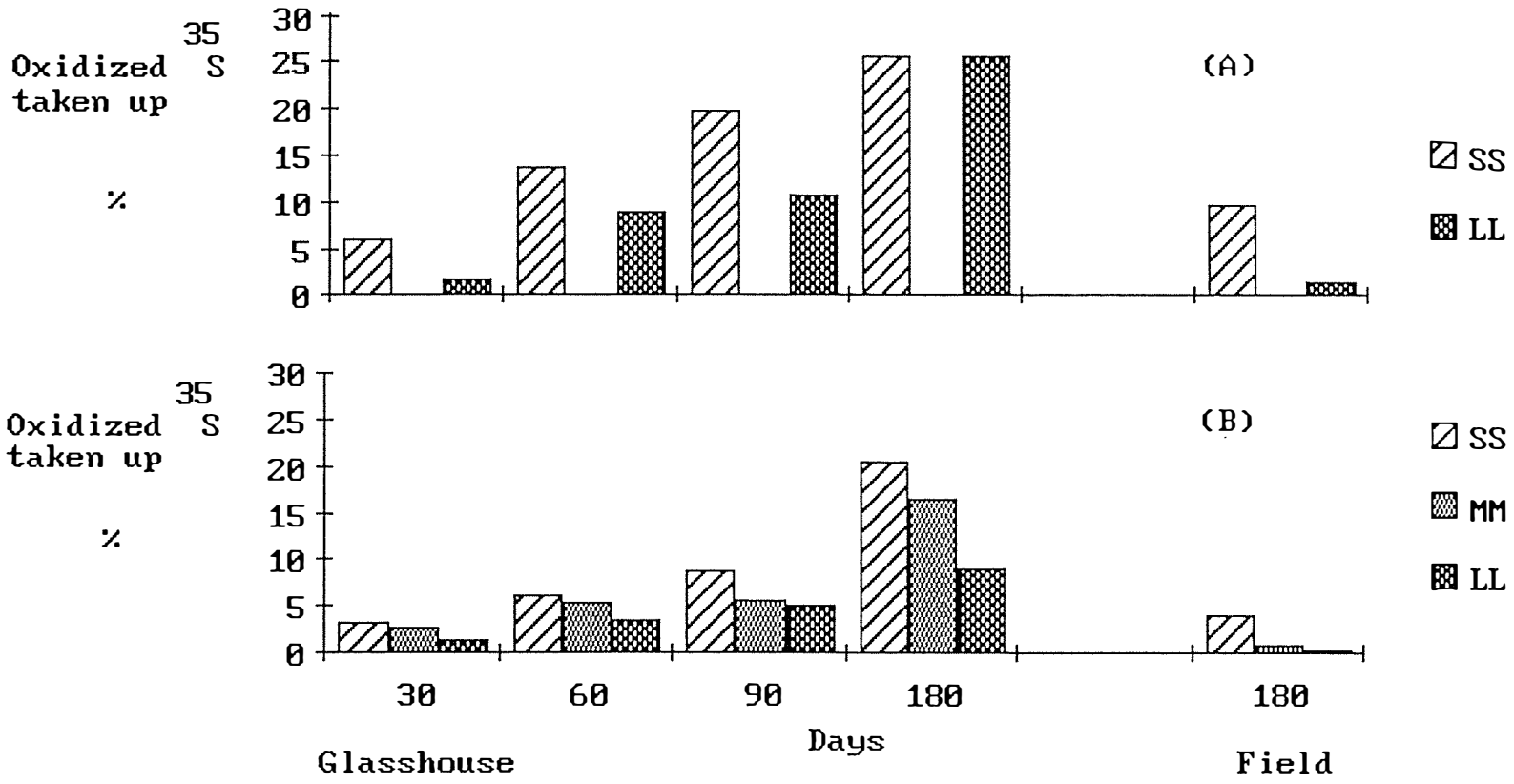


Figure 7.9 The effect of $^{35}S^{o}$ particle size on the percentage of oxidized $^{35}S^{o}$ cumulatively taken up by pasture on Ramiha (A) and Tokomaru (B) soils.

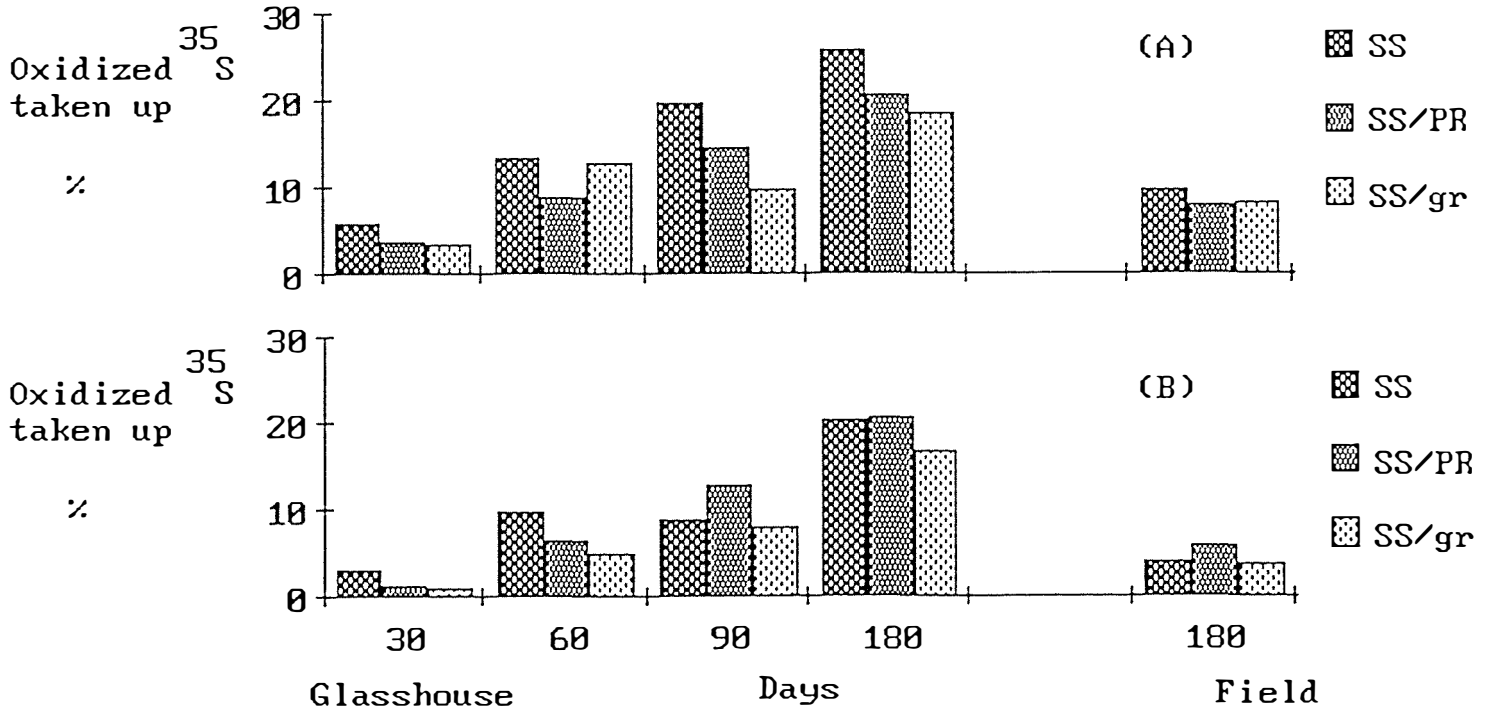


Figure 7.10 The effect of granulation of S^0 with or without phosphate rock on the percentage of oxidized $^{35}S^0$ cumulatively taken up by pasture (SS/PR and SS/gr compared with SS).

7.4.2.3 Residual $^{35}S^{o}$ activity and S^{o} (acetone extracts), extractable S and ^{35}S (CaP-S) activity in soil cores

Negligible amounts of $^{35}S^{o}$ activity were detected in acetone extracts of soil samples taken below 3 cm depths from some selected glasshouse and field soil cores. The percentage of applied $^{35}S^{o}$ recovered in the top layer (0-3 cm) soil cores are presented in Table 7.12A. At sampling dates up to 60 days, the residual activity of $^{35}S^{o}$ was not significantly different in cores fertilized with S^{o} different particle size. The reason for this lack of significance was large variability in measurement of $^{35}S^{o}$ by acetone extraction and total ^{35}S determination in soil samples. In Tokomaru soils, at least 60-90 days of incubation were required before differences in the activity of $^{35}S^{o}$ remaining in soil fertilized with different particle size treatments were evident. For both soils the rate of oxidation of S^{o}, as indicated by residual $^{35}S^{o}$ activity in soils from glasshouse and field trials, appeared to be approximately similar, ranging from 10 to 14 $\mu g\ S^{o}\ cm^{-2}$ (specific oxidation rate per unit surface area) for the SS particle size in Tokomaru and Ramiha soils, respectively (Chatupote, 1990). In general, by 180 days, the amount of S^{o} oxidized increased significantly with a decrease in S^{o} particle size.

In general, the percentage recoveries of $^{35}S^{o}$ in the Ramiha soil were lower than those in the Tokomaru soil. This was attributed to a slightly faster oxidation rate of S^{o} in the Ramiha soil as suggested by Chatupote (1990). The faster oxidation rate of S^{o} in the Ramiha soil may have resulted from the higher available moisture content in this soil (Moser and Olsen, 1953; Wainwright, 1984) and higher organic matter content (Wainwright *et al.*, 1986).

After 180 days the extent of S^{o} oxidation as determined from residual $^{35}S^{o}$ (Table 7.12A), was similar in both glasshouse and field trials. Oxidation rates as measured by the amount of S^{o} remaining in soil (Table 7.13A) showed similar trends to results obtained from the amount of $^{35}S^{o}$ activity remaining (Table 7.12A). At early samplings, however, the $^{35}S^{o}$ provided a more sensitive (less variability in replicate data) measure of S^{o} oxidation than measuring the amount of S^{o} residues (Table 7.12A).

In general, data on the amount of $^{35}S^{o}$ activity (Table 7.12A) and S^{o} (Table 7.13A) remaining in the soil cores fertilized with granulated materials SS/gr and SS/PR did not show significantly different S^{o} oxidation rates in both glasshouse and field trials.

Percentages of $^{35}S^{o}$ biologically oxidized into phosphate extractable ^{35}S (CaP-^{35}S, HI-reducible ^{35}S) forms in the 0-10 cm soil cores are shown in Figures 7.11, 7.12, Table 7.12B and Appendix 7.7. Throughout the 180 days, the finer particle size (SS granulated or ungranulated) produced larger amounts of extractable ^{35}S and by 180 days, in the glasshouse trial, about 12 and 18% of applied elemental $^{35}S^{o}$ appeared in extractable ^{35}S in Tokomaru and Ramiha soils, respectively. The percentage of $^{35}S^{o}$ transformed into phosphate extractable ^{35}S in field trials was approximately 50% of that in the glasshouse trial. Given that

S^o oxidation rates in glasshouse and field soils were similar, these results suggested that the net immobilization of ^{35}S labelled fertilizer was greater in field soils. This supported the greater recovery of added ^{35}S as organic S in field soils at 180 days (Table 7.14B). It may be that the warmer glasshouse temperature induced greater re-mineralization of previously immobilized ^{35}S which would maintain higher ^{35}S activity in the CaP-S extracts.

Very low amounts of ^{35}S activity remained in the CaP-extract of the MM and LL treatments suggesting that the rate of S^o oxidation in these treatments did not exceed the rate at which solution $SO_4^=$ was immobilized by synthesis of plant and soil organic S.

As shown in Appendix 7.7, the majority of ^{35}S activity recovered by CaP-extracts was in the 0-3 cm soil depth. Only in this depth was the effect of particle S^o size noticeable. The data indicated that there was little movement of ^{35}S down the profile. In field conditions more extractable ^{35}S was measured at lower soil depths of Ramiha soil as compared to the Tokomaru soil as evident by significant difference between the amounts of extractable ^{35}S in the SS and LL treatment in the lower layer of the Ramiha field soil. This probably reflects a greater leaching of sulphate S and a greater amount of adsorbed sulphate (CaP extractable) in the lower depths of the Ramiha soil (Appendix 7.8 compared to the Tokomaru soil, Appendix 7.9). As finer particle sizes of S^o, whether granulated or not, increased the ^{35}S activity in the extractable S pool so did they maintain higher extractable S levels (Appendix 7.8 and 7.9) in the upper part (top 0-3 cm) of the glasshouse and field Ramiha and Tokomaru soils. This was particular noticeable in the earlier harvests. Extractable S values in the 3-6 (middle) and 6-10 (bottom) cm soil depths showed no effect of S fertilizer form although in the bottom depths of the glasshouse cores extractable S concentrations tended to increase at final harvest. At no time during the experiment did the top soil extractable S values decrease to a level which would be expected to limit plant growth (i.e. below 10 mg S kg^{-1}, Sinclair and Saunders, 1984).

In general, the Ramiha soil had larger amounts of extactable ^{35}S than the Tokomaru soil in both glasshouse and field trials. This was consistent with the cumulative ^{35}S uptake, %SDFF and the percentage of oxidized $^{35}S^o$ taken up by pasture, as discussed Section 7.4.2.2. above. These data reflected greater S oxidation rate in the Ramiha soils which resulted from greater soil moisture and soil organic matter as mentioned above. The Ramiha soil fertility status (Table 7.1 and 7.2), in general, was higher than the Tokomaru soil; i.e. higher extractable P and S contents. This indicated that S oxidation was favoured by higher soil fertility status which provide more essential nutrients for the S oxidizing micro-organisms allowing their population to increase.

The data on extractable ^{35}S activity (CaP-S) in the soil cores revealed some effects of granulation and phosphate rock (Figure 7.12 and Appendix 7.7). In the glasshouse trial, initially, up to 60 days, granulation of S^o (SS/gr, SS/PR) produced slightly less extractable ^{35}S but thereafter had less effect on the activity of ^{35}S in CaP extracts particularly in field soils (Figure 7.12, Table 7.12B and Appendix 7.7).

Addition of ground phosphate rock at granulation did increase the release of extractable ^{35}S from granules (SS/PR cf. SS/gr) in the soil cores (0-10 cm), throughout the trial period in both the glasshouse and field trials (Figure 7.12 Table 7.12B and Appendix 7.7). Data on the amounts of $^{35}S^{o}$ (acetone extract) remaining in the top layer of soil cores were presented in Table 7.13A. These data did not show that phosphate rock increased S^{o} oxidation as did the extractable ^{35}S data as mentioned above (see Table 7.12B). An explanation for increased Ca-^{35}S in the presence of PR when extractable S (Table 7.13B and Appendix 7.8 and 7.9) did not increase with time is that addition of PR stimulated remineralization of soil organic ^{35}S. The accumulation or reduction of organic ^{35}S (as discussed below), however, does not indicate this trend.

7.4.2.4 Immobilization into organic matter

There was no significant effect of S^{o} particle size on the immobilization of $^{35}S^{o}$ into soil organic S (Table 7.14B) and by 180 days 19-55% of the added $^{35}S^{o}$ had been immobilized in the glasshouse Ramiha soil and 30-51% in the glasshouse Tokomaru soil. In field soils, the extent of immobilization was greater being 51-55% in Ramiha soil and 70-76% in the Tokomaru soil.

The majority of the immobilization appeared to occur during the first 30 days after fertilization. There are, however, a number of discrepancies between the activity of ^{35}S in organic S and that remaining as acetone extractable S^{o}. For example, it is impossible to incorporate 35% and 38% of the $^{35}S^{o}$ label (LL treatment) into organic S by 30 days in Tokomaru and Ramiha soil, respectively when only 10% and 17% of the $^{35}S^{o}$ could be accounted for as acetone extractable $^{35}S^{o}$ (Table 7.12A). Obviously, sampling and analysis error involved in determining activities of ^{35}S in acetone extractable S^{o} and total ^{35}S ($NaHCO_3 + Ag_2O$ digestion) compound to produce large error in estimating the activity of ^{35}S in organic S. Bearing these errors in mind, little can be said about the rate of ^{35}S incorporation into organic S or the effect of S particle size of granulation on the rate of incorporation.

There was no significant effect of particle size, granulation with and without phosphate rock on the immobilization of $^{35}S^{o}$ into organic S (Table 7.14B) and this may also be due to inaccuracies in the determination of $^{35}S^{o}$ in acetone extraction and total ^{35}S ($NaHCO_3$+Ag_2O digestion) in soil samples (Table 7.14A).

The greatest single fate of $^{35}S^{o}$ in this experiment was immobilization into soil organic S.

7.4.2.5 Movement of ^{35}S down the profile and leaching losses

As discussed earlier in Section 7.4.2.1, 75-100% of the ^{35}S remained in the soil cores and was not taken up by plants or leached. The vertical distribution of this total ^{35}S in the Ramiha (Figure 7.13) and Tokomaru (Figure 7.14) glasshouse soils after 180 days showed that little of the ^{35}S moved beyond the upper 0-3 cm of the undisturbed soil columns. These results and others for other harvest dates and fertilizer treatments are presented in Appendix 7.10. Notably, in the field cores there was greater movement of S isotope to depth.

The main mechanisms that would move isotope to depth are either leaching of $^{35}SO_4^=$, translocation of ^{35}S down roots or through the action of earthworms. There was evidence of earthworms casting activity in all soil cores. A lack of S^o oxidation would prevent movement by these mechanisms unless earthworms ingested small particle of unoxidized $^{35}S^o$.

In the field trials leaching losses during the 180 days of the experiments could be estimated from the amount of total ^{35}S not recovered (soil plus cumulative pasture uptake) from the undisturbed soil cores (0-10 cm) and data are presented in Tables 7.10 and 7.11. Results indicated that in spite of larger pasture uptake, slightly larger amounts of $^{35}S^o$ labelled fertilizers were unaccounted for on the Ramiha soil in relation to Tokomaru soil and approximately 10-16% of ^{35}S appeared to move beyond the 10 cm depth. Despite the greater overall loss of isotope from the Ramiha soil there was more movement of ^{35}S to the middle and bottom depths of field cores in the Tokomaru soil (Figures 7.14, 7.16 and Appendix 7.10). A small fraction of ^{35}S lost from the Ramiha soil cores could have been lost laterally, particularly if some of the heavier rainfall had caused particle run-off.
By 180 days, in the glasshouse trial, about 5 and 1% and 15 and 5% (mean of seven replications) of the total ^{35}S applied were found in the middle and bottom layer of the Ramiha and Tokomaru soils, respectively. No significant difference was found among the total ^{35}S within the lower soil layers of the soil cores treated with three particle sizes of the ^{35}S labelled S^o.

Smaller amounts of total ^{35}S in the top layer of the SS (<0.150 mm particle size) treated soil cores during the 90 and 180 sampling times resulted from greater pasture uptake rather than movement down the profiles.

In the field soils, at 180 days, a similar trend to the glasshouse trials was observed. The total ^{35}S recovered from the <0.150 mm S^o (SS) treated top layers In both soils were significantly less than those treated with the 0.250-0.500 mm S^o (LL). No significant differences between

the amounts of total soil ^{35}S were found in the middle and the bottom layers treated with different S^o particle sizes. At 180 days larger amounts of total soil ^{35}S appeared in the lower depths of the field cores in relation to glasshouse data. In the field trials more than 25% of labelled fertilizers moved beyond the top layer (0-3 cm) where it was initially placed. Only data from the Ramiha soil showed any effect of particle size in the lower layers (see Appendix 7.10).

Granulation and addition of phosphate rock did not show any effect on the movement of $^{35}S^o$ labelled fertilizer as evident by similar amounts of ^{35}S activity in the lower layers in both soils in the glasshouse and field trials (Figures 7.15, 7.16 and Appendix 7.10).

Analysis of the leachates from the glasshouse cores showed that no significant differences were detected in the amounts of HI-reducible ^{35}S in leachates from soil cores treated with different particle sizes and granules of ^{35}S labelled S^o (Table 7.15A). Total amounts of $^{35}S^o$ labelled fertilizer in leachates in each event were considered negligible amounting to less than one percent of the applied ^{35}S, even though during the leaching period up to 56 days, approximately 65% and 29% of the SS particle size had oxidized in the Ramiha and Tokomaru soils respectively (Table 7.12). The small amount of HI-reducible ^{35}S present in the leachates is mostly attributable to rapid immobilization of ^{35}S into non-leachable organic S and the sulphate adsorption capacity of both soils (see Appendix 7.14) since little ^{35}S was taken up by plants.

The retention of soil sulphate against leaching loss due to rapid incorporation of fertilizer ^{35}S into soil organic S was also reported by Gregg (1976), Gregg and Goh (1978 and 1979) and Goh and Gregg (1982a).

The amounts of HI-reducible S in leachates were also measured and the results are presented in Table 7.15B. No significant effect of S^o particle size was observed due to large variation in the observed data. The concentration of HI-reducible S in leachates from each leaching event was less than one $\mu g\ ml^{-1}$ (data not shown). Similar concentrations of S were also observed in leachates collected from a mole-drained field plots of Tokomaru soil (Smith, 1979; Heng, 1991). Total amount of HI-reducible S in leachates from five leaching event were about 0.3-0.8 mg S $core^{-1}$ which is equivalent to 0.17-0.45 kg S ha^{-1}.

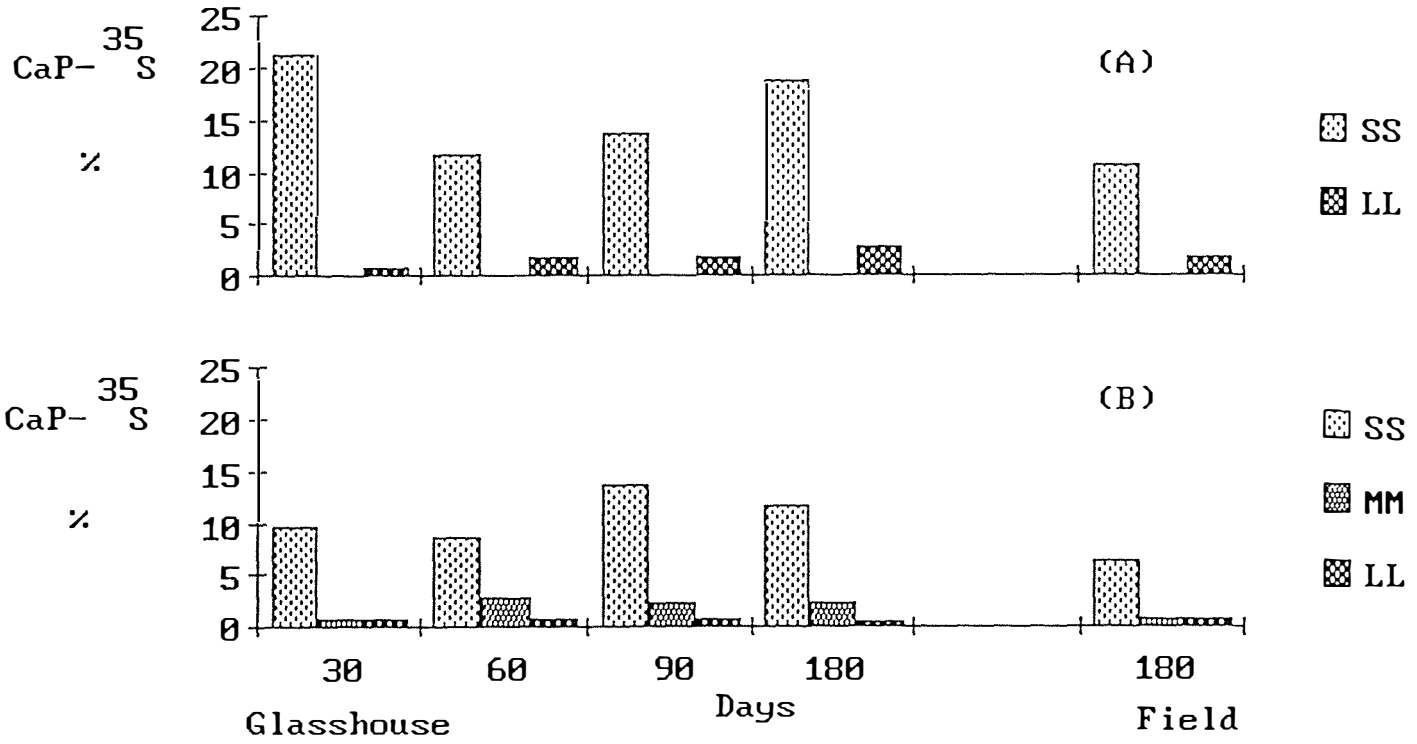


Figure 7.11 The effect of S^0 particle size on the recovery of extractable ^{35}S (CaP-^{35}S) in soil cores (0-10 cm) in Ramiha (A) and Tokomaru (B) soils.

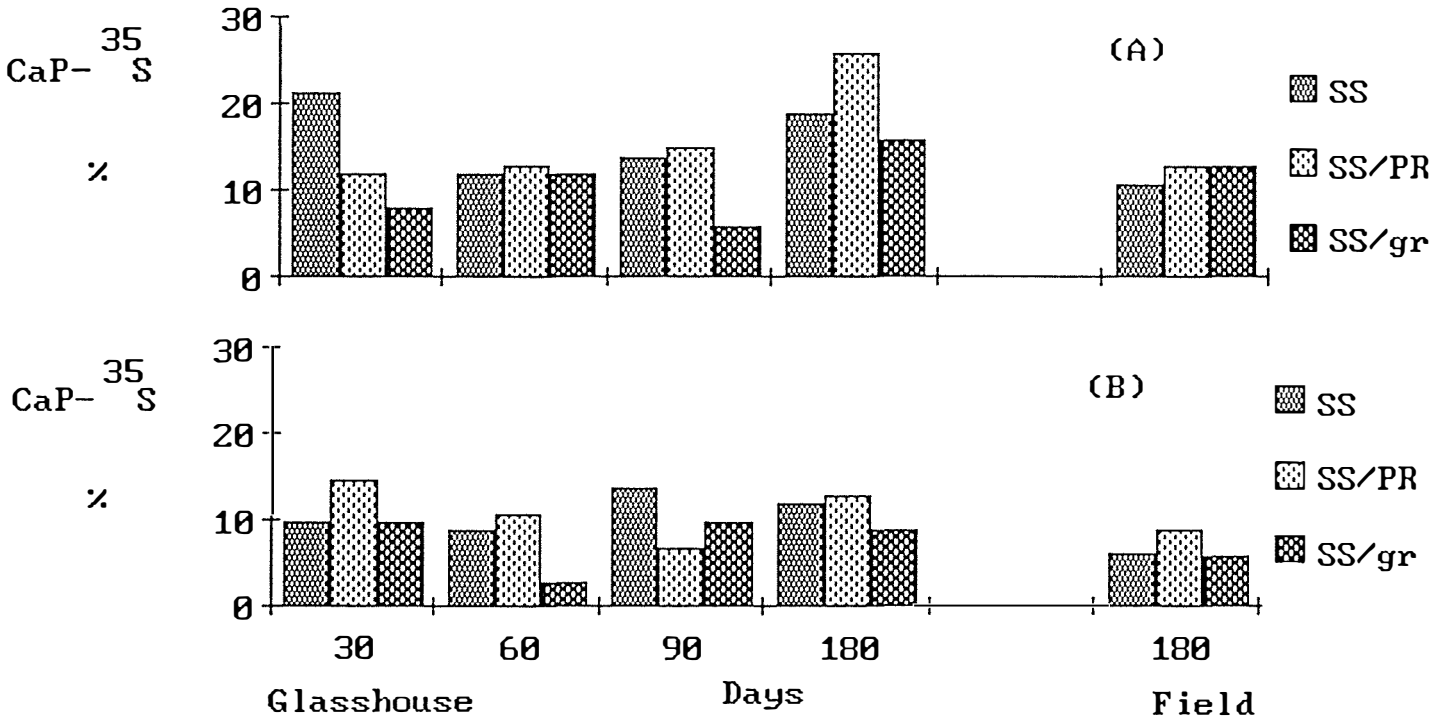


Figure 7.12 The effect of granulation of S^0 with or without phosphate rock on the recovery of extractable ^{35}S (CaP-^{35}S) in soil cores (0-10 cm) (SS/PR and SS/gr compared with SS) in Ramiha (A) and Tokomaru (B) soils.

Table 7.12 Percentage of applied ^{35}S recovered as residual $^{35}S^{o}$ (acetone extracts), in 0-3 cm soil depth (PART A) and extractable ^{35}S (CaP-S) (PART B) in soil cores (0-10 cm) in glasshouse trials and after 180 days in the field trials (average of three replications).

Treatments		Days after fertilizer application: 30	60	90	180[1]	Field 180
PART A, RESIDUAL ELEMENTAL $^{35}S^{o}$						
		% $^{35}S^{o}$ recovered (acetone extract)				
Ramiha	SS	58.7	45.4^{b}	42.3^{b}	21.4^{b}	21.7^{b}
	LL	82.7	94.5^{a}	84.6^{a}	75.6^{a}	63.2^{a}
	SS/PR	63.4	52.5^{b}	52.1^{b}	25.5^{b}	18.7^{b}
	SS/gr	66.9	53.8^{b}	63.4^{b}	21.2^{b}	22.3^{b}
F-test		ns	**	*	**	**
C.V. %		18.5	10.4	8.8	30.8	17.7
Tokomaru	SS	64.8	61.8	50.6^{b}	30.1^{b}	31.4^{c}
	MM	86.5	70.0	61.9^{a}	69.8^{a}	49.9^{b}
	LL	90.4	75.9	79.6^{a}	69.9^{a}	64.3^{a}
	SS/PR	69.5	66.3	65.5^{b}	35.6^{b}	29.4^{c}
	SS/gr	68.1	75.6	43.9^{b}	34.3^{b}	28.3^{c}
F-test		ns	ns	**	**	*
C.V. %		20.1	16.8	19.7	19.1	13.3
PART B, CaP-S EXTRACTABLE ^{35}S						
		.... % ^{35}S recovered as CaP-S				
Ramiha	SS	21.4^{a}	12.0^{a}	13.9^{a}	18.5^{b}	10.9^{a}
	LL	1.1^{c}	1.6^{b}	1.9^{c}	3.0^{c}	2.3^{b}
	SS/PR	12.4^{b}	12.8^{a}	15.3^{a}	25.5^{a}	12.9^{a}
	SS/gr	8.4^{ab}	11.6^{a}	5.9^{b}	15.5^{b}	12.7^{a}
F-test		**	**	***	***	***
C.V. %		44.8	24.9	24.6	27.7	27.8
Tokomaru	SS	10.3^{a}	9.0^{a}	13.6^{a}	12.1^{a}	6.4^{b}
	MM	1.2^{b}	3.2^{b}	2.4^{d}	2.4^{c}	1.3^{c}
	LL	1.2^{b}	1.1^{b}	1.3^{d}	0.8^{c}	0.9^{c}
	SS/PR	14.5^{a}	11.1^{a}	10.2^{b}	12.7^{a}	9.4^{a}
	SS/gr	10.7^{a}	2.8^{b}	6.6^{c}	8.7^{b}	6.4^{b}
F-test		**	*	**	***	***
C.V. %		50.2	46.5	27.2	34.3	27.2

, ** and * = significant at 5, 1 and 0.1% level, respectively; ns = not significant; mean separation by DMRT at 5% level denoted by letters*

[1] *average of seven replications*

Table 7.13 Percentage of applied S^0 recovered as residual S^0 in 0-3 cm layers (PART A) and total amounts of extractable S, CaP-S, (PART B) in soil cores (0-10 cm) in glasshouse trials and after 180 days in the field trials (average of three replications).

Treatments		Days after fertilizer application 30	60	90	180[1]	Field 180
PART A, ELEMENTAL S RECOVERED						
				.....%S^0 recovery		
Ramiha	SS	61.7	57.5^{b}	54.2^{c}	22.5^{b}	20.7^{b}
	LL	93.2	115.1^{a}	100.8^{a}	48.7^{a}	41.5^{a}
	SS/PR	70.6	66.4^{b}	64.2^{b}	18.1^{c}	15.8^{b}
	SS/gr	80.0	81.3^{b}	79.1^{b}	22.5^{b}	15.0^{b}
F-test		ns	*	**	**	**
C.V. %		15.1	16.9	9.3	31.7	26.3
Tokomaru	SS	79.1	68.9	55.5	35.5^{b}	38.5^{b}
	MM	100.0	76.6	68.3	58.4^{a}	54.8^{b}
	LL	102.3	80.6	81.1	77.7^{a}	73.2^{a}
	SS/PR	87.7	68.7	63.4	40.4^{b}	32.7^{b}
	SS/gr	72.6	81.3	48.7	41.7^{b}	34.3^{b}
F-test		ns	ns	ns	**	**
C.V. %		13.5	15.2	19.3	18.0	23.8
PART B, EXTRACTABLE S (CaP-S)						
				..... mg S core^{-1}		
Ramiha	SS	48.1	58.3^{bc}	52.8^{a}	65.5^{ab}	50.3^{ab}
	LL	37.2	52.0^{c}	36.8^{ab}	53.5^{c}	41.6^{c}
	SS/PR	50.8	68.5^{a}	47.3^{ab}	75.2^{a}	54.1^{a}
	SS/gr	46.9	57.7^{bc}	44.3^{ab}	68.1^{ab}	45.5^{bc}
	PR	39.7	61.6^{ab}	35.0^{b}	57.7^{c}	44.2^{bc}
	Ctrl	46.9	54.2^{c}	46.5^{ab}	36.5^{d}	40.8^{c}
F-test		ns	*	**	**	**
C.V. %		14.8	7.0	18.8	16.8	7.3
Tokomaru	SS	32.8	32.8	43.4^{a}	39.8^{ab}	32.0^{ab}
	MM	29.2	36.2	25.1^{bc}	33.7^{c}	24.9^{bc}
	LL	27.6	25.8	22.5^{bc}	32.9^{c}	26.5^{bc}
	SS/PR	40.3	37.8	25.3^{bc}	43.3^{a}	35.1^{a}
	SS/gr	48.9	32.3	31.4^{b}	41.2^{a}	33.5^{a}
	PR	30.0	29.6	23.5^{bc}	34.5^{c}	30.9^{ab}
	Ctrl	23.9	25.8	18.9^{c}	35.1^{ab}	33.3^{a}
F-test		ns	ns	**	**	**
C.V. %		30.2	17.2	18.7	12.5	9.9

, ** and * = significant at 5, 1 and 0.1% level, respectively; ns = not significant; mean separation by DMRT at 5% level denoted by letters;* [1] *average of seven replications*

Table 7.14 Percentage recovery of total ^{35}S (PART A) and organic ^{35}S (PART B) in soil cores (0-10 cm) at six sampling times in glasshouse trials and after 180 days in the field trials (average of three replications).

Treatments		Days after fertilizer application 30	60	90	180[1]	Field 180
PART A, TOTAL ^{35}S				.....% recovered		
Ramiha	SS	114.9	91.6	91.3	75.5	74.5
	LL	131.9	119.8	109.9	97.9	87.7
	SS/PR	104.3	101.9	98.4	99.2	81.3
	SS/gr	98.6	88.3	87.6	80.2	72.1
F-test		ns	ns	ns	ns	ns
C.V. %		14.4	15.2	12.3	11.2	11.4
Tokomaru	SS	115.1	100.9	88.4	92.9	95.0
	MM	136.9	132.1	113.7	101.8	100.4
	LL	126.7	122.6	141.3	104.4	105.5
	SS/PR	116.3	111.4	109.4	99.9	101.4
	SS/gr	120.6	107.5	86.3	87.3	91.0
F-test		ns	ns	ns	ns	ns
C.V. %		13.7	15.2	12.7	13.7	20.7
PART B, ORGANIC $^{35}S_a$				.....% recovered		
Ramiha	SS	34.8	34.2	35.1	37.4	51.9
	LL	48.2	23.7	23.9	18.9	50.6
	SS/PR	28.6	36.5	31.1	52.5	56.1
	SS/gr	23.2	23.3	17.8	34.3	55.0
F-test		ns	ns	ns	ns	ns
C.V. %		40.1	68.8	43.8	56.4	13.5
Tokomaru	SS	40.1	30.2	24.2	50.8	70.5
	MM	49.2	58.9	48.6	29.5	72.4
	LL	35.1	45.6	60.1	33.7	68.9
	SS/PR	32.3	33.8	37.5	50.5	76.3
	SS/gr	41.2	29.3	32.1	43.5	69.9
F-test		ns	ns	ns	ns	ns
C.V. %		52.1	39.4	50.0	21.1	14.8

ns = not significant; [1] *average of seven replications;*

a calculated as: Soil organic ^{35}S=(total ^{35}S) - (residual $^{35}S^{o}$) - (CaP-^{35}S), as described in Section 3.3.5

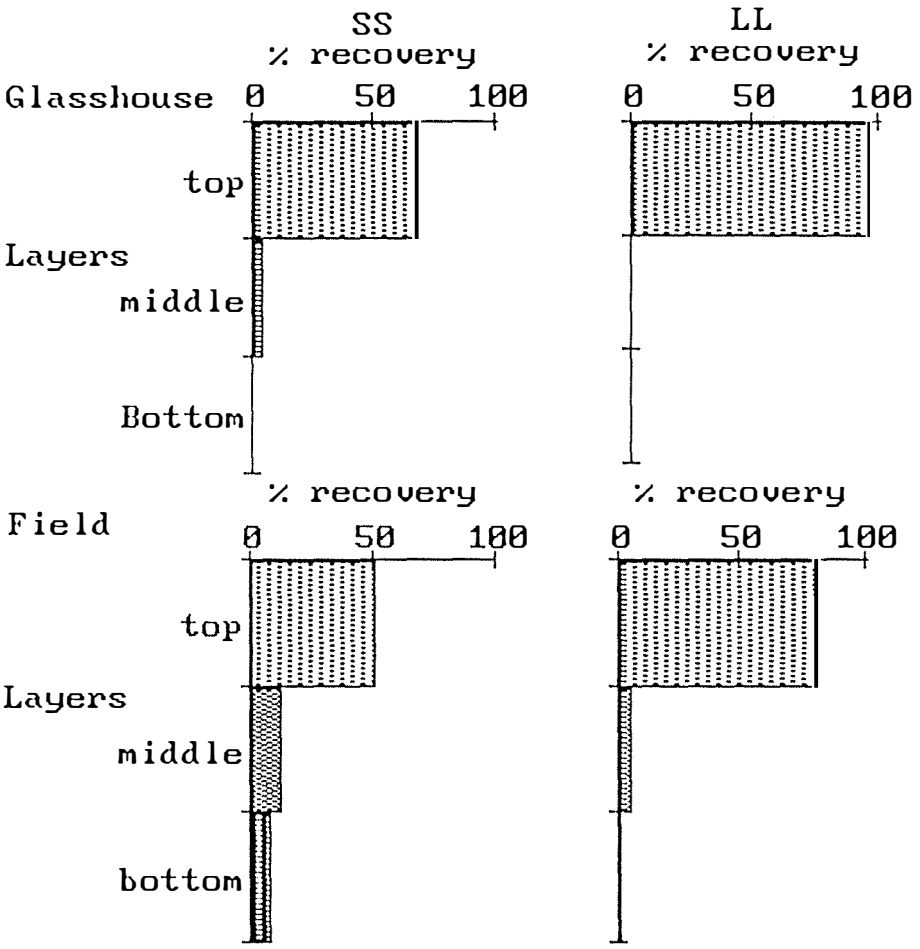


Figure 7.13 Total recoveries of ^{35}S in three layers of Ramiha soil 180 days after fertilization with two particle sizes of $^{35}S^{o}$.

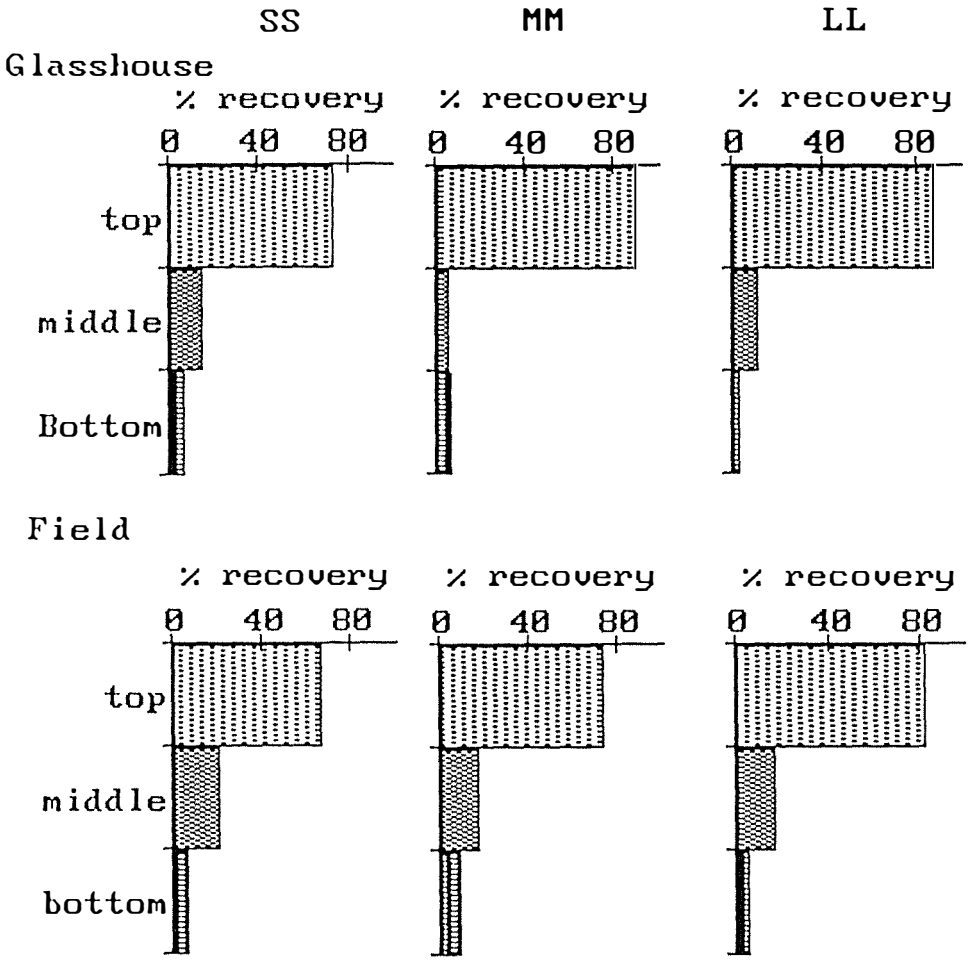


Figure 7.14 Total recoveries of ^{35}S in three soil layers of Tokomaru soils 180 days after fertilization with three particle sizes of $^{35}S^{0}$.

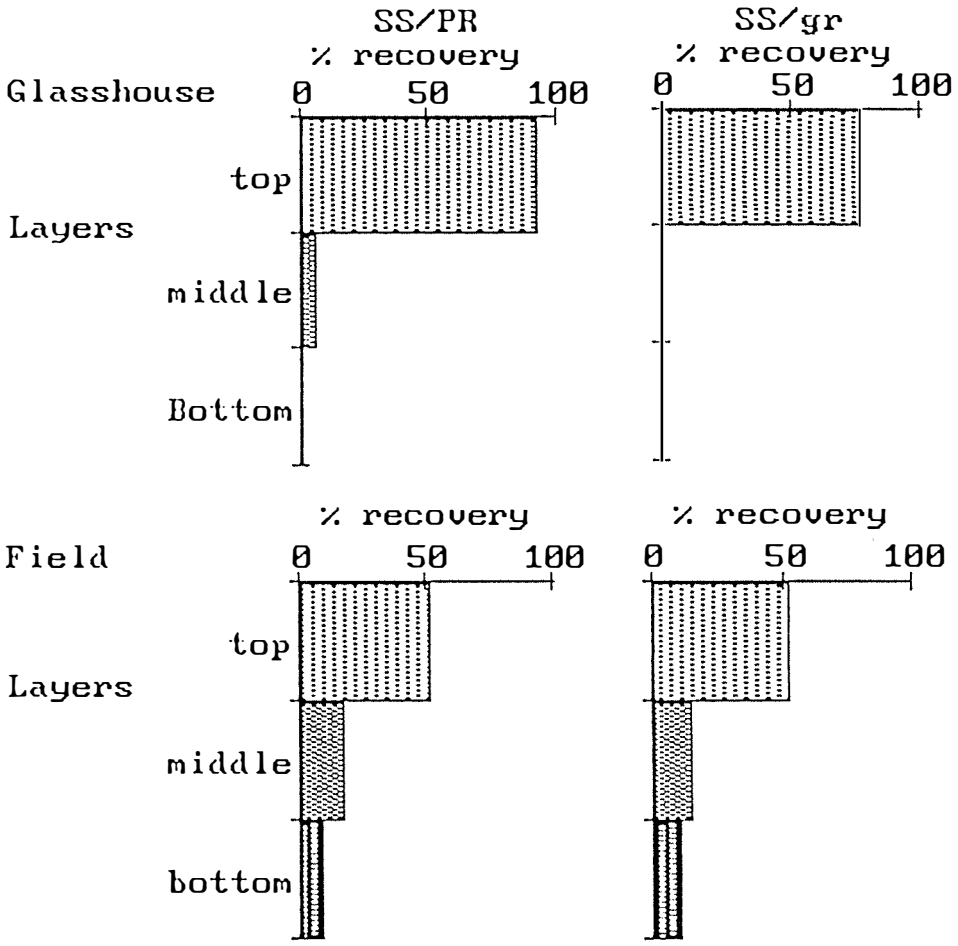


Figure 7.15 Total recoveries of ^{35}S in three soil layers of Ramiha soil 180 days after fertilization with fine $^{35}S^{o}$ (SS) granulated with and without phosphate rock.

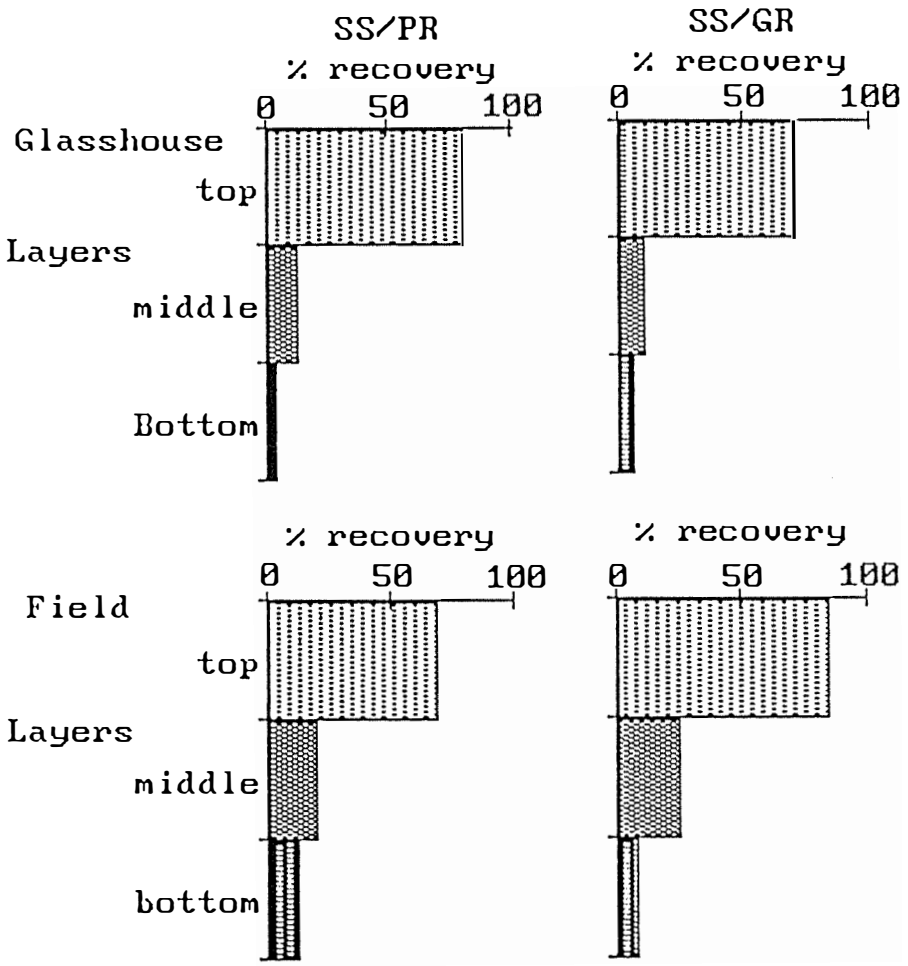


Figure 7.16 Total recoveries of ^{35}S in three soil layers of Tokomaru soil 180 days after fertilization with fine $^{35}S^{o}$ (SS) granulated with and without phosphate rock.

Table 7.15 Percentage recovery of ^{35}S in leachates (PART A) and total amounts of sulphur and HI-reducible S (PART B) in leachates at five leaching events in glasshouse trials.

Treatments		Days after fertilizer application 7	14	21	44	56	
PART A, ^{35}S RECOVERY				..... % recovery			Total
Ramiha	SS	0.0014	0.026	0.017	0.064	0.163	0.272
	LL	0.0003	0.001	0.001	0.001	0.015	0.018
	SS/PR	0.0047	0.019	0.007	0.112	0.110	0.254
	SS/gr	0.0003	0.001	0.003	0.003	0.059	0.058
F-test		ns	ns	ns	ns	ns	ns
C.V. %		160.6	228.6	247.7	203.6	101.8	82.1
Tokomaru	SS	0.0120	0.006	0.009	0.074	0.333	0.434
	MM	0.0076	0.007	0.008	0.038	0.016	0.206
	LL	0.0032	0.002	0.006	0.001	0.009	0.019
	SS/PR	0.0094	0.031	0.026	0.012	0.749	0.155
	SS/gr	0.0120	0.014	0.013	0.073	0.119	0.231
F-test		ns	ns	ns	ns	ns	ns
C.V. %		102.6	123.6	87.6	409.9	140.3	310.1
PART B, HI-reducible S				... mg S 10^{-3} event^{-1}			Total
Ramiha	SS	94.0	53.0	49.8	181.5	90.7	469.4
	LL	62.9	45.0	73.6	100.5	32.5	314.6
	SS/PR	95.6	86.6	75.9	238.5	102.1	598.8
	SS/gr	67.7	64.3	52.0	123.3	74.9	380.9
	PR	60.6	63.2	77.9	49.3	72.2	324.3
	Ctrl	60.2	51.2	77.5	88.3	65.8	342.9
F-test		ns	ns	ns	ns	ns	ns
C.V. %		34.1	30.6	32.5	120.9	138.5	120.1
Tokomaru	SS	90.5	87.9	92.9	204.0	409.9	885.3
	MM	91.4	90.5	127.6	216.9	52.4	578.4
	LL	64.4	82.8	108.5	80.0	53.6	389.4
	SS/PR	67.7	91.0	108.6	147.0	79.1	502.2
	SS/gr	69.3	62.2	51.2	190.3	121.7	494.7
	PR	117.8	142.9	93.8	58.9	34.4	447.9
	Ctrl	76.4	162.3	77.9	66.0	19.0	403.4
F-test		ns	ns	ns	ns	ns	ns
C.V. %		120.1	209.0	45.1	54.9	138.6	40.1

ns = not significant at 5% level

7.4.2.6 Summary

Despite the fact that for much of the experimental period plant S concentrations in field and glasshouse experiments were well below optimum level, only glasshouse soils fertilized with the finer S^0 (SS) particle size consistently had higher S uptake, but this was not translated into increased dry matter production. However, N and P deficiency may have limited pasture growth.

There was always a large influence of available soil-S or organic S mineralization on the amounts of plant S taken up and the amount of CaP extractable soil S, therefore the non radioactive data show little or no effect of added S^0 fertilizers on plant S uptake and soil S status as measured by CaP.

Measurement of the fate of ^{35}S derived from fertilizer showed clearly that the extent of S oxidation, plant uptake of S and the percent of oxidized S^0 taken up by plants were always higher with the finest particle size of S^0. Except for the study of Lee *et al.,* (1987) previous experiments in New Zealand soils have only inferred these fertilizer effects from differences in plant S uptake or levels of extractable $SO_4^=$ in S fertilized vs. non-fertilized soils (Boswell and Swanney, 1988; Smith and McDougall, 1988; Swanney *et al.,* 1988; Boswell and Swanney, 1991). Recently published work by Nguyen and Goh (1991) has also shown similar effects. Notably the percent of oxidized S taken up by plants was greater in glasshouse soil than field soils where evapotranspiration rate were higher. This result suggests that available S is used more efficiently when water used by plant is high. Such evidence agrees with the concept that sulphate uptake by plants occur mostly by mass flow of soil water to roots (Barber, 1984).

The finer particle size of S^0 produced greater amounts of soil extractable ^{35}S (CaP-S) and the majority of this fraction remained in the top layer (0-3 cm). The amounts of ^{35}S leached were negligible and the majority of fertilizer S remained in the top soil layer (0-3 cm), more than 50-90%, mostly present in organic form.

No significant effects of particle size or granulation of the fine S^0 with and without phosphate rock on the amounts of ^{35}S leached or immobilized into organic S were observed. Initially, 0-60 days, the amounts of fertilizer S^0 and ^{35}S leached beyond 10 cm soil depth were negligible. The failure to detect differences in the rate of transformation of ^{35}S into organic S caused by fertilizer form were as much due to the inaccuracy of total ^{35}S determination ($NaHCO_3$ + Ag_2O digestion) and total $^{35}S^0$ (acetone extraction) when particulate S^0 was present in small soil samples.

In general, granulation of fine S^0 with and without phosphate rock slightly reduced the efficient use of the oxidized S^0 by plants. But this effect was not consistent in both soils and in the Tokomaru soil, addition of phosphate rock (PR) appeared to give improved uptake of oxidized S compared to S^0 granulated alone.

Data on the percent of plant S derived from fertilizer (%SDFF) and the percentage of oxidized $^{35}S^0$ cumulatively taken up by plants, indicated that the efficiency of S^0 in providing S for pasture plant was higher in the Ramiha soil which had a higher soil fertility status than the Tokomaru soil.

Data on acetone extraction for residual S^0 and $^{35}S^0$ activity revealed similar trends of S^0 oxidation rate. However, accuracy in determination of the residual S^0 and total S in the S^0 treated soil samples needs further improvement.

7.4.3 *FATE OF SULPHATE-BASED FERTILIZERS*

This sub-experiment was carried out for the first 90 days of the glasshouse experiment described above. Previously, in Chapter 5, the fate of sulphate-based (SSP) and microfine S^0 fertilizers has been investigated under field conditions in the undisturbed soil cores in the Tokomaru soil. In this experiment two sulphate-based fertilizers were investigated in glasshouse conditions. The main objective was to compare the fate of ^{35}S labelled gypsum and superphosphate in soils contrasting in organic matter content, S content, and P retention with that of S^0.

7.4.2.1 Recovery of ^{35}S in soils and plants

The cumulative uptake of ^{35}S radioactivity by plants and that remaining in the top 10 cm soil in the glasshouse after 90 days are shown in Table 7.16 for both Ramiha and Tokomaru soils.

Cumulative plant uptake of ^{35}S under glasshouse conditions accounted for approximately 20% of the isotope which was greater than that of S^0 fertilized soils, 6-12%, during the same period (see Appendix 7.4). The majority (67-80%) remained in the soil. The amount of isotope leached beyond the 10 cm soil cores was about 4% for both soils (Table 7.19) being more than double the percentage of $^{35}S^0$ that was leached (Table 7.15).

7.4.3.2 Plant uptake of S and ^{35}S and percentage of plant sulphur derived from fertilizers (%SDFF)

There were no significant differences in S concentration and cumulative S uptake (soil plus fertilizer S) of pastures between pastures grown on soil treated with GP and SSP (Figure 7.17B, Appendix 7.11). In terms of S concentration and cumulative S uptake, pasture grown on GP and SSP responded very well to the application of these two fertilizers. Both S concentration and cumulative S uptake were significantly higher than the control soil cores. The increased S uptake only slightly increased dry matter yield of pasture grown on the Ramiha soil but significantly increased the dry matter yield in the Tokomaru soil (Figure 7.17A and Appendix 7.11). The lack of dry matter yield response in the Ramiha soils might be due to larger phosphate extractable S in soil in relation to the Tokomaru soil (Table 7.2 and Appendix 7.13)

Pasture treated with ^{35}S labelled GP and SSP cumulatively took up the same amounts of ^{35}S on both soils and also showed a similar trend in the %SDFF (Figures 7.18A, 7.18B and Appendix 7.12). However, cumulative ^{35}S uptake from soils treated with ^{35}S labelled GP and SSP were much higher than those treated with the <0.150 mm elemental $^{35}S^{o}$ (SS).

In Ramiha soil, initially both ^{35}S labelled GP and SSP treated cores had higher %SDFF (34-50%) than the <0.150 mm (SS) treated soil cores (24-28%), as presented in Figure 7.18B and Appendix 7.12. But thereafter comparable %SDFF values were observed. Notably, the %SDFF was not always a good indicator for a comparison on the relative efficiencies of use between the two sources, sulphate and S^{o} (i.e. GP or SSP vs. finer particle of S^{o}, SS). This contrasts to the discussion in section 7.4.2.2, since the GP and SSP fertilized cores yielded larger amounts of ^{35}S uptake. The greater S uptake in the GP and SSP probably indicated that environments in the GP and SSP soil cores were more favourable for S uptake than that of SS soil cores. The SS treatment still provided less S for plant demand than the GP and SSP treatments. Therefore both %SDFF and the amount of ^{35}S uptake should be considered together in comparison of two sources of fertilizer S (see also the discussion in section 7.4.5 and 7.4.2.2).

7.4.3.3 Immobilization into organic matter

The percentage of applied $^{35}SO_4^{=}$ converted into soil organic ^{35}S in the soil cores (0-10 cm) treated with ^{35}S labelled gypsum and superphosphate were similar (Tables 7.17 and 7.18). However, larger amounts of organic ^{35}S occurred in the Tokomaru soil (49-53%) than In the

Raimha soil (39-40%). In comparing these results to those of previous experiment in Chapter 5 (see Tables 5.6 and 5.8), extents of applied ^{35}S incorporation into organic S were similar and occurred at similar rates.

The proportion of soil organic ^{35}S (value expressed as percentage of total S remaining in the soil cores) in this experiment were less than those reported in Chapter 5 (Table 5.8). In Chapter 5, during the same period (90 days) about 90% of ^{35}S from ^{35}S labelled superphosphate remained as organic S while in this experiment only 52 and 60% were observed. The reason may be attributed to the lower leaching losses in the glasshouse experiment. Even after five leaching events, which were approximately equivalent to 0.7 pore volume, about 35 to 44% of the ^{35}S was accounted for in the phosphate extractable ^{35}S fractions mostly in the top soil indicating that much of the drainage water must bypass the soil pores containing the majority of the $^{35}SO_4^=$ or that significant quantities of surface adsorbed organic ^{35}S are extracted by calcium phosphate (Watkinson and Perrott, 1990; Watkinson *et al.*, 1991) .

As shown in Table 7.18 when the amounts of organic ^{35}S were expressed as the percentage of the total ^{35}S remaining in the soil cores, the majority of total ^{35}S in the middle and bottom layer were in organic form (about 70 to 80%).

Despite the large experimental errors involved in the comparison, it appeared that at 90 days more ^{35}S had been converted into organic S in the soil fertilized with gypsum and SSP (Table 7.17) than in the same soil fertilized with $^{35}S^o$ (Table 7.14). Over this period (0-90 days) it is expected that the rate of ^{35}S immobilization would be limited by the extent of S oxidation, however, because of the large errors involved in measuring total ^{35}S and residual $^{35}S^o$ in the presence of particulate ^{35}S this relationship is not always clearly shown. For example, at 30 days in LL treated Tokomaru soil the percentage of ^{35}S in acetone extractable ^{35}S (Table 7.12A) was 90.4% and organic ^{35}S (Table 7.14B) was 35.1% giving a total of 140% whereas for all SS treatments there appeared to be a better mass balance of isotope.

7.4.3.4 Movement of ^{35}S down the profile and leaching losses

Vertical distribution of soil ^{35}S fractions in each soil layer, presented in Table 7.18, demonstrate the movement of ^{35}S released from labelled gypsum and superphosphate in the intact soil cores. Data presented were the results of soil samples taken after 90 days which was about 40 days after the last leaching events (Table 7.19) were applied. Results indicated that both treatments exhibit similar ^{35}S movement in soil cores. About 50, 10 and 5% of the

^{35}S released form labelled fertilizer were present in the top, middle and bottom layers, respectively and the majority were in organic forms.

Five leaching events were also applied to both soil cores as described in Section 7.3.6. No significant difference occurred between the amounts of HI-reducible ^{35}S in the leachates from the two soils (Table 7.19). About 0.4 to 1.6% of ^{35}S labelled fertilizer was recorded in each event during the leaching. On average, the total amounts of the HI-reducible ^{35}S leached beyond the 10 cm depth was about 4% of total applied ^{35}S labelled fertilizers. This represents a larger leaching loss of fertilizer S than in the S^o treatments discussed in Section 7.4.2.5.

As mentioned in Section 7.4.3.3 above, the majority of ^{35}S in the middle and bottom layer were incorporated into organic forms by microbial processes (Freney *et al.*, 1971, 1975; Gregg, 1976). Again, this process along with the sulphate retention capacity of the soils (see Appendix 7.14) will play a role in reducing leaching losses of the $^{35}S^o$ labelled fertilizers in the leaching events.

The amounts of HI-reducible S in the leachates were also measured (Table 7.19B). There was no significant difference in the amount leached from GP or SSP treated soil but there were large variations in the data for each event. Much larger amounts of HI-reducible S, equivalent to 0.9-1.0 kg ha^{-1}, were leached from the GP and SSP treated cores when compared to control and the S^o treated cores (particularly the <0.150 mm, SS cores, Section 7.4.5.2).

7.4.3.5 Summary

Both GP and SSP increased extractable soil S (CaP-S) and raised pasture S concentration above the 'critical level' and in turn generated dry matter yield increases for pasture grown on Tokomaru soil with a low level of extractable soil S (Tokomaru soil)

The efficiency of plant use of S from GP and SSP were similar. In these two soil materials there was no significant effect of the phosphate in SSP on the amount of S taken up by plants, leached down soil profile or immobilized into organic S.

The majority of radioisotope (total S, extractable S and organic S) remained in the top 3 cm soil layer with the majority being converted to organic S in 90 days experiment. Further experiments on the fate and residual value of fertilizer S should concentrate on this top layer rather than inclusion of soil at deeper depths, because the observed rates of ^{35}S immobilization indicated high biological turnover of nutrients in this zone. There may, however,

be strong interaction between the amount of fertilizer S leached and soil status because in contrast to the result in the field trial on higher S status, Tokomaru soil (Chapter 5), less radioactivity was apparently leached. This agrees with the findings of Saggar *et al.* (1990a, 1990b) who reported greater S leaching loss from soil with high S status.

7.4.4 Comparison of sulphate-based fertilizers and elemental S

To facilitate comparisons of the efficiency of use of sulphate and S^o fertilizers (Figure 7.19), simple relationships between S and ^{35}S taken up by pastures and time were constructed using the combined means of the GP and SSP treatments and the individual means of the SS and control treatments during the first 90 days of the glasshouse experiment.

Plant uptake of S and ^{35}S from sulphate-based fertilizer (GP and SSP) was more readily available to pasture plants than the S in the finer S^o particles (SS). Plants utilized greater amounts of fertilizer S in the GP and SSP fertilized cores than in the SS fertilized cores. However, the utilization of oxidized $^{35}S^o$ was similar to the soluble $^{35}SO_4^=$ from GP and SSP in the Ramiha soil, but on average lower in the Tokomaru soil. The results in this Chapter indicated a superiority of GP and SSP over S^o in providing fertilizer S for pasture plants in the short term. To be as effective as sulphate S fertilizer in providing short term available S for pastures, the particle size of S^o must be as small as microfine S^o, discussed in Chapter 5. In Chapter 5, the microfine S^o was as effective as SSP. One problem with the microfine S^o is that apparent losses from the plant-soil system were approximately 45% (Chapter 5).

In this Chapter where losses of ^{35}S were small there was no evidence to support the finding reported in Chapter 5, that there was greater immobilization of $^{35}S^o$ into soil organic matter than ^{35}S from SSP. Such results may vary with soil S status and climate and S particle size. Daily uptake of S by pasture (as indicated by slope of regression lines in Figure 7.19) in this experiment was much lower when compared with the results discussed in Chapter 5 (Figure 5.2 and Table 5.5 compared with Figure 7.19 and Appendix 7.11). The mean daily S uptake for pasture fertilized with suphate-based (GP and SSP), S^o (SS) fertilizers and Ctrl were about 0.18, 0.1 and 0.09 kg ha^{-1} day^{-1}, respectively, (compared with field results of SSP and microfine S^o treated cores in Chapter 5 which were about 0.25 and 0.28 kg ha^{-1} day^{-1}, respectively). Pasture S concentrations on GP and SSP treated cores were similar in both of these experiments (in Chapter 5 and 7). Dry matter production and the absolute amounts of S taken up were influenced by the general fertility of each site. The second site, although the same soil type, had a poor fertilizer history reflected in the total soil S content (395 mg S kg^{-1} soil, Table 7.2) compared to the fertilized site in Chapter 5 (540 mg S kg^{-1} soil, Table 5.3). In

these legume-based pastures regular fertilizer history leads to increase N fixation. Nitrogen availability is a primary factor limiting pasture yield and probably limited pasture growth. For example, the percent of plant S derived from S^0 in the higher fertility site (Chapter 5, Appendix 5.2) was at least 50% lower than observed at the lower fertility site (Figure 7.8). These results will reflect the faster oxidation rate of microfine S^0 and greater leaching loss of S observed at the first site and the lower availability of soil S at the second site. Similar differences were observed for SSP and GP but the comparison between field results in Chapter 5 (Appendix 5.2) and the glasshouse results in Chapter 7 (Figure 7.18B) are less valid because of the much higher water use of the glasshouse grown plants.

It is important therefore to consider the fertility status of soil and climate when attempting to extrapolate results pertaining to the efficiency of fertilizer use to other sites and situations even on the same soil type as shown by Gregg (1976).

7.4.5 Comparison of glasshouse and field trials

Cumulative S and ^{35}S uptake, dry matter yield, S concentration of pasture plants, percentage of oxidized S^0 taken up by plants and CaP-^{35}S were higher in the glasshouse trial. Only the %SDFF's from S^0 fertilizers were similar.

The difference in plant growth conditions in the glasshouse and field produced some large differences in some measurements of plant growth and S uptake (Chapter 7 only) but not the %SDFF. Such data suggest that the climate differences (temperature and soil moisture content) between glasshouse and field soil cores had parallel effects on S^0 oxidation, mineralization of soil organic S and plant S uptake as the ratio at which soil S and $^{35}S^0$ were made available stayed constant. This effect needs investigating further because they indicate that glasshouse studies using undisturbed soil cores may be relevant to field conditions when a fertilizer with biologically controlled release rate (S^0 oxidation) is being evaluated.

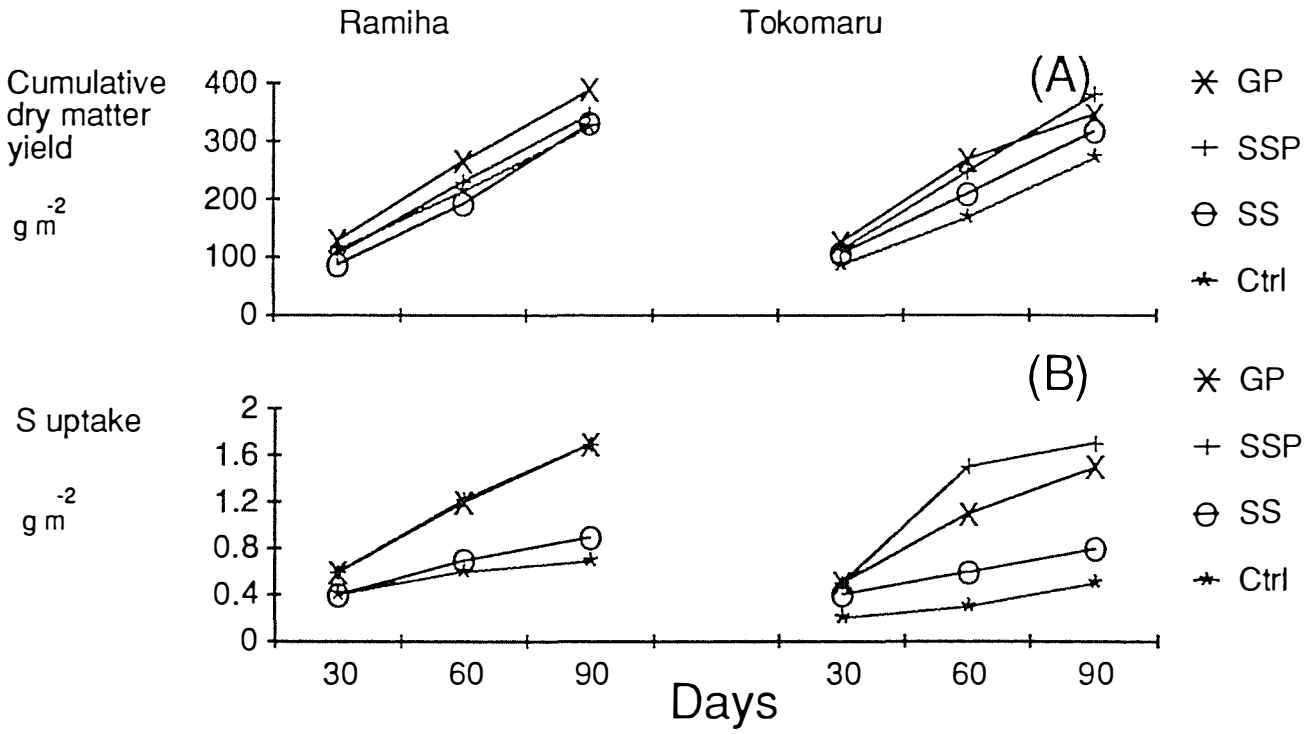


Figure 7.17 Cumulative dry matter yield (A) and S uptake (B) of pasture on Ramiha and Tokomaru soils; GP and SSP vs. SS.

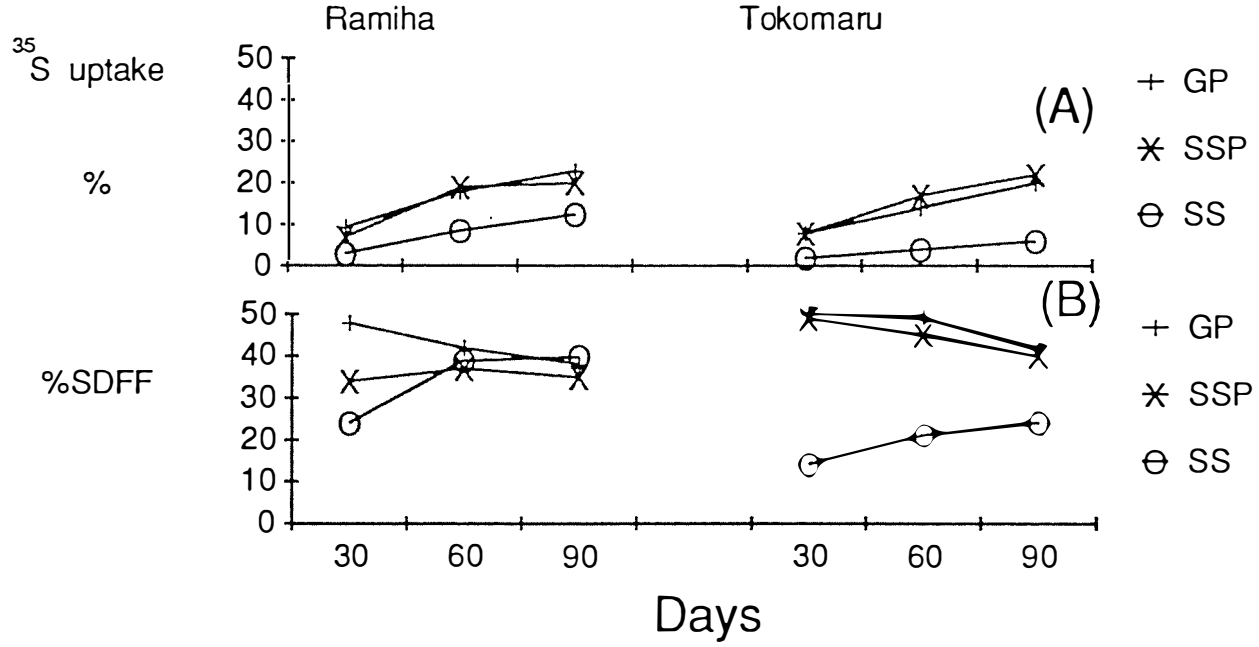


Figure 7.18 Cumulative ^{35}S uptake (value expressed as percentage of ^{35}S applied) by pasture (A) and %SDFF (B); ^{35}S labelled GP and SSP vs. $^{35}S^{O}$ labelled SS.

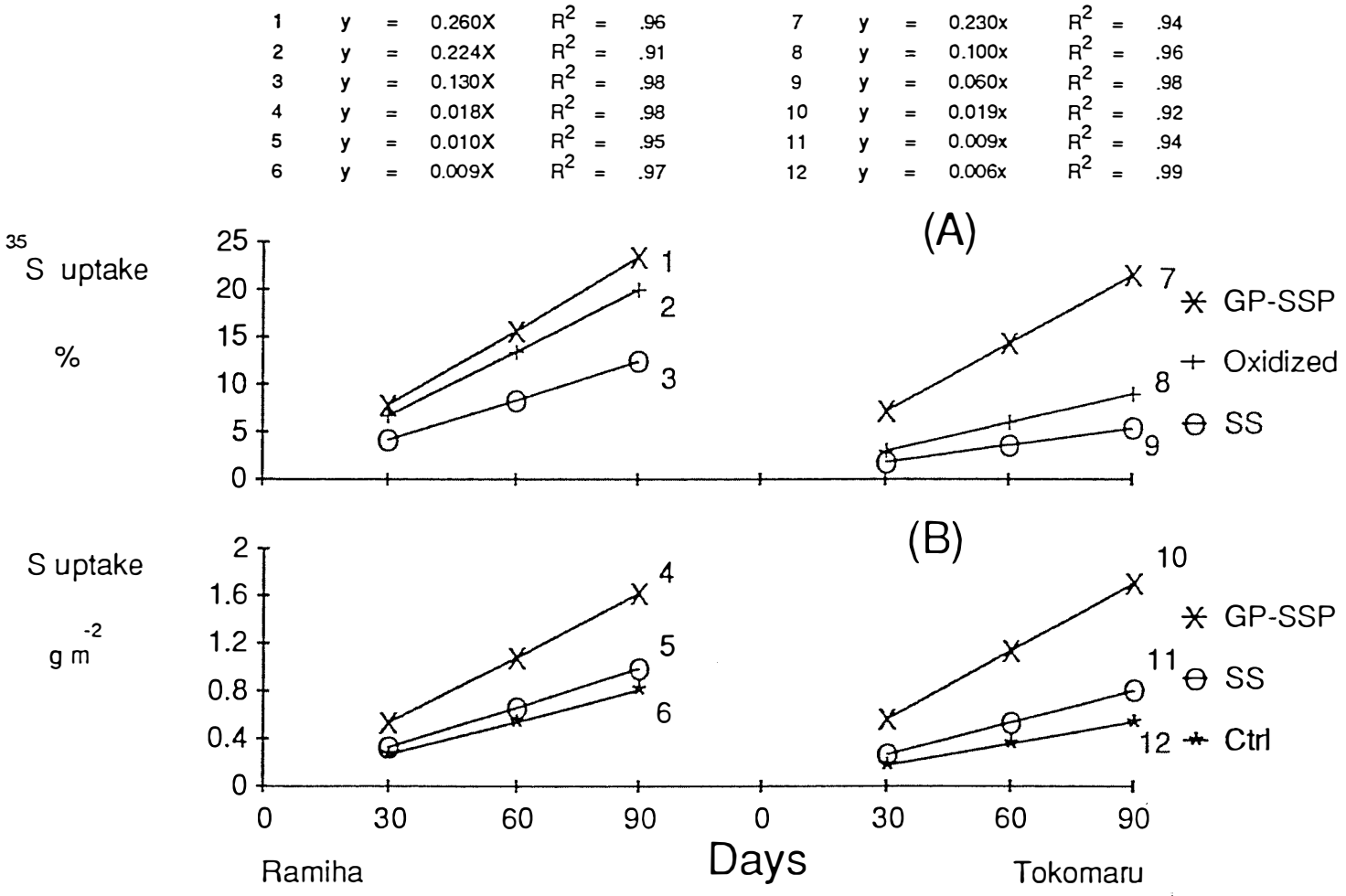


Figure 7.19 Simple relationships between cumulative ^{35}S uptake (A) (value expressed as percentage of ^{35}S applied) and S uptake (B) by plants against time (a comparison between sulphate-S and elemental S fertilizers).

Table 7.16 Total recovery of ^{35}S in soil and plant in two soils resulting from applications of ^{35}S labelled gypsum and superphosphate after 90 days.

Treatments		..... Soils ..... Ramiha %	Tokomaru %
PLANT	GP	22.6	19.4
	SSP	19.6	22.4
F-test		ns	ns
C.V. %		10.8	16.7
SOIL	GP	69.7	81.4
	SSP	67.7	80.2
F-test		ns	ns
C.V. %		14.9	8.2
TOTAL	GP	92.3	100.8
	SSP	87.4	102.7
F-test		ns	ns
C.V. %		11.36	4.3

ns = not significant

Table 7.17 Fractions of ^{35}S in soils (% recovery) 90 days after applying ^{35}S labelled gypsum (GP) and superphosphate (SSP).

Soils	Treatments	Total ^{35}S %	Organic ^{35}S %	Extractable ^{35}S %
Ramiha	GP	69.7	39.1 (56.1)	30.6 (43.9)
	SSP	67.4	40.3 (59.6)	27.4 (40.1)
F-test		ns	ns	ns
C.V. %		14.9	19.1	19.5
Tokomaru	GP	81.4	52.7 (64.7)	28.4 (34.8)
	SSP	80.2	49.4 (61.6)	30.8 (38.3)
F-test		ns	ns	ns
C.V. %		8.2	7.4	8.4

numbers in brackets were proportion expressed as the percentage of total S remained in the soil cores; ns = not significant

Table 7.18 Distribution (% recovered) of total ^{35}S (TT), organic ^{35}S (Org) and phosphate extractable ^{35}S (CaP-S) in three soil depths of two soils 90 days after application of ^{35}S labelled gypsum (GP) and superphosphate (SSP).

		Ramiha			Tokomaru		
Fertilizers	Layers	TT	Org	CaP-S	TT	Org	CaP-S
			... % ...			... % ...	
GP	Top	55.5	28.4	27.1	62.1	37.9	24.2
	Middle	10.3	7.9	2.5	12.8	10.1	2.7
	Botom	4.1	2.7	1.4	6.5	5.0	1.4
SSP	Top	53.3	30.6	22.6	67.5	38.7	28.9
	Middle	9.1	6.3	2.8	8.0	6.8	1.9
	Bottom	5.3	3.4	1.9	4.7	3.9	0.7
F-test							
Fertilizers		ns	ns	ns	ns	ns	ns
Within fertilizer[1]		10.7	7.2	6.8	5.8	3.2	3.7
C.V. %		31.5	37.1	47.8	14.5	14.3	25.7

ns = not significant at 5% level; [1] Lsd at 5% level

Table 7.19 Percentage recovery of ^{35}S labelled fertilizer sulphur in leachates (PART A) and HI-reducible S in leachates (PART B) at five leaching events after ^{35}S labelled gypsum (GP) and superphosphate (SSP) applications.

		Days after fertilizer application					
treatments		7	14	21	44	56	
PART A, ^{35}S RECOVERY							
				....% ^{35}S recovered ...			Total
Ramiha	GP	1.4	1.5	0.4	0.8	0.2	4.2
	SSP	0.6	0.8	1.6	1.2	0.1	4.3
F-test		ns	ns	ns	ns	ns	ns
C.V. %		127.5	168.6	38.9	88.5	74.8	57.1
Tokomaru	GP	1.2	0.4	1.0	0.9	0.2	3.6
	SSP	2.3	0.6	0.6	1.2	0.2	4.9
F-test		ns	ns	ns	ns	ns	ns
C.V. %		101.5	84.5	127.5	67.2	75.9	45.9
PART B, HI-REDUCIBLE S							
				... mg S event^{-1} ...			Total
Ramiha	GP	0.33	0.52	0.14	0.40	0.20	1.60[a]
	SSP	0.10	0.30	0.43	0.60	0.15	1.55[a]
	Ctrl	0.06	0.05	0.08	0.09	0.07	0.30[b]
F-test		ns	ns	ns	ns	ns	*
C.V. %		143.1	129.1	165.8	83.1	75.7	44.5
Tokomaru	GP	0.33	0.15	0.34	0.50	0.27	1.60[a]
	SSP	0.54	0.21	0.20	0.52	0.10	1.55[a]
	Ctrl	0.08	0.02	0.08	0.06	0.02	0.40[b]
F-test		ns	ns	ns	ns	ns	*
C.V. %		95.9	44.9	111.8	68.7	94.1	35.5

** = significant at 5% level; ns = not significant at 5% level; mean separation by DMRT at 5% level*

7.5 CONCLUSIONS

The implications of the findings in this Chapter are that the efficiency of plant use of S^0 can be improved by decreasing S^0 particle size. Unlike the findings in Chapter 5 using higher fertility soil, S derived from S^0 was not conserved to a greater extent in soil organic matter than S derived from SSP. This suggests that the particle size of S^0, climate effects and soil fertility status have a large impact on transformations of S in soil.

Granulation with or without ground phosphate rock, slightly decreased the oxidation rate or the efficiency of finely ground S^0 in supplying S to plants in the short term. Granule breakdown and S^0 dispersion will be an important factor to consider when evaluating granular S^0 fertilizers.

The use of ^{35}S tracer has confirmed that S^0 (coarser particle SS, MM, LL) is more suitable as a maintenance fertilizer for maintaining the S status in soils and plants rather than rapidly increasing pasture yield. Superphosphate and gypsum are more suitable for this.

It is recommended that experiments are carried out to examine closely the effects of existing soil fertility status on the transformations and loses of fertilizer S in pasture soils. Some such studies have been undertaken by Sakadevan (1991). In such studies, techniques need to be developed to overcome the varability inherent in analysing mixtures of particulate fertilizers and soils, particularily, measurements of total S and residual S^0. The inablility to remove this variability in the experiments discussed above has limited the interpretation of the results.

CHAPTER 8

MODELLING THE FATE OF SULPHUR IN THE PASTURE SYSTEM

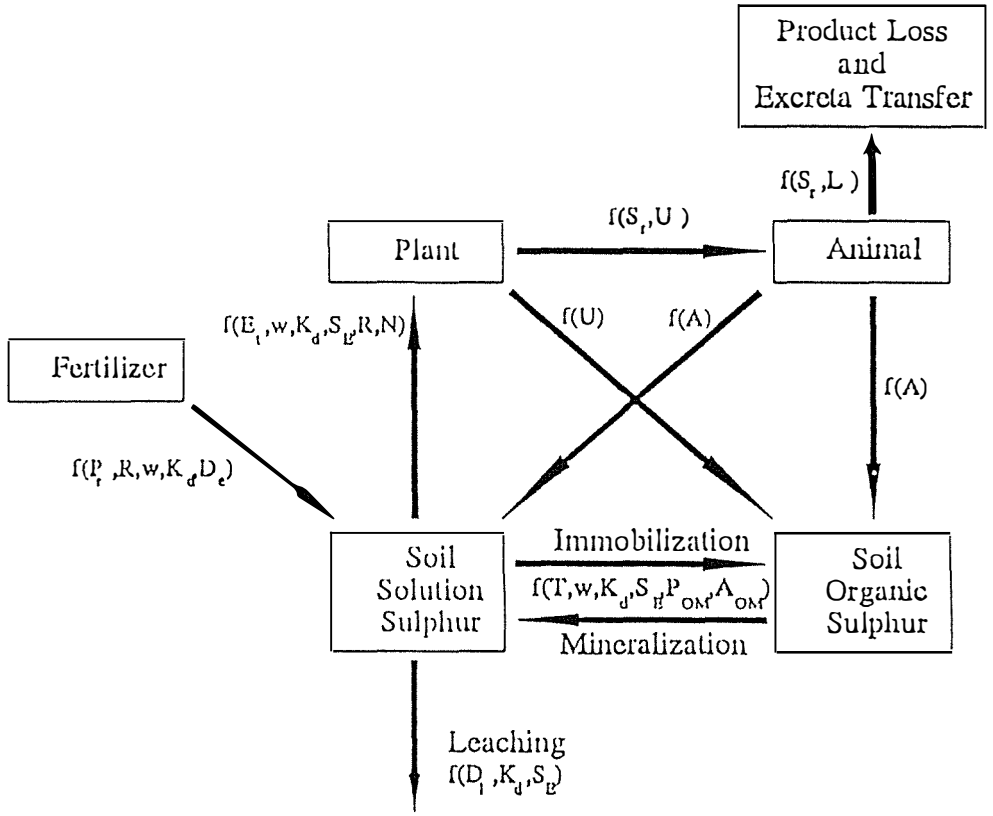
8.1 INTRODUCTION

Recognized methods for calculating the fluxes of isotopically labelled compounds or nutrients from one 'pool' to another in 'open' systems (Shipley and Clarke, 1972) cannot be applied to aquic soil environments when the added labelled nutrient (in this case $^{35}SO_4^=$) or a product of the added labelled (i.e. $^{35}S^o$ oxidized to $^{35}SO_4^=$) is susceptible to randomly occurring drainage events that vary in their intensity and specific activity of S leached is not easily quantified. For example after $^{35}SO_4^=$-based fertilizer addition to soil the specific activity of the soil CaP-S pool will increase as the fertilizer enters the soil and then decrease as mineralization transforms unlabelled soil organic S into $SO_4^=$, which is then removed by plant uptake, immobilization and leaching processes. Leaching, being an intermittent process, will transfer S of varying specific activity to lower soil depths depending on when it occurs after isotope addition in soil. In order to use isotopic labelling techniques to calculate actual mineralization and immobilization rate of S in field soils, measurement of soil solution $^{35}SO_4^=$ specific activity would need to be made at very frequent time intervals, particularly after rain, at a number of soil depths. Alternately, if there were long time intervals between measurements then a model of daily events that influence S movement and transformation (e.g. rainfall and plant uptake) is required to interpret the significance of measurements of S pool size and specific activity made at each time interval.

The objective of this Chapter is two fold, (1) to develop a model, which if it accurately describes the observed movement and transformation of S and ^{35}S in Chapter 5, will be used to calculate the actual rates of mineralization and (2) to summarize information gained in this thesis and from other studies on the soil-plant part of S cycle in a form which is useful for indicating future research directions.

A simple computer simulation model recently constructed by Sakadevan (1991) was modified to describe the movement, transformation and losses of S from the soil-plant system studied in Chapter 5. The conceptual S pools are outlined in Figure 8.1. These concepts are explained in more detail in Section 8.3 and 8.4. The model output is compared to field observations made in Chapter 5.

Fig. 8.1. A Simple Conceptual Dynamic Model Showing the S - Cycle



R = Rainfall
K_d = Soil Sulphate Adsorption Coefficient
T = Temperature
E_t = Transpiration
S_r = Stocking Rate
P_{OM} = Plant Organic Matter, Quality
A_{OM} = Animal Organic Matter, Quality
P_r = Particle Size plus Oxidation or Solubility Constant
S_B = Extractable Sulphate
D_l = Drainage
A = Animal Excretion Patterns, including S_r and L
w = Soil Volumetric Water Content
N = Growth Limiting Nutrient
U = Pasture Utilisation
f = function of a variable listed
L = Landslope
D_e = Diffusion Coefficient of Sulphate

8.2 MATERIALS AND METHODS

Methods employed in making the field observations were discussed in Chapter 5.

8.3 MODEL DEVELOPMENT

The simulation model developed in Section 8.3.1.1 to 8.3.3.3 builds on a model developed by Sakadevan (1991) and for clarification much of his discussion of the model development is repeated and modified where appropriate.

8.3.1 Water balance

8.3.1.1 Calculating drainage volumes.

The vector for the movement of plant nutrients in the soil and loss through leaching is the soil water. A drainage model developed by Sakadevan (1991) was used to predict the movement of water through the soil. Daily drainage water volumes for a soil column of known depth were predicted using the following simple balance equations:

$$W_f = W_i + R - EP \qquad 8.1$$

where

W_i = the initial depth of water (mm) in a soil column of specified depth.
W_f = the final depth of water (mm) in a soil column of same depth.
R = daily rainfall (mm day^{-1}).
EP = daily potential evapotranspiration (mm day^{-1}) calculated from daily air temperatures and sun light hours by the method of Prestley and Taylor (1972).

To keep the model simple, it was assumed that rainfall intensity did not exceed the infiltration rate of water into the soil and that run-off did not occur. The amount of drainage (D, mm day^{-1}) leaving a certain soil depth (Z) is given by the equation

$$D = W_f - W_{fc} \qquad 8.2$$

where

W_f = depth of soil water (mm) to soil depth Z (mm)
W_{fc} = depth of soil water at field capacity (mm) in soil depth Z (mm)

In a field trial where actual drainage volumes were measured (Sakadevan, 1991), this equation explained 98% of the variation (1:1 relationship) in the observed drainage volumes

8.3.1.2 Estimating actual daily evapotranspiration.

The actual evapotranspiration (***AEP***) from these pasture soils will be used to estimate the flux of water from the soil through the plant. With complete cover of the soil by pasture plants 100% of evapotranspiration (***EP***) can be considered to occur from the leaves of pasture plants (Coulter, 1973; Jensen, 1973; McNaughton *et al.*, 1979; Payne, 1988). This will be the vector which carries soil solution sulphate into the plant (Figure 8.1) to predict plant S uptake (Section 8.3.3).

The effect of decreasing soil water content is to decrease the rate of evapotranspiration (Payne, 1988; Gregory, 1988). This effect can be approximated by the relationship given by Scotter *et al.* (1979) and Rickard *et al.* (1986). Rickard *et al.* (1986) considered that for the Lismore stony silt loam plant growth and actual evapotranspiration (***AEP/EP*** = 0 at permanent wilting point, **PWP**) completely stops when the soil water deficit was approximately 70% of the maximum soil water deficit and the potential evapotranspiration (***EP***, ***AEP/EP*** = 1) could be achieved when the soil water deficit was less than 50% of maximum. In the absence of data on the relationship between pasture growth and soil water deficit, for the Tokomaru field site, the relationship derived by Rickard *et al.* (1986) was modified and applied. At the Tokomaru site W_{fc} was measured at approximately 45 mm for the top 100 mm of soil and the maximum observed deficit was approximately $0.3W_{fc}$ = **PWP** (see below). Actual evapotranspiration (***AEP***) was allowed to equal the potential evapotranspiration (***EP***) at soil water contents between W_{fc} and $0.65(W_{fc})$. The ratio of ***AEP/EP*** was allowed to decrease linearly, however, when the soil water content declines from $0.65(W_{fc})$ to $0.3(W_{fc})$ which was considered appropriate for the Tokomaru silt loam (Heng, 1991. personal communication). The soil water depth at which the soil water deficit is zero (W_{fc}) were estimated as 15 mm, 15 mm, 15 mm and 91 mm for 0-33 mm, 33-66 mm, 66-100 mm and 100-300 mm (Heng, 1991, personal communication) soil depths, respectively. Using this relationship the ***AEP*** was calculated from a knowledge of daily ***EP*** and the soil water content using a computer program written in QuickBASIC (Microsoft Corporation, 1987) by Heng (1991).

A detailed description of the movement of soil water through the plant-soil system is given by Gregory (1988). In the simple model presented in this Chapter, however, it is assumed that the amount of water removed by the plant from the soil depends only upon the distribution of root weight in each soil depth. Evidence that this method provides a reasonable description of

water removal from different soil depths is presented by Payne (1988). Williams (1988) showed that, the root mass distribution of a ryegrass-clover dominant sward on Tokomaru silt loam was 55%, 20%, 15% and 10% for 0-33 mm, 33-66 mm and 66-100 mm and 100-300 mm soil depths, respectively. The total ***EP*** was partitioned to draw water from each depth relative to the percentage root distribution in each soil layer. ***EP*** from i^{th} soil layer was calculated as follows:

$$EP_{,i} = EP\,(r_i) / (\Sigma r_T) \qquad 8.3$$

where

Σr_T = total root mass for all the soil layers (i = 1 to 4)
r_i = root mass for the i^{th} layer.

EP from each depth was then converted to ***AEP*** based on the relationship between ***AEP/EP*** and soil water deficit in each depth.

8.3.2 A single sulphate pool for plant uptake, immobilization and leached sulphate.

The simple configuration in Figure 8.1 assumes that there is one available sulphate pool from which S is taken up by plant and lost by leaching (Syers and Curtin, 1987; McCaskill and Blair, 1988). In the experiment described in Chapter-6, evidence was provided that the specific activity of plant ^{35}S formed during a period t_n to t_{n+1} was similar to that of CaP extractable ^{35}S over the same period. Sakadevan (1991) has also shown that the specific activity of ^{35}S leached from mini-lysimeters for time t_n to t_{n+1} were similar to that taken up by plants (plant $^{35}S/S$ = 0.73 leached $^{35}S/S$) over the same period. The reasons given by Sakadevan (1991) for slightly higher specific activities of leached $SO_4^=$ were: That early leaching of $^{35}SO_4^=$ immediately after labelled SSP application, moved $^{35}SO_4^=$ to soil depths where root activity and $SO_4^=$ turnover were slower than in upper more densely rooted soil horizons. Subsequent rainfall events were suspected of leaching some of the less diluted ^{35}S from the lower soil depths. Also in the plant rhizosphere, which includes micropores exploited by fine roots and root hairs (Barber, 1984), the rate of sulphate turnover may be more rapid than in the bulk soil or in macropores. More leached S may be derived from the larger pores (mobile phase) rather than micropores (immobile phase). Plant root hairs normally derive S from the immobile phase by diffusion (Barber, 1984). The sulphate in the immobile phase may be diluted rapidly because of increased mineralization associated with root activity. Thus, in general, the specific activity of sulphate in larger pores may be diluted at a slower rate than that in areas of intense root activity.

Evidence indicates that both plant S and leached S are derived from a pool with similar specific activity, but the results of Sakadevan (1991) indicated that a robust leaching and mineralization/immobilization ($SO_4^=$ turnover) model is required before the specific activities of measurable outputs of the plant-soil system can be adequately predicted. Sakadevan (1991) concluded that for a computer model to simulate combined plant uptake, organic turnover and $SO_4^=$ leaching it would be wise to partition the soil into defined zones of differing root activity. In this respect a vertically layered model with root activity and mineralization decreasing down the soil profile would seem acceptable if root activity is known.

8.3.3 Modelling the fluxes of S between various pools

The following discussion represents the S mass balance central to modelling changes in the size of the extractable S pool in the soil. The amount of sulphate present in the soil solution for a given layer (i), which is potentially available for plant uptake and loss by leaching at any time 't' (Δt normally = 1 day) is given by the balance equation

$$E_{s,t} = E_{s,tn-1} - P_s - L_s + M_s + I_s + F_s \qquad 8.4$$

where

$E_{s,t}$ = sulphate (kg S ha^{-1}) present in the plant available pool solution at any time t for i^{th} layer

$E_{s,tn-1}$ = sulphate (kg S ha^{-1}) present in the plant available solution at time t-1 for i^{th} layer

P_s = sulphate taken up by plant (kg S ha^{-1} day^{-1}) during the period from t-1 to t from the i^{th} layer

L_s = sulphate lost by leaching (kg S ha^{-1} day^{-1}) during the period from the ith layer

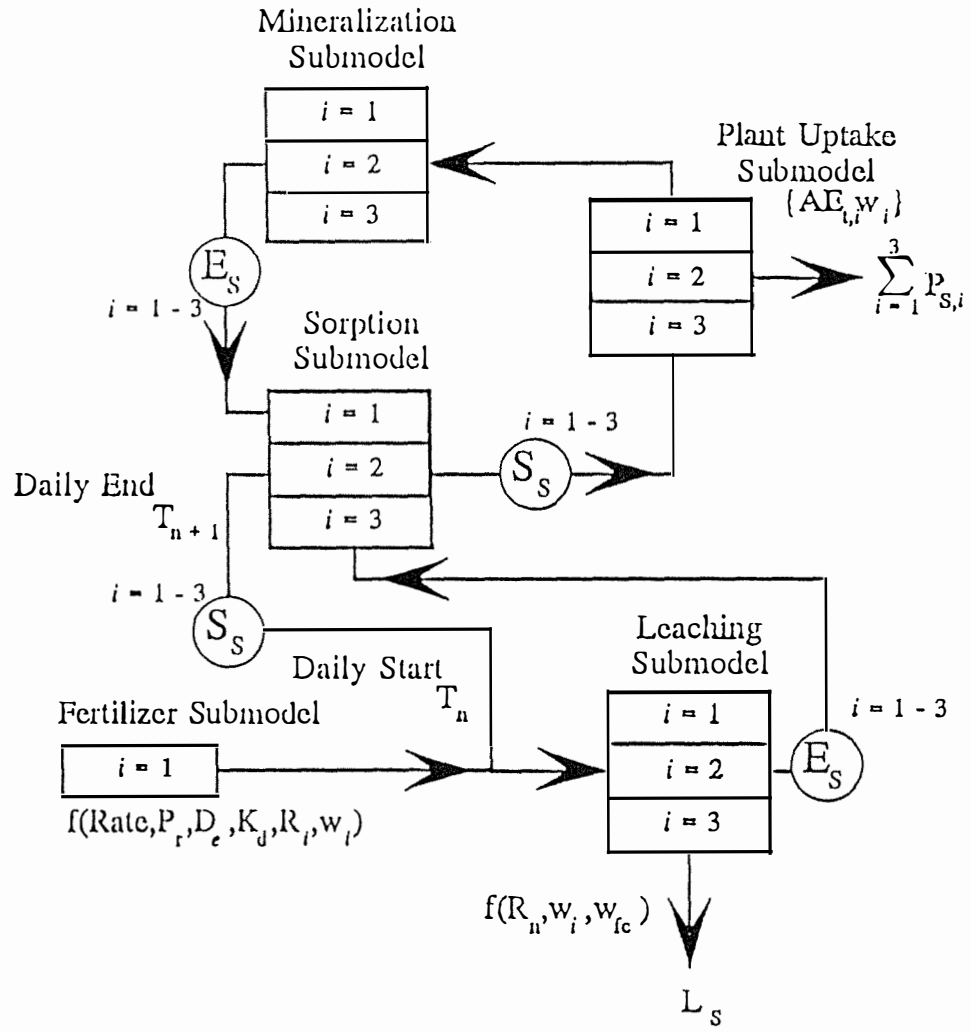
M_s = amount of sulphate (kg S ha^{-1} day^{-1}) mineralized to the sulphate pool from soil organic matter in one day in i^{th} layer.

I_s = amount of S (kg S ha^{-1} day^{-1}) entering the sulphate pool in the i^{th} layer from rainfall or other solution inputs

F_s = amount of sulphate (kg S ha^{-1} day^{-1}) entering the sulphate pool from fertilizer.

At the field site, estimated S input through rainfall is not high(<2 kg S ha^{-1} y^{-1}, Smith, 1979; Heng, 1991; Ledgard and Upsdell, 1991) The majority of the S input to the sulphate pool is from the mineralization of soil organic matter, which includes the decomposition of animal excreta, plant litter, root and older soil organic matter, and the amount of fertilizer S, if applied. In the model plant S, immobilized S and leached S are derived from solution sulphate and the order in which the processes occur on a daily basis is firstly leaching followed by, plant uptake and mineralization/immobilization (Figure 8.2). The following sections construct submodels to describe the dynamic features of the components of equation 8.4

Fig. 8.2. Hierarchical Order of the Processes in the S Cycle



S_S = Soil Solution Sulphate
E_S = Extractable Soil Sulphate
Rate = Rate of Fertilizer Application
i = Soil Layer 1, 2, or 3
For definition of symbols see Figure 8.1.

8.3.3.1 Predicting the soil solution sulphate concentration.

The method used here was similar to that used by Heng (1991) and Sakadevan (1991) using the relationships between laboratory determined CaP extractable S and field-soil solution $SO_4^=$ concentrations. For a model to predict the amounts of S taken up by plants or leached (Figure 8.1) the soil solution sulphate must be estimated on a regular basis. Ideally, functions are required to describe the relationship (sorption isotherm) between adsorbed and solution sulphate in field soils. It is difficult to measure the soil solution sulphate concentrations in field soils, particularly when the soil moisture content is lower than field capacity. In dry periods the soil solution cannot be collected using porous, porcelain cups and suction sampling. It is however, relatively easy to core sample soils and carry out laboratory measurements. The common soil test to measure solution plus adsorbed sulphate in the soil is to extract the soil with 0.01 M or 0.04 M $Ca(H_2PO_4)_2$ (Searle, 1979; Saunders *et al.,* 1981). Some of the S extracted from pasture top soils is in organic form (Watkinson and Perrott, 1990; Watkinson *et al.,* 1991).

The slopes of the Freundlich isotherm describing the relationships between CaP-S and the solution sulphate concentration for soil samples taken from the Tokomaru silt loam pasture soil are presented in Table 8.1. Heng (1991) also used a Freundlich 'isotherm' to described the relationship between the $SO_4^=$ concentration in soil water sampled from 200 mm soil depth in the Tokomaru soil and the CaP-extractable S concentration in cores taken from that depth (Table 8.1). Notably the 'field' isotherms (Heng, 1991) differ markedly from the 'laboratory' isotherms for approximately the same soil depth. The laboratory isotherms show an increasing $SO_4^=$ sorption power as the soil depth increase. Estimated field isotherms for the 0-33 mm and 33-66 mm soil depths were produced by changing slope of field isotherms in the same ratio that the laboratory isotherms changed with soil depth.

As discussed by Sakadevan (1991) the relationships were not strictly adsorption isotherms, because the extractable sulphate measured using 0.04 M $Ca(H_2PO_4)_2$ contains solution, adsorbed and some organic S (Watkinson and Perrott, 1990; Watkinson *et al.,* 1991). For this reason true adsorption isotherms were not generated using plots of adsorbed sulphate vs. solution sulphate. The Freundlich isotherms represented the relationships between extractable S and soil solution sulphate for all the three soil layers (Table 8.1) as described above.

Table 8.1 Freundlich type equation fitted to laboratory and field mesured isotherms.

Isotherm method	Soil layers (mm)	Fitted[1] equations $C_s = aC_l^b$	Model depths (mm)	Equations[1] used in model $C_s = aC_l^b$
Laboratory batch method (Heng, 1991)				
	0-50	$2.87C_l^{0.379}$	0-33	$2.3C_l^{0.627}$
	50-300	$2.87C_l^{0.379}$	33-66	$2.3C_l^{0.627}$
	300-450	$3.78C_l^{0.382}$	66-100	$3.03C_l^{0.627}$
Field method (Heng, 1991)				
	0-200	$3.03C_l^{0.627}$	100-200	$3.03C_l^{0.627}$

1 C_s = extractable soil S (kg S ha^{-1} mm^{-1} soil depth)
C_l = solution $SO_4^=$ concentration (kg S ha^{-1} mm^{-1} soil depth)

a and b = empirical constants

8.3.3.2 Accounting for leaching of sulphate

8.3.3.2.1 Layered drainage model with mobile and immobile water phases.

Only a few mechanistic models have attempted to describe sulphate leaching from pastures (Heng, 1991; Sakadevan, 1991). The approach used in this study was that of Sakadevan (1991). As sulphate adsorption capacities for each soil depth (measured by Heng, 1991) increasing with depth and because, in this study, forms of soil S had been measured in three soil layers (0-33 mm, 33-66 mm and 66-100 mm, Chapter 5), a three layered leaching model was considered an appropriate way of dealing with the S sorption differences and the soil S data available.

The main assumption in this three layer model is that an input of water either rainfall or drainage from the layer above (I-1)(R, mm) moves into a layer I of the soil profile, once the soil water depth (W_i, mm) is equal to soil water depth at field capacity (W_{fc}, mm) the incoming water displaces an unknown proportion of native soil water in this layer in relation to the input volume (the condition when R exceeds W_{fc} is discussed later).

This assumption simulates the condition where some of the input water, $R\alpha$ mm (a fraction α) will move through larger pores interacting little with the native soil water, while in smaller capillaries native soil water will be displaced ahead of the incoming water, $R(1-\alpha)$ mm. A value for α of 0.3 was determined for Mahoenui silt loam (Sakadevan, 1991) by iteratively fitting a drainage model, similar to that discussed below, to the amounts of $SO_4^=$ leached from an undisturbed soil core subjected to simulated rainfall. In the absence of a fitted value of α for the Tokomaru silt loam, a value for α of 0.3 was used in this study.

The soil solution sulphate concentration ($C_{l,i}$ kg S mm^{-1} soil depth ha^{-1}) for a given amount of extractable sulphate ($E_{S,i}$) for i^{th} layer before input water passes through the layer is given by the equation

$$C_{l,i} = (E_{s,i} / a_i)^{(1/bi)} \qquad 8.5$$

where

$E_{S,i}$ = the amount of extractable sulphate (kg S ha^{-1}) present in i^{th} layer

a_i and b_i = coefficients of the Freundlich isotherms (empirical constant) explaining the relationship between extractable sulphate and the soil solution sulphate for the i^{th} layer.

The functions for all the three layers were given in Table 8.1. Prior to reaching field capacity (W_{fc} mm) for the layer i = 1 (i.e, $R_i + W_i \geq W_{fc}$) then the new soil solution concentration for layer i = 1 is given by the equation

$$C_{l,i} = ((E_{s,i} + I_{s,i} * R_i) / a_i)^{(1/bi)} \quad 8.6$$

where

$I_{s,i}$ = the concentration of sulphate (kg S mm^{-1}) in the incoming water.
R_i = the amount of input water to the i = 1 layer day^{-1}.

The remaining parameters have been described earlier. In the case of the surface soil layer (i = 1) the incoming water is rainfall and the concentration of S is negligible, except when fertilizer particles are present on the soil surface (see later discussion). Under such conditions the concentration of sulphate in the soil solution is the one given in equation 8.5 (i.e. the change in concentration of sulphate in the soil solution of layer i = 1 due to incoming rainwater is assumed to be negligible unless leaching occurs).

When the soil water depth of the i^{th} layer (W_i, mm) has reached W_{fc}, then the drainage volume (D_i, mm ***day-1***) is equal to the input water volume (R_i, mm day^{-1}) and the total amount of S lost by leaching ($L_{s,i}$, kg S ha^{-1} day^{-1}) from the i^{th} layer is sum of two components.

1. The amount of native soil solution sulphate displaced ($L_{d,i}$, kg S ha^{-1} day^{-1}) by the slow moving input volume water ($D_i(1-\alpha)$) entering the layer which is given by the equation.

$$L_{d,i} = [(E_{s,i} / a_i)^{(1/bi)}] * D_i(1-\alpha) \quad 8.7$$

2. The amount of S in the mobile fraction ($L_{m,i}$, kg S ha^{-1} day^{-1}) of the incoming water ($D_i\alpha$) which passes directly through the soil layer. When the input water passes rapidly through the surface layer without interacting with the native soil water its drainage path to lower soil depths is presumed to be un-impeded. Therefore this water will pass through the rest of the layers without any interaction with the native soil water. For the purposes of the model W_i, for the layers i = 2 and 3, is only increased by the water volume $D(1-\alpha)$ displaced from the i-1 layer. Also this means that drainage $D_{i=1}$ α occurs from the whole soil profile as soon as $R_{i=1} + W_{i=1} > W_{fc,i=1}$, irrespective of the $W_{i=2}$ and $W_{i=3}$.

The input water sulphate concentration ($I_{s,i}$) has have two distinct components: (a), the fast moving drainage water volume $D_i\alpha$ with S concentration $I_{s,}\alpha$, and (b), slow moving drainage

water $D_i(1-\alpha)$ with S concentration $I_{s(}1-\alpha)$. The amounts of S in the mobile leached volume would be given by the equation

$$L_{m,i} = I_{s,i} * \alpha * D_i \quad 8.8$$

Therefore

$$L_{s,i} = L_{d,i} + L_{m,i} \quad 8.9$$

In the case of rain water at the Tokomaru site which contains negligible sulphate, $I_{s,i}$ is zero then $L_{m,i}$ is equal to zero. At other sites closer to coast $I_{s,i}$ would require a positive value (Ledgard and Upsdell, 1991).

If the displaced drainage water volume ($D(1-\alpha)_{i-1} + W_i - W_{fc}$) is negative then $D(1-\alpha)_i = 0$ and no leaching occurs. i.e. $L_{s,i}$ is zero in equation 8.9. After leaching, the equilibrium between adsorbed and solution sulphate was allowed to establish prior to plant uptake.

The amount of extractable sulphate (kg S ha^{-1}) remaining in the i^{th} soil layer after an input event or a drainage event is given by the size of initial extractable sulphate pool, $E_{s,i}$ plus the amount of sulphate leached from the layer above ($L_{s,i-1}$) minus the sulphate leached from that layer ($L_{s,i}$).

$$E_{s,i(t+1)} = E_{s,i,t} + L_{s,(i-1)} - L_{s,i} \quad 8.10$$

where

$E_{s,i(t+1)}$ = the amount of extractable sulphate (kg S ha^{-1}) present after leaching.

The new concentration of sulphate in the solution is

$$C_{l,i(t+1)} = (E_{s,i(t=1)} / a_i)^{(1/bi)} \quad 8.11$$

where

t = a time (normally = 1 day) or volume step (see discussion later).

This is the solution concentration that is used to calculate plant uptake on a day when leaching occurs (Section 8.3.3.3).

8.3.3.3 Plant uptake of sulphur

The amount of S removed from each of the three layers depends upon plant uptake, immobilization and drainage. Drainage was considered to be immediate (instantaneous) relative to plant uptake (during periods of transpiration) and mineralization/immobilization (continuous processes). Therefore in this model leaching of S followed by the re-establishment of the equilibrium between adsorbed and solution S prior to plant uptake, immobilization and mineralization takes place.

The second step in the model process is the plant uptake of S from soil solution. The amount of any nutrient taken up by plant for a particular period of time is a function of the amount of water removed by the plant (Scotter *et al.*, 1979; Hayman and Stocker, 1982; McAneney and Judd, 1983; Rickard *et al.*, 1986; Martin, 1990) and the soils ability to supply the nutrient to the soil solution. The amount of water removed by plant can be considered to be the actual evapotranspiration (***AEP***) from pasture soils (see discussion 8.3.1.2). So, assuming that most sulphate moves to plant roots by mass flow (Barber, 1984) the amount of S removed by plant (P_S, kg S ha^{-1}) is proportional to the ***AEP*** (mm) multiplied by the soil solution sulphate concentration (C_l, kg S mm^{-1} ha^{-1}). This assumes that C_l in the Tokomaru soil never reaches concentrations higher than that which can be adsorbed by pasture roots. Even in campsites of very high S status, Sakadevan (1991) found that ***AEP*** * $C_{l,i}$ gave a good prediction of observed plant uptake.

The amount of water removed from each layer was calculated from daily ***AEP*** multiplied by the soil solution sulphate concentration (calculated from equations 8.5 or 8.6 or 8.11 in each layer) to give the amount of S removed from each layer. Initially it has been assumed in this model that all of the S removed by the plant was moved to the root zone by mass flow. Thus when drainage occurs from the soil, the amount of S removed from the particular layer by plant uptake will be lower than that removed without drainage.

Mathematically, the amount of S removed by plant from i^{th} layer is given by the equation

$$P_{s,i} = C_{l,i} * AEP_{t,i} \qquad 8.12$$

Where

$P_{s,i}$ = the amount of S (kg S ha^{-1} day^{-1}) removed by plant from the i^{th} layer,

$AEP_{t,i}$ = the volume of ***AEP*** removed from the i^{th} layer (mm day^{-1})

$C_{l,i}$ = discussed earlier or if drainage occurs $C_{l,i}$ is recalculated using equation 8.10 and 8.11.

As mentioned in Section 8.3.3.2; there exists an equilibrium between the soil solution sulphate concentration and adsorbed sulphate. As the soil solution sulphate is decreased by plant uptake, sulphate will be released into soil solution from the amount of sulphate on the surface

of the soil. This equilibrium was allowed to re-establish on a daily basis after mineralization and immobilization fluxes of S to and from the extractable S pool had also been accounted for. Uptake of S by roots was accounted for as part of below ground immobilization.

8.3.3.4 Accounting for immobilization and mineralization of soil organic sulphur

Immobilization of S into soil organic matter and its subsequent mineralization were considered to be continuous root and microbial processes which extract S from and input S into the extractable S (E_S) pool.

As reported in the literature review (Section 2.3) the net difference between these two processes is highly dependent on the C : S ratio of organic substrates available for soil micro-organism growth and the soil solution sulphate concentration. Other than experiments examining net $SO_4^=$ release or immobilization in laboratory studies, except that from experiment carried out by May and co-workers (May *et al.,* 1972; May *et al,.* 1973; Till, 1979) on dry land Australian pastures, little information is available on individual rates of immobilization and mineralization in field soils. Immobilization requires a source of carbon. The dominant sources of carbon in subsurface layers of field soils are plant roots. Thus the extent of immobilization of $SO_4^=$ in any soil layer was considered to be proportional to the sulphate uptake activity of roots in that layer which is already dependent upon soil moisture content, maximum/minimum temperatures, sunshine hours and root mass (which are embodied in the calculation of AEP_i). This can be conveniently expressed as:

$$\Delta\text{Immobilization} / \Delta t = K_{imm} * P_{s,i} \qquad 8.13$$

where

Δimmobilization	=	amount of S immobilized during time Δt
K_{imm}	=	an immobilization constant, unitless, proportionally constant
$P_{s,i}$	=	plant uptake from soil layer i per day as discussed earlier

Other authors, however, Gregg (1976) and May *et al.,* (1972, 1973) have noted that after addition of $SO_4^=$ to field soils, there was initial rapid immobilization of sulphate, indicating that above certain soil sulphate levels (C_{ak}) there is additional immobilization stimulated by high soil solution sulphate concentration. Thus equation 8.13 was expanded with a pre-condition this:

IF $C_{l,i}$ > $(C_{ak}) * K$ THEN 8.14

$K_{imm} = K_{imm} * ((C_{l,i} - C_{ak})$ 8.15

where

C_{ak} = threshold $E_{s,i}$ above which immobilization increases in proportion to the difference between the threshold C_{ak} and current E_s

Mineralization of soil organic S is the major process supplying S to pasture plants in aquic environments of the central north island, New Zealand. The development of a pasture index (PDI) to account for pasture S supply from organic S (Sinclair *et al.*, 1985) in fertilized pastures reflects this. The importance of mineralization in supplying both plant S and leached S in New Zealand, North Island hill pasture has been confirmed recently by Sakadevan (1991). Therefore it can be deduced that in a long established permanent pasture in this environment the extent of mineralization in each soil layer must be closely related to the root activity in each layer. Unlike immobilization, however, mineralization may not always be positively related to root S uptake because mineralization may be stimulated by lower soil $SO_4^=$ concentrations and inhibited by higher soil solution $SO_4^=$ levels increased through fertilizer addition. As discussed in Chapter 2, Section 2.3, Tabatabai and co-workers have shown that sulphatase enzyme levels are inhibited in the presence of high $SO_4^=$ concentrations. For the purpose of developing a preliminary model it was considered that mineralization rate was proportional to the soil organic S content (O_i, kg S soil layer ha^{-1}), the root mass, temperature and soil water availability (i.e. AEP_i) and inversely proportional to the soil sulphate concentration in each soil layer. This was expressed as:

$$\Delta\text{mineralization} / \Delta t = K_{min} * AEP_i * O_i / E_s \quad 8.16$$

where

K_{min} = a mineralization constant kg S ha^{-1} mm^{-1} day^{-1}
t = a length of time

At the end of each day (t = 1 day) the new amount of S (kg S soil layer ha^{-1}) in the extractable pool was calculated by subtracting P_s and Δimmobilization

8.3.3.5 Executing the model

The whole model was executed by writing a program in QuickBASIC (Microsoft Corporation, 1987) and executing the program on an IBM compatible personal computer. The input data required to run the model discussed above were those appropriate to the experimental conditions in Chapter 5, they include:

climatic data consisting of Julian day, daily rainfall (mm), daily maximum and minimum temperature and sunshine hours; initial soil water content data consisting of soil volumetric water contents (mm) at field capacity and initial soil volumetric water contents (mm) for each soil layer of depth z mm; the percentage distribution of plant roots in each soil layer and the total organic S (Table 5.3) and the initial $E_{s,i}$ value, i.e. calcium phosphate extractable S (freeze-dried preparation technique, Table 5.3 and 6.3A) in each soil layer (kg S ha^{-1} $layer^{-1}$); The Freundlich isotherm constant for the 'isotherms' describing the distribution of $SO_4^=$ between soil solution and soil surfaces (Table 8.1).

The mobile water volume α remained at 0.3, that determined by Sakadevan (1991) for Mahoenui silt loam, because there was insufficient time to conduct such experiments on undisturbed cores of Tokomaru silt loam soil.

8.4 RESULTS AND DISCUSSION

8.4.1 Prediction of plant S uptake on unfertilized soil

To simulate 150 days of S transformation, the model takes 15 seconds to run on an IBM compatible personal computer. Several iterations were conducted adjusting only K_{min} and K_{imm} values until the model accurately predicted the plant uptake on the unfertilized control plots described in Chapter 5 (Table 5.5) and gave what were considered acceptable readout for the changes in soil CaP-S levels (equivalent to E_s in the previous discussion) in all depths. The model output and observed plant S uptake values are compared in Figure 8.3A and 8.3B. The model gave a remarkably accurate prediction of changes in plant S uptake with time (Figure 8.3A). The CaP-S values (not presented) however could not be compared directly with those measured at 30 and 60 days (Table 5.12) which, unlike the initial soil test CaP-S (Appendix 5.3), had been determined on oven dried, not freeze-dried, soil. The model output was not very sensitive to small changes in α (±10%) so that the value α = 0.3 was retained. Plant S uptake and S leaching loss (data not shown) were dependent upon the relative size of K_{min} and K_{imm}. Changes in the difference between them rapidly increased or decreased the size of the CaP-S-pool, which influenced plant uptake. The final K_{min} and K_{imm} values selected to give the best fit of plant uptake indicated that actual rate of mineralization and immobilization were 1.5 to 2 fold greater than the rate of plant uptake from any particular soil depth.

8.4.2 Prediction of plant S uptake on superphosphate fertilized soil

To predict plant S uptake on fertilized soils required that a submodel be added which could predict the release of sulphate from superphosphate into soil. The non-mechanistic approach of McCaskill and Blair (1989) was considered but it was decided that a mechanistic approach may be more portable between soils.

8.4.2.1 Movement of sulphate from superphosphate into soil

Superphosphate (SSP) can be considered to contain gypsum, monocalcium phosphate, less soluble phosphates and minor impurities.

When a granule of SSP is applied to moist soil, moisture absorbed by the granule forms a fertilizer solution which is similar in Ca(1.3-1.5 M) and P(3.4-3.8 M) concentration to the metastable triple point solution (MTPS) when an excess of monocalcium phosphate is dissolved in water (Williams, 1971a). This solution has a pH between 1.6-1.8 and initial S concentration of 0.02-0.07 M. This concentration will remain higher than that of saturated solution of gypsum (0.014 M) while the pH of the fertilizer solution remains low. Sulphate will diffuse from this fertilizer solution into the soil and Williams (1971b) has observed the hemispherical nature of the sulphate concentration gradient below a SSP particle.

In order to model the rate and extent of $^{35}SO_4^=$ movement into soil cores fertilized with SSP in Chapter 5 this diffusive flux of $SO_4^=$ was simulated in the following manner:

Consider a SSP particle (12% S, density of ρ = 1900 mg cm^{-3} and radius r_n) sitting on a soil surface. The soil has a volumetric moisture content of θ at field capacity, a tortuosity (or impedance) factor (Nye and Tinker, 1977; Barber, 1984) of f and a soil sulphate buffer capacity $\delta C_S/\delta C_l$ (where $\delta C_S/\delta C_l = a * b * C_l^{(b-1)}$, where a and b are the coefficients of a Freundlich sorption isotherm representing the relationship between adsorbed soil sulphate (C_S) and the soil solution sulphate concentration (C_l). The equation describing the diffusive flux (F mg cm^2 s^{-1} of sulphate from the fertilizer solution at the particle surface across distance X cm to the soil solution can be written as:

$$F = D * \theta * f * \delta C_l / \delta C_x / [a * b * C_l^{(b-1)}] \qquad 8.17$$

where

$\delta C_l/C\delta_x$ = the concentration gradient of $SO_4^=$ between the fertilizer solution and the soil solution (mg cm^{-3} cm^{-1})

D = diffusion coefficient of sulphate in water $1 * 10^{-5}$ cm^2 s^{-1} (Barber, 1984)

For the purposes of simulation this relationship can be solved by using a finite difference method similar to that used by Kirk and Nye (1986) to model the dissolution and diffusion of P away from particles of dicalcium phosphate. In the case of the superphosphate particle (described above) it is considered to have its lower hemispherical surface ($2\pi r^2$) in contact with the soil water film. Diffusion of $SO_4^=$ away from the particle can be considered to occur in a number (n = 2 to 6) of distance steps through a series of hemispherical shells (volume $2/3\pi$-(r^3_{n+1} - r^3_n) where the width of each shell ($r_{(n+1)}$ - r_n) = dx. Each time step (*t*) a new concentration of sulphate per unit soil volume ($C_S + \theta * C_l$, mg cm^{-3}) and soil solution concentration (C_l) are calculated:

$$(C_s + \theta * C_l)_{L,\, t+1} = (C_s + \theta * C_l)_{L,t} + Flux_{(L-1,\, t)} - Flux_{(l,t)} \qquad 8.18$$

The finite difference solution of equations 8.17 and 8.18 were written in QuickBASIC programming language (Microsoft Corporation., 1987) and executed using IBM compatible personal computer. Time steps of 1 *day* and hemispherical shell widths of 0.1 cm were used with initial soil solution $SO_4^=$ concentrations of $7.5 * 10^{-4}$ mg S cm^{-3} and the fertilizer solution at the surface of a granule was 0.64 mg S cm^{-3} in the Tokomaru silt loam soil while $\theta = 0.45$, f = 0.3 and the Freundlich sulphate sorption isotherm is that of top soil layer calculated in Table 8.1. Each step, the flux of S into the soil was subtracted from the particle mass and a new radius calculated. Three simulations were conducted with particle radius ranging from 0.15 to 0.05 cm at an application rate of 30 kg S ha^{-1}. The simulation model indicated that particle radius would have a marked effect on the rate of movement of fertilizer into the soil by diffusion (Table 8.2). The most distant radii of the hemispherical shell of S penetration into soil are less than those observed by Williams (1971b) possibly because the sorption isotherm for the Tokomaru soil is steep in the region of soil solution concentration, $7.5 * 10^{-4}$ mg cm^{-3}.

Field observations conducted during the normal addition of fertilizer in field trials on winter wet Tokomaru soils indicated that 3 mm SSP granules disappeared in less than the 128 days indicated in Table 8.2 (M.J. Hedley, personal communication). Additional mechanisms explaining the disappearance of granules are coverage of granules by earthworm casts (Syers and Springett, 1984) and the impact of rainfall (McCaskill and Blair, 1989) on the granule itself. The effect of rainfall was simulated as follows:

Table 8.2 The simulated time taken for 30 kg S ha^{-1} to diffuse into Tokomaru silt loam and depth of sulphate movement (radius of outer hemispherical shell from center of granule).

Initial particle radius	Diffusion only		Rainfall plus diffusion
	Days taken for 30 kg S ha^{-1} to enter soil	Radius of final hemispherical[a] shell	Days taken for 30 kg S ha^{-1} to enter soil[b]
cm	days	cm	days
0.15	128	1.6	73
0.10	68	0.9	46
0.05	26	0.3	22

a = i.e. maximun soil depth penetrated.
b = distance travelled > 100 mm due to leaching.

A term was added to the diffusion model to estimate the volume of rain (cm^3) impacting on the remaining granule surface area (πr_n^2). The volume of rain was allowed to form a saturated solution of superphosphate S, concentration = 0.64 mg S cm^3, before passing into the soil by mass flow. The combined diffusive and mass flow flux of S leaving the granule accelerated the decrease in granule size (Table 8.2). If the soil was already at field capacity, a fraction (α, mobile volume discussed in Section 8.3.3.2.1) of the SSP solution derived from the rain was allowed to move freely through the soil as macropore flow to be included as leached sulphur. The addition of rainfall rapidly increased the rate of SSP dissolution and depth of entry into the soil (see model output Figure 8.4 to 8.7). The combined rainfall diffusion model was used to simulate the input of S into the surface layer (I = 1) of the SSP fertilized soil.

8.4.2.2 Prediction of plant uptake on SSP fertilized plots

The rainfall diffusion submodel was added to the simulation model discussed in Section 8.4.2.1 and the simulation model was executed using the same parameters described previously. The simulated entry of fertilizer S into the soil (Figure 8.4) indicates how the onset of rain rapidly accelerates fertilizer dissolution and movement. The comparison of plant S uptake and CaP-S in each soil layer on the SSP fertilized cores with the observed values are presented in Figures 8.3B and 8.5. The prediction of actual plant S uptake was very good (1:1 r^2 = 99.7) but prediction of CaP-S concentrations during the period of the trial were less accurate (Figure

8.5), in particular, observed CaP-S concentration during the first 30 days were double those that could be predicted by the model. Conversely after 60 days while there was a reasonable prediction of CaP-S level in the top 0-33 mm of soil there was overestimation at lower depths. The model-predicted CaP-S levels were sensitive to major drainage events (cf. Figure 8.4 and 8.5), reflecting leaching of S, particularly severe just after 120 days from fertilizer application. Field observations by Ghani *et al.,* 1990 also showed that CaP-S decreased markedly after recent rainfall. The total accumulated leaching loss 21 kg S ha^{-1} (Figure 8.4) compared favorably with leaching losses of S (8-70% of that applied annually) that occur from hill country pastures in the North Island of New Zealand (Saggar *et al.,* 1990a, 1990b). This was equivalent to an average drainage water sulphate concentration of approximately 12 ppm S which is within the range (6-14 ppm S) measured by Heng *et al.*, (1991) for SSP fertilized paddocks on a Tokomaru soil

8.4.3 Prediction of ^{35}S movement and transformation in undisturbed soil cores

One main objective of constructing this model was to overcome the difficulties that are normally encountered in predicting the fate of isotopic tracers in open systems where the rate and intensity of isotope loss is haphazard and limits the interpretation of ^{35}S isotope to determine actual rates of soil organic S mineralization. The movement of isotope into soil was simulated in the model by using the rainfall/diffusion simulation of SSP input into layer $I = 1$ to add ^{35}S at a rate proportional to the amount of S released (i.e. S release rate multiplied by SSP ^{35}S specific activity). The specific activity of fertilizer ^{35}S released into the soil was immediately diluted by the amount of CaP-S present in layer $I = 1$. This is to simulate plant uptake of non-labelled and labelled S from the soil layers and to accommodate the fact that soils were sampled in 33 mm layers and thoroughly mixed prior to analysis. It is not expected that ^{35}S would normally mix with all the CaP-S in field soil without the soil being artificially mixed. Leaching, plant uptake and immobilization removed ^{35}S from this pool in proportion to the amount of S removed multiplied by the CaP-S ^{35}S specific activity. Leached ^{35}S entered the next layer $I = 2$ (3 or 4) where the process of mixing and removal were repeated. Immobilized S and ^{35}S in each layer entered the organic S pool in each soil layer. The specific activity of mineralized organic S was simulated by calculating a running total of the S and ^{35}S in the organic pool. The predicted and observed percentages of added isotope present in soil CaP-S fractions, plant S and soil organic S are presented in Figures 8.6-8.8.

Whereas the model simulates well the percentages of added ^{35}S remaining in the CaP-S fractions from all soil depths. The model seriously over predicts plant ^{35}S uptake and the percentage of added ^{35}S remaining in the top soil as organic S. The main reason for this is

that the current parameters of the model fail to predict the large observed loss of isotope from the soil cores during the first 30 days (Appendix 5.4). Although the model predicted that drainage occurred at 11, 12 and 23 days, leaching was insufficient to remove large amounts of ^{35}S from the core. Irrespective of increasing α to accommodate larger leaching loss of fertilizer solution on days when rainfall caused leaching, it was not possible to simulate such large losses of isotope by 30 days.

The relative distribution of ^{35}S between soil and plant S forms predicted by the model were ranked in the same order as the observed values for variation between forms and soil depths (i.e. observed and predicted ^{35}S recovery was the greatest in soil organic matter > plant > CaP-S and the end of the first 30 days and throughout the remainder of the experiment and also organic S in the top soil layers was greater than the middle and lower soil depths). This comparative similarity between observed and predicted ^{35}S distribution gives some support to using root activity as a modifier of mineralization and immobilization rates to describe the relative extent of these processes in different soil depths.

The large amounts of unaccounted for ^{35}S activity at 30 days (Appendix 5.4) presumed leached remain a problem in evaluating the model. Uncertainty concerning the actual fate or cause of isotope loss (leaching or lateral movement from the core) means that confidence cannot be placed in adjusting fertilizer release rate (or rainfall solution rate of SSP) and leaching parameters to account for this loss. The data in Chapter 7 concerning the fate of $^{35}S^{o}$ applied to the Tokomaru soil provide a set of data in which most added $^{35}S^{o}$ was accounted for and could be used to further test the simulation model. A sub model predicting $^{35}SO_4^{=}$ release rates from oxidizing S^{o} developed by Chatupote (1991) could be used to simulate ^{35}S input into soil layer *l*. Unfortunately time has not allowed this to be completed prior to submitting this thesis.

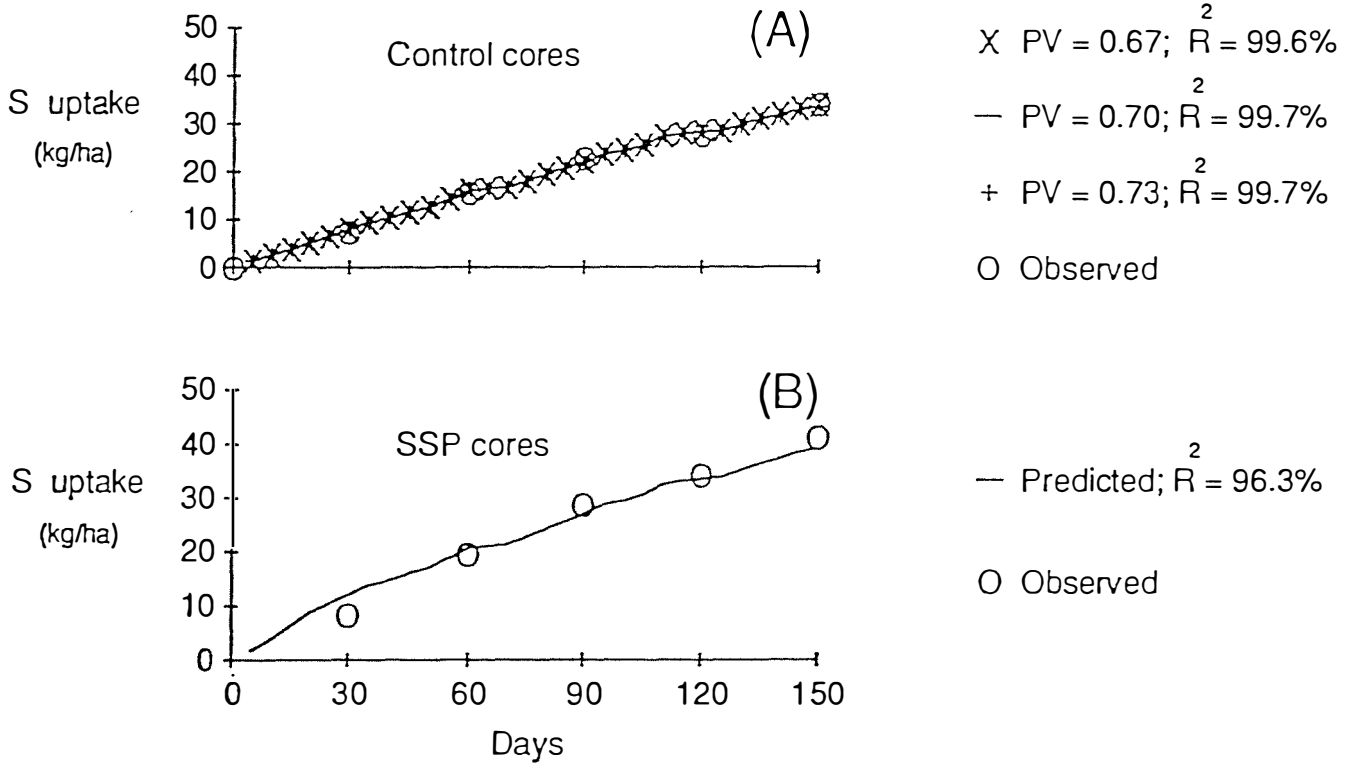


Figure 8.3 Comparison of predicted and observed plant S uptake on (A) control (unfertilized) and (B) SSP fertilized soil cores (experimental details Chapter 5). The coefficient of determination (R^2) represents the variation in observed data accounted for by the model prediction (PV stands for the less mobile fraction of soil water; 1-α).

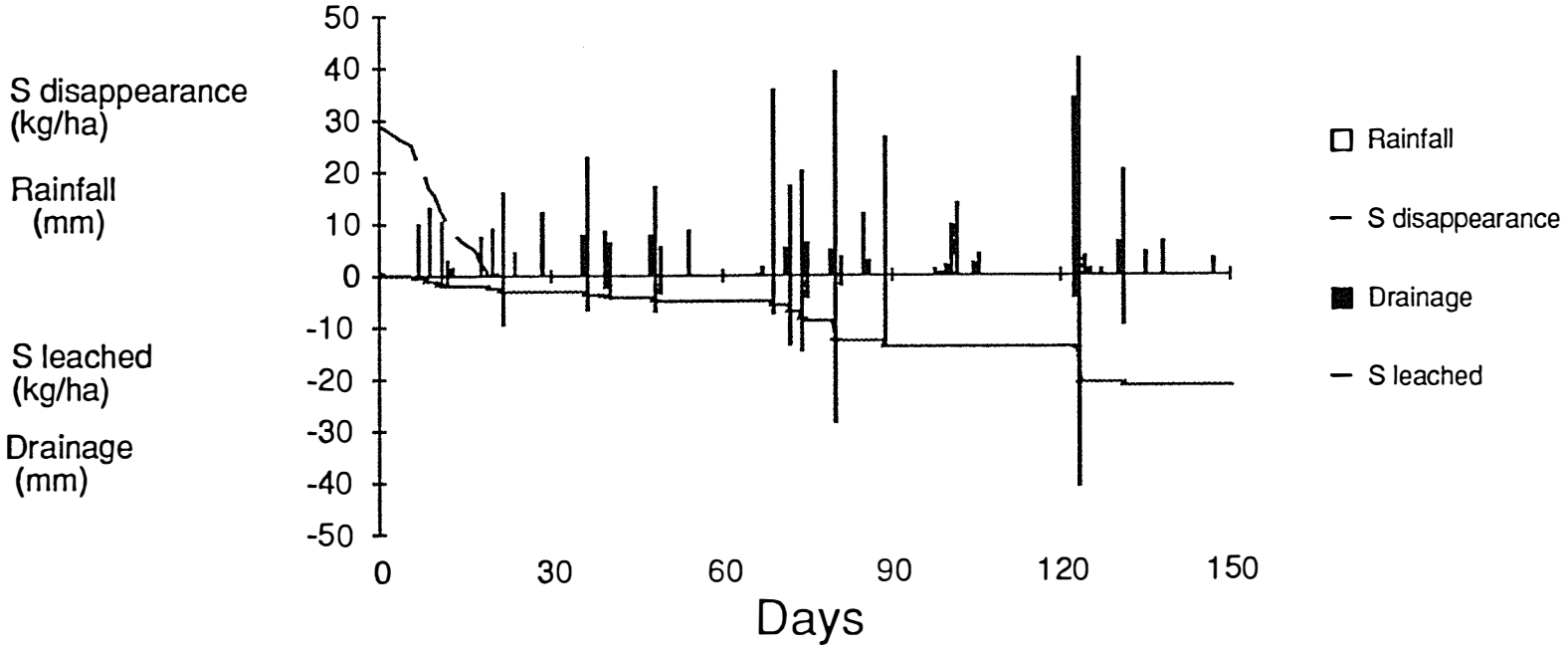


Figure 8.4 The measured rainfall and predicted drainage occurring from the top 100 mm of Tokomaru soil during the experiment conducted in Chapter 5. The dotted line shows the disappearance of surface applied superphosphate (30 kg S ha^{-1}) as it dissolves and moves into soil. The solid line shows the predicted accumulated leaching loss of S from top 100 mm.

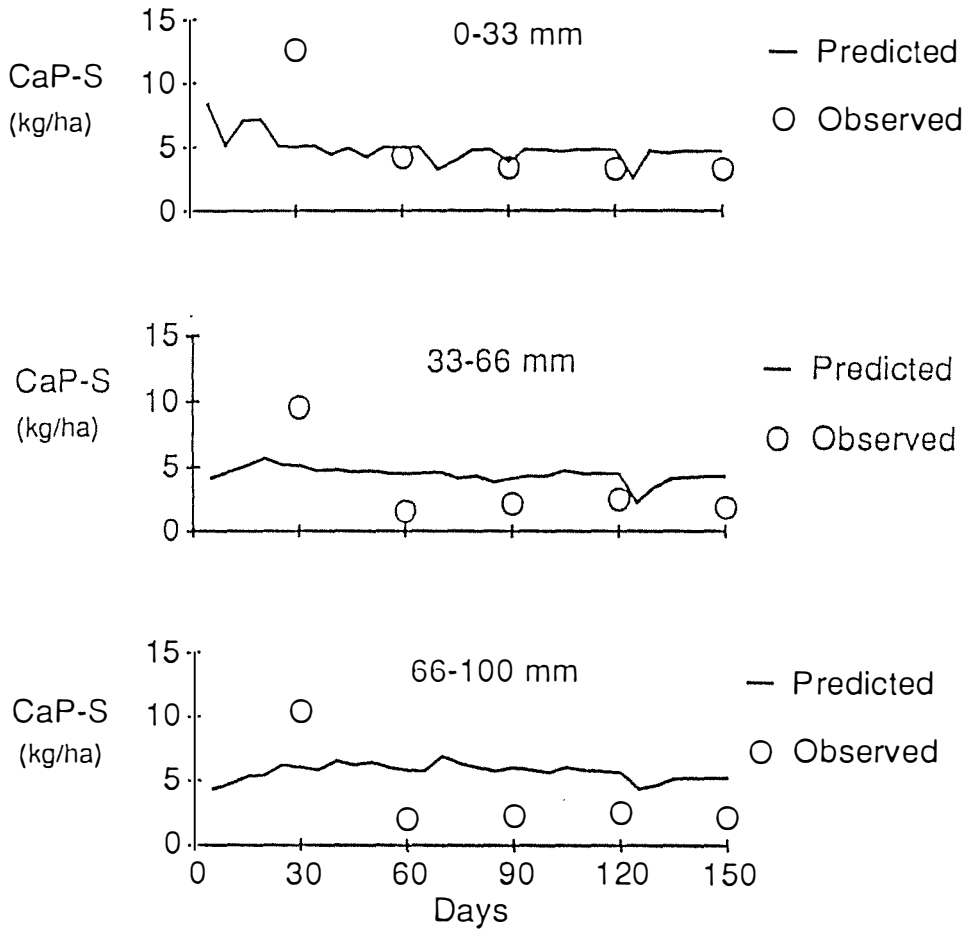


Figure 8.5 Comparison of predicted (output every 5 days) and observed amounts of CaP-S in each soil depth in SSP fertilized cores.

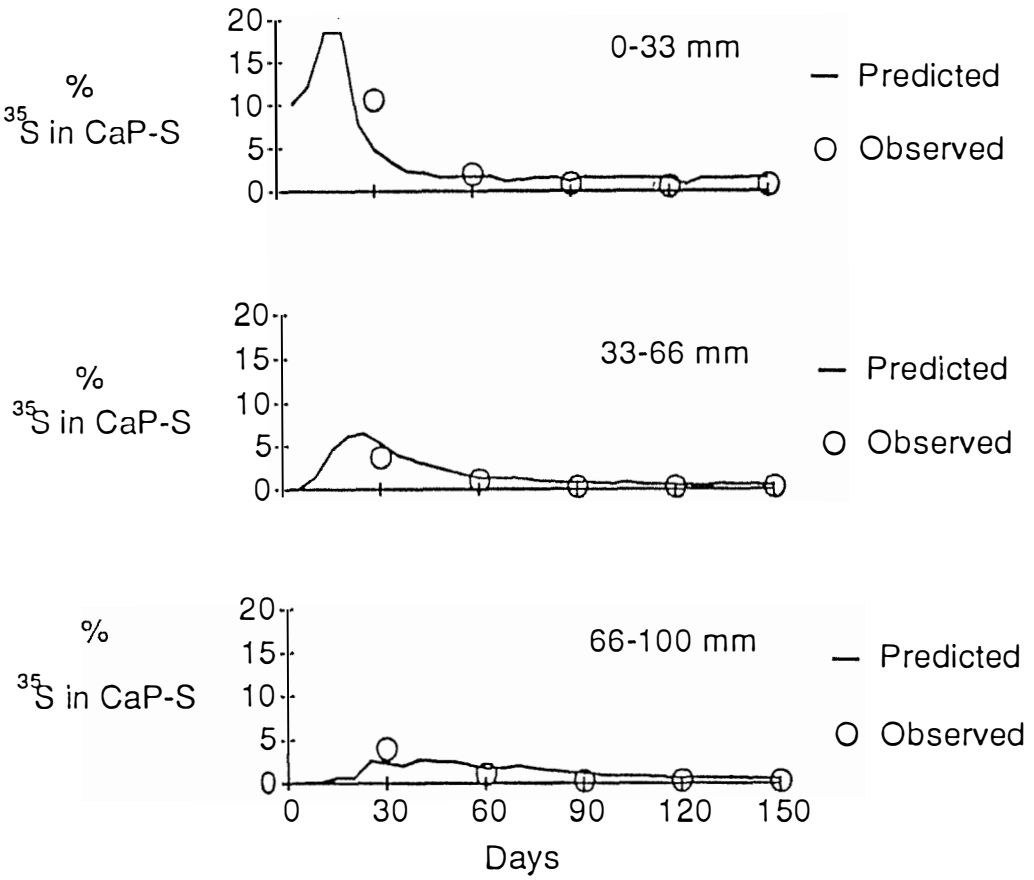


Figure 8.6 Comparison of predicted and observed percentages of added ^{35}S recovered in CaP-S fractions from different soil depths.

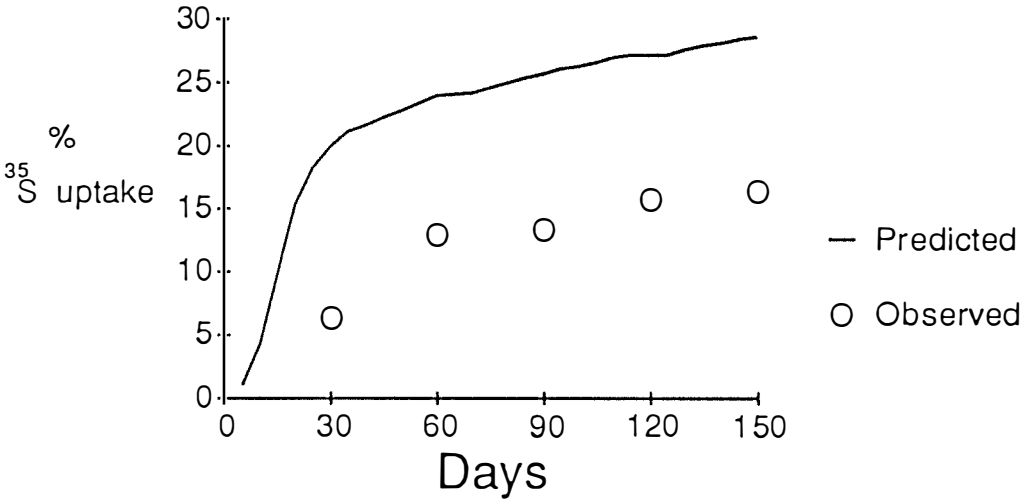


Figure 8.7 Comparison of predicted (output every 5 days) and observed percentage of added ^{35}S recovered in pasture plants.

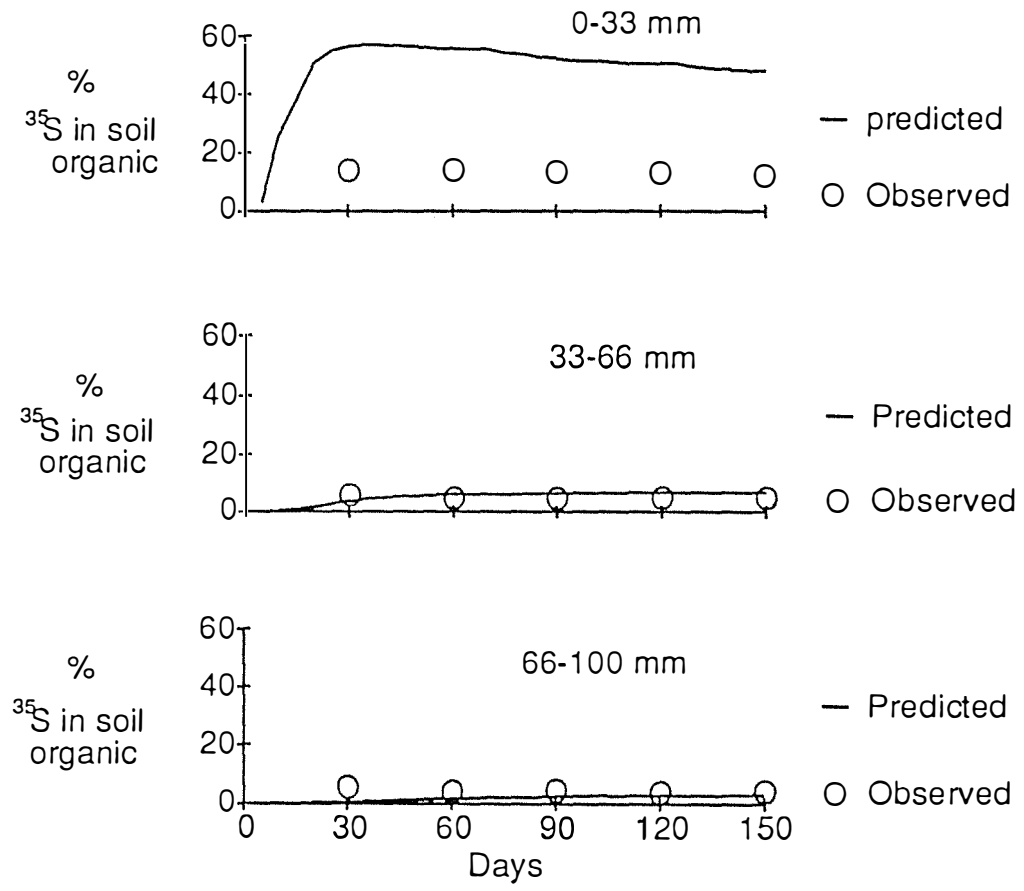


Figure 8.8 Comparison of predicted and observed percentage of added ^{35}S recovered as organic S in different soil depths.

8.5 CONCLUSIONS

The simulation model provided a very accurate method of predicting plant uptake of S from both S fertilized and unfertilized soil cores over 150 days of plant growth. Due to the lack of quantitative information on factors influencing plant uptake, immobilization and mineralization rates of S in field soils many unsubstantiated assumptions were made to construct the simulation model developed in this Chapter. If all the assumptions made to construct the model are true the model indicates that, on an average, actual rates of mineralization and immobilization exceed root uptake of S by 1.5 to 2 fold (mg S turned over per unit of S taken up by plants).

The model was less accurate at predicting the changes in the CaP-S extractable pool size and was relatively inaccurate at predicting the transformations of ^{35}S tracer added to the soil as SSP. The observed relative distribution of ^{35}S between organic soil S and CaP-S at different soil depths and plant S forms and the relative distribution predicted by the model were similar, which gave support to the concept of using root activity as a modifier of plant uptake, and organic S turnover at different soil depths. This concept may be useful in other studies on the fate of nutrients in pasture systems. There was great uncertainty, however, concerning the quantity of ^{35}S tracer data to test the model because of the large unaccounted for losses of ^{35}S which had been presumed to result from leaching. The model fail to predicted large leaching losses in the first 30 days.

It was not possible, therefore, to test with any confidence many of the assumptions made in constructing the model. Output from the current model suggested that, because of the small initial distance that S will move into soils from SSP or fertilizer in general, emphasis on the study of S transformation processes particularly immobilization and mineralization should concentrate on studying narrow horizontal soil slices close to the soil surface (a point also raised by Till, 1979). Various outputs from this model are sufficiently close to observed values that further research should be undertaken to test its various assumptions.

In the current model the role of litter return in immobilization of S at the soil surface was not considered. To complete the cycle observations on litter and the return of grazing animal dung and urine detailed by other authors (Gillingham, 1980; Gillingham *et al.,* 1980; Boswell, 1983; Saggar *et al.,* 1990a, 1990b) could be added in order to test the ability of the model to predict plant S uptake over longer periods of time and under grazing conditions.

CHAPTER 9

SUMMARY

9.1 REVIEW OF LITERATURE

Sulphur is an important major nutrient, particularly important in the nutrition of legume-based pastures. During the S cycle, highly mobile sulphate is formed which is susceptible to leaching losses. Because of this, the abundance of S in grazed-pasture systems in aquic environments decreases with time until pasture production becomes limited by S deficiency. Sulphur fertilizers are, therefore, required to maintain pasture productivity.

The persistence of plant available soil S in pasture grazed by sheep is influenced by several factors. These include: the rate and form of fertilizer input, pasture growth rate and its S status, the amount of pasture consumed by animals, uneven returns of S in dung and urine, losses from the removal of animal products, rainfall, surface run-off, sulphate retention characteristics of soil and leaching, and the extent to which the applied fertilizer S is immobilized into soil organic S reserves.

New Zealand's traditional pasture fertilizer for S and P has been superphosphate (SSP). Recent studies have shown large leaching losses of S from SSP fertilized pastures. Sulphur fertilizer applied as elemental S (S^0) is non-water soluble and is a slow $SO_4^=$ release form that has been shown to be more efficiently used by plants than $SO_4^=$-based fertilizers in soil environments where leaching is severe. Whereas this slow-release mechanism may reduce S leaching loss from S^0 fertilizer in the short-term, it is unclear whether reduced S leaching can be achieved in the long term because S leaching is a function of the rate of S cycling which is a function of pasture productivity (i.e. stocking rate). Knowledge of the comparative fate of sulphate and S^0 based fertilizers in soils is required to formulate future fertilizer application strategies.

This study was undertaken with the main objective of tracing the short-term fate of both S^0 and sulphate-based fertilizers applied to undisturbed grazed pasture soil cores. A method for radioactively labelling fertilizer sulphur was developed. Different $^{35}S^0$ particle size diameters, ranging from microfine particles of less than 0.010 mm to particles of 0.500 mm, were employed in a study in conjunction with the sulphate-based fertilizers, gypsum and superphosphate. Transformations of these products were studied in a series of field and

glasshouse trials using undisturbed cores of pasture soil. The effect of sheep dung on the short term immobilization of soil and fertilizer S was also measured. Finally, a simple computer simulation model was constructed to explain the fate of fertilizer $^{35}SO_4^=$ in pasture soil.

9.2 LABELLING TECHNIQUES

Methods for manufacturing radioactively labelled (^{35}S) sulphur fertilizers (S^o of different particle size, SSP and gypsum) were developed. The level of enrichment of fertilizers with ^{35}S activity was based on concepts regarding the fate of S in the plant and soil system being studied. These included dilution of the label by soil and herbage S, the sensitivity of detection of ^{35}S, sample size and the amount of fertilizer needed. These techniques, which do not involve using any specialized equipment other than a small Geiger counter monitoring for safety, gave fertilizer materials which were uniformly labelled with more than 95% of the radioactive ^{35}S recovered in the fertilizers.

9.3 THE EFFECT OF SHEEP DUNG ON THE SHORT-TERM IMMOBILIZATION OF SOIL AND FERTILIZER S

A field study showed that in a short term (from June 27 to October 30, 1985) the pasture dry matter yield in the area directly adjacent to the dung was insignificantly influenced by the nutrients P and S contained in the dung. This result was influenced by the high soil fertility of the experimental sites which historically, had been well maintained with fertilization to ensure an optimum pasture production. Very small amounts of plant P and S (about 2-5%), within 10 cm of the dung, were derived from the dung. The majority of plant P and S were derived from soil sources, a large part of which will be derived from slowly mineralizing dung that has accumulated with time. Furthermore, the presence of the dung did not significantly influence the immobilization of soil or fertilizer derived phosphate and sulphate.

This study confirms findings of others that the short-term effect of dung on nutrient availability was small. Therefore, the role of dung is one of a slow release component as it is decomposed by soil organisms and is converted into soil humus which in turn continues to release S over a longer period of time.

It was concluded that in short-term (up to one year) field trials examining the fate of fertilizer S in well developed permanent pastures, it is not necessary to apply sheep dung to experimental

plots in order to simulate the effect of dung return because probably this will have little effect on the size of the 'bank' of decomposing dung and soil humus in pasture soils. For long term (several years) experiments on permanent or less well developed pasture where soil fertility is very low, animal dung should be deposited to maintain soil organic S reserves.

9.4 THE FATE OF S FROM S^o AND SSP IN SOIL

For a period of 150 days after applying ^{35}S labelled microfine S^o and SSP, to separate undisturbed cores of pasture soil, the transformation and movement of ^{35}S to plant and soil S forms was monitored. During the first 30 days of the experiment, there were large unaccounted for losses of ^{35}S from all soil cores. At the time this was presumed to result from leaching. A drainage model developed in Chapter 8, however, suggested that there was insufficient drainage to generate such a large leaching loss. If it is assumed that the unaccounted for losses of ^{35}S were due to leaching then leaching losses of S beyond the 10 cm soil layer were larger in the SSP treated cores. The large unaccounted for loss of ^{35}S, however, reduced the confidence of using the measured transformation of ^{35}S to calculate amounts of soil and fertilizer S transformed into plant and soil S fractions. Despite this large loss of isotope, clear trends in the relative rates of transformation of ^{35}S added as SSP or S^o were evident.

Within 30 days there was rapid oxidation of the microfine S^o to calcium phosphate extractable sulphur and movement of ^{35}S from both S^o and SSP to plants and deeper soil depths. During 150 days, plant uptake accounted for 13.6% of the S^o and 16.3% of the SSP. Incorporation of ^{35}S labelled fertilizer S into the soil organic fraction was the process that conserved the largest amounts (22-40%) of ^{35}S labelled fertilizer in the soil. Notably in all experiments the major organic S form labelled with ^{35}S was carbon-bonded S, particularly in upper soil zones. More of the microfine $^{35}S^o$ was transformed into the organic S than ^{35}S from SSP, indicating that more soil organic S reserves may be formed through the use of S^o fertilizer rather than SSP. Such an indication has not been reported previously.

An inverse dilution technique where undisturbed soil cores were uniformly labelled (rather than the fertilizer) indicated that carbon-bonded S was likely to be the source of mineralized organic S. Both results from the inverse dilution and labelled fertilizer techniques were consistent in this respect.

In terms of plant S uptake, the microfine S^o was initially slightly less effective than SSP. Over a longer period (90-150 days), however, both fertilizers showed a similar performance,

reflecting the larger conservation of ^{35}S in the root zone of the S^0 treated plot. One problem with the rapidly oxidizing microfine S^0 was that apparent losses of S from the plant-soil system were only slightly lower than that from SSP.

9.5 MEASUREMENT OF PLANT AVAILABLE SOIL SULPHUR

Using soil samples, labelled with different forms of ^{35}S (derived from the experiment described in Chapter 5), a series of studies were undertaken to evaluate soil preparation and extraction techniques. The objective was to find a technique where the specific activity of S in the soil extract was the same as S taken up by pasture. The initial concept applied was that if the extract : plant ^{35}S specific activity ratio was close to unity then the extract and the plant must draw S from a similar biologically active pool.

Similar ^{35}S specific activity does not confirm, however, that the S in the extract is the precursor of S taken up by plants. Confirmation that CaP quantitatively extracts plant available S requires a quantitative assessment of plant S uptake relative to depletion of CaP-S pools. The CaP-S pool is dynamic and requires a consideration of all factors influencing its magnitude.

Of the two soil preparation techniques (extraction of moist-crumbled soil or freeze-dried and ground soil) the average ^{35}S specific activity in a CaP-S extract from a freeze-dried sample of the top 6 cm of pasture soil was most closely related to that of S taken up by plants over several periods of plant growth. Calcium chloride (CaCl-S) extracts from freeze-dried soil and CaCl-S and CaP-S extracts from moist soils had ^{35}S specific activities that were generally higher than that of S taken up by plants. Freeze-drying and grinding caused significantly more S, but not ^{35}S, to be extracted from soil samples. Therefore, freeze-drying exposes a form of soil S to extraction that was not freely exchangeable with added sulphate-^{35}S even during 150 days of plant growth.

Results indicated that plant roots had access to forms of soil S that could not be accessed by soil water and exchangeable sulphate ions (i.e. extracted by $CaCl_2$ from moist soil) during 150 days of plant growth. Such forms are likely to be: (a) organic S that is mineralized during plant growth or (b) aggregate-protected, adsorbed sulphate ($SO_4^=$), which becomes accessible to the penetrating roots or root hairs during plant growth but not to $CaCl_2$ extraction of moist soil.

9.6 INFLUENCE OF FERTILIZER FORM ON THE FATE OF S IN SOIL

The influence of S^o particle size and granulation of S^o with and without phosphate rock on the transformations of S in soil were studied using undisturbed soil cores and ^{35}S labelled fertilizer. In addition, the relative fate of S from SSP and gypsum was also studied. Some of the soil cores were removed to a glasshouse where controlled simulated rainfall events were applied. Both glasshouse and field studies continued for 180 days. As other authors have observed, there was a marked decreased in the rate of S^o oxidation as S^o particle diameter increased from <0.150 mm to 0.150-0.250 to 250-500 mm. These findings also indicated that the efficiency of plant use of S^o can be improved by decreasing S^o particle size.

Most of the $^{35}SO_4^=$ oxidized from S^o remained in the top 33 mm of soil and was transformed into soil organic S. Negligible $^{35}SO_4^=$ was leached from glasshouse or field cores which contrasted with the observations in Chapter 5.

Granulation of the finest S^o particle size (<0.150 mm) with or without phosphate rock had little practical longer-term effect on its rate of oxidation or fate in field or glasshouse soils. This indicated that provided granules can disperse into their component particles, granular S^o products should be acceptable for agronomic use.

Under the simulated environmental conditions of the glasshouse study, plants took up more fertilizer S from gypsum and SSP than from fine S^o (<0.150 mm particle size). The fate in soil of the S derived from either gypsum, SSP or fine S^o were essentially similar, with most fertilizer S being converted to soil organic S. There was marginally more leaching of S to the 6-10 cm soil depths with the sulphate-based fertilizers.

The main indication from this study and that conducted in Chapter 5 is that unless severe leaching occurs immediately after the application of sulphate-based fertilizers then the fate in soil of the S applied either as sulphate or S^o will be similar. The major fate pathway will be incorporation into soil organic matter. In the short term, plant uptake of fertilizer S will be greater, the more quickly the fertilizer becomes soluble, providing of course that conditions are suitable for plant growth.

9.7 MODELLING THE SHORT-TERM FATE OF FERTILIZER S IN SOIL

A preliminary mechanistic computer simulation model was modified to described S transformations in the soil-plant system of the pasture studied. It provided a very accurate

method of predicting plant uptake of S from both $SO_4^=$-S fertilized and unfertilized soil cores. If all the assumptions made to construct the model are true the model indicated that on average actual rates of mineralization and immobilization exceed root uptake of S by 1.5 to 2 fold (mg S turned over per unit of S taken up by plants).

The assumptions made in the model were to be tested by attempting to predict the redistribution of fertilizer ^{35}S between plant and soil S forms using the measured data from Chapter 5. There was only a relative similarity between observed and predicted proportional ^{35}S distribution between soil and plant S forms. The model was relatively inaccurate at predicting the actual amounts of isotope present in each form and could not simulate the large loss of isotope that had previously (Chapter 5) been attributed to leaching. It was not possible, therefore, to verify any of the assumptions made in the model or the indicated rate of mineralization. The relative similarity between observed and predicted ^{35}S proportional distribution between soil S forms gives some support to using root activity as a modifier of mineralization and immobilization rates to describe the extent of this process in different soil depths.

9.8 SUGGESTIONS FOR FURTHER RESEARCH

As S is a much more mobile nutrient in soil than P, understanding S transformations in soil remains the key to understanding the sustainability of New Zealand legume-based pasture systems.

Techniques need to be developed to overcome the variability inherent in analyzing mixtures of particulate fertilizers and soils, particularly, measurements of total S and residual S^o. The inability to remove this variability in the experiments discussed above has limited the interpretation of the results. A sequential extraction procedure may be appropriate for determining the S^o, CaP-S, total S and organic S on the same soil sample. In this study, CaP-extracts of freeze-dried soil reflected the same S transformations (same time dependent-^{35}S specific activity) as S taken up by plants, however, more studies of undisturbed soils are required to determine the actual size of the labile S pool which CaP and plants sample.

Results suggest that the climate differences (temperature and soil moisture content) between glasshouse and field soil cores had parallel effects on S^o oxidation, mineralization of soil organic S and plant S uptake as the ratio at which soil S and $^{35}S^o$ were made available stayed constant. This effect needs investigating further because they indicate that glasshouse

studies, using undisturbed soil cores, may be directly relevant to field conditions when a fertilizer with biologically controlled release rate (S^0 oxidation) is being evaluated.

Further research on field transformations (incorporation into soil organic S and leaching) of applied fertilizer S should focus on changes in these processes during the first 30 days after fertilizer application because much transformation of the applied S into soil organic S mainly occurred during this period. Studies should be undertaken in soils of different fertility status, soil textures and rainfall. Furthermore, unless leaching is severe, studies should be concentrated separately on the top 0-30 mm soil layer, rather than inclusion of soil at deeper depths, because the observed rates of ^{35}S immobilization indicated high biological turnover of nutrients in this zone. This will result in a better estimation of the rate of immobilization of applied S fertilizers.

Many assumptions made in the short term fate model need further clarification. The relationships between soil solution S and extractable S need further investigation to test and refine the model, however, modelling plant uptake of S by evapotranspiration appears to accurately describe temporal changes in actual plant uptake both in this study and that of Sakadevan (1991). Care should be taken in soils of low S status where $SO_4^=$ diffusion to root may be more important than in soils of high S status.

The importance of litter and dung return needs investigation and could be used to modify the model in order to test its ability to predict plant S uptake over longer periods of time.

BIBLIOGRAPHY

Al-Khafaji, A.A. and Tabatabai, M.A. 1979. Effect of trace elements on arylsulfatase activity in soil. *Soil Science* 127: 129-133.

Alexander, M. 1977. *Introduction to Soil Microbiology*. John Wiley & Sons Inc., London.

Andrew, C.S. 1975. Evaluation of plant and soil sulphur tests in Australia. In *Sulphur in Australasian Agriculture* (Ed. by K.D. McLachlan). Sydney University Press, pp 196-200.

A.O.A.C., 1984. Sulfur in fertilizers. Association of Analytical Chemists, 14th edition, AOAC Inc., Arlington, Verginia, pp 32-34.

Asher, C., Blamey, F. and Mamaril, C.P. 1983. Sulfur nutrition of tropical annual crops. In *Sulphur in South East Asian and South Pacific Agriculture* (Ed. G.J. Blair), pp 54-64.

Attoe, O.J. and Olson, R.A. 1966. Factors affecting rate of oxidation in soils of elemental sulfur and that added in rock phosphate-sulfur fusions. *Soil Science* 101: 317-325.

Autry, A.R. and Fitzgerald, J.W. 1991. Potential for organic sulfur accumulation in a variety of forest soils in saturating sulfate concentration. *Biology and Fertility of Soils* 10: 281-284.

Aylmore, L.A.G. and Karim, M. 1968. Leaching of fertilizer ions in soil columns. Transactions of 9th International Congress of Soil Science. 1: 143-155.

Barber, S.A. 1962. A diffusion and mass-flow concept of soil nutrient availability. *Soil Science* 93: 39-49.

Barber, S.A. 1984. *Soil Nutrient Bioavailability, A mechanistic approach.* John Wiley and Sons. 398 p.

Barber, S.A., Walker, J.M. and Vassey, E.H. 1963. Mechanisms for the movement of plant nutrients from soil and fertilizer to plant root. *Journal of Agricultural and Food Chemistry* 11: 204-207.

Bardsley, C.D. and Kilmer, V.J. 1963. Sulfur supply of soils and crop yields in Southeastern United States. *Soil Science Society of America Proceedings* 27: 197-199.

Barley, K.P. 1964. Earthworms and decay of plant litter and dung: A review. *Proceedings of the Australian Society of Animal Production* 5: 236-240.

Barrow, N.J. 1960a. The effect of varying the nitrogen, sulphur, and phosphorus content of organic matter on its decomposition. *Australian Journal of Agricultural Research* 11: 317-330.

Barrow, N.J. 1960b. Stimulated decomposition of soil organic matter during the decomposition of added organic material. *Australian Journal Agricultural Research* 11: 331-338.

Barrow, N.J. 1960c. A comparison of the mineralization of nitrogen and of sulphur from decomposing organic materials. *Australian Journal of Agricultural Research* 11: 960-969.

Barrow, N.J. 1961a. Studies on mineralization of sulphur from soil organic matter. *Australian Journal of Agricultural Science* 12: 306-319.

Barrow, N.J. 1961b. Mineralization of nitrogen and sulphur from sheep faeces. *Australian Journal of Agricultural Research* 12: 644-650.

Barrow, N.J. 1967a. Studies on extraction and availability to plants of adsorbed plus soluble sulfate. *Soil Science* 104: 242-249.

Barrow, N.J. 1967b. Some investigation of the effect of grazing on the nutrition of pasture. *The Journal of Australian Institute of Agricultural Science* 33: 254-262.

Barrow, N.J. 1968. Determination of elemental sulphur in soils. *Journal of Science, Food and Agriculture.* 19: 454-456.

Barrow, N.J. 1969a. Effects of adsorption of sulfate by soils on the amount of sulfate present and its availability to plants. *Soil Science* 108: 193-201.

Barrow, N.J. 1969b. The accumulation of soil organic matter under pasture and its effect on soil properties. *Australian Journal of Experimental Agriculture and Animal Husbandry* 9: 437-444.

Barrow, N.J. 1970. Sulphate adsorption by soil as a determinant of sulphur status and methods of supplying sulphur on non-adsorbing soils. Proceedings of 11th International Grassland Congress. 370-373.

Barrow, N.J. 1975. Reaction of fertilizer sulphate in soils. In *Sulphur in Australasian Agriculture.* (Ed. K.D. McLachlan) Sydney University press, pp 50-54.

Barrow, N.J. 1985. Reaction of anions and cations with variable-charge soils. *Advance in Agronomy* 38: 183-230.

Barrow, N.J. and Lambourne, L.J. 1962. Partitioning of excreted nitrogen, sulphur and phosphorus between the faeces and urine of sheep being fed pasture. *Australian Journal of Agricultural Research* 12: 461-471.

Barrow, N.J., Spencer, K. and McArthur, W.M. 1969. Effect of rainfall and parent material on the ability of soils to adsorb sulfate. *Soil Science* 108: 120-126.

Bates, C.J. 1971. Factor affecting critical nutrient concentrations in plants and their evaluation - A review. *Soil Science* 122: 116-130.

Beaton, J.D. 1987. Desirable characteristics of sulphur containing fertilizers suitable for Asia and the Pacific region. *Proceeding of the Symposium on Fertilizer Sulphur Requirements and Sources in Developing Countries of Asia and the Pacific*, Bangkok, pp 100-111.

Beaton, J.D., Burns, G.R. and Platou, J. 1966. *Determination of sulphur in soil and plant material.* The Sulphur Institute. Technical Bulletin No. 14. 56 p.

Beaufils, E.R. 1973. Diagnosis and recommendation integrated system (DRIS). Soil Science, Bulletin No. 1: 1-132, University of Natal, Pietermaritzburg, South Africa.

Beckman Liquid Scintillation systems. Model LS 1801/3801/5801, Operating Manual.

Bettany, J.R., Saggar, S. and Stewart, J.W.B. 1980. Comparison of the amounts and forms of sulphur in soil organic matter fractions after 65 years of cultivation. *Soil Science Society of America Journal* 44: 70-75.

Bettany, J.R., Stewart, J.W.B. and Halstead, E.H. 1973. Sulfur fractions and carbon, nitrogen and sulfur relationships in grassland, forest and associated transitional soils. *Soil Science Society of America Proceedings* 37: 915-918.

Bettany, J.R., Stewart, J.W.B. and Halstead, E.H. 1974. Assessment of Available soil sulphate in an ^{35}S growth chamber experiment. *Canadian Journal of Soil Science* 54: 309-315.

Bettany, J.R., Stewart, J.W.B. and Saggar, S. 1979. The nature and forms of sulfur in organic matter fractions of soils selected along an environmental gradient. *Soil Science Society of America Journal* 43: 981-985.

Biederbeck, V.O. 1978. Soil organic sulfur and fertility. In *Soil Organic Matter*. (Eds. M. Schnitzer and S.U. Khan) Elsevier Scientific Publising Co., New York, pp 273-310.

Bird, P.R. 1971. Sulphur metabolism and excretion studies in ruminants, 2. organic and inorganic sulphur excretion by sheep after intraruminalor intraduodenal infusion of sodium sulfate. *Australian Journal of Biological Science* 24: 1329-1339.

Blair, G.J. 1979. *Sulfur in the tropics*. The Sulphur Institute. 69 p.

Blair, G.J., Chinoim, N., Lefroy, R.B.D., Anderson, G.C. and Crocker, G.J. 1991. A soil sulphur test for pastures and crops. *Australian Journal of Soil Research* 29: 619-626.

Blakemore, L.C., Collie, T.W. and Metson, A.J. 1968. *Sulphur*. New Zealand Soil Bureau Bulletin No 26(2), pp 103-109.

Blakemore, L.C., Ludecke, T.E., Leamy, M.L and Metson, A.J. 1969. A study of the pattern of sulphur response shown by dry-land lucerne on some brown-grey earths. *New Zealand Journal of Agricultural Research* 12: 333-351.

Blanchar, R.W. 1986. Measurement of sulfur in soils and plants. In *Sulphur in Agriculture*. (Ed. M.A. Tabatabai), Agronomy series number 27, American Society of Agronomy, pp 455-490.

Bloomfield, M. 1967. Effect of some phosphate fertilizers on the oxidation of elemental sulphur in soil. *Soil Science* 103: 219-223.

Bohn, H.L., McNeal, N.L. and O'Conner, G.A. 1979. *Soil Chemistry*. John Wiley & Sons, New York. 329 p.

Bohn, H.L., Barrow, N.J., Rajan, S.S.S., and Parfitt, R.L. 1986. Reaction of inorganic sulfur in soils. In *Sulphur in Agriculture*. (Ed. M.A. Tabatabai), Agronomy series number 27, American Society of Agronomy, pp 233-250.

Bolan, N.S., Scotter, D.R., Syers, J.K. and Tillman, R.W. 1986. The effect of adsorption on sulphate leaching. *Soil Science Society of America Journal* 50: 1419-1424.

Bolan, N.S., Syers, J.K. and Sumner, M.E. 1991. Calcium induce sulfate adsorption by soil. in press.

Bolan, N.S., Hedley, M.J., Syers, J.K. and Tillman, R.W. 1987. Single superphosphate-reactive phosphate rock mixtures 1. Factors affecting chemical composition. *Fertilizer Research* 13: 223-239.

Bolan, N.S., Syers, J.K., Tillman, R.W. and Scotter, D.R. 1988. Effect of liming and phosphate application on sulphate leaching. *Journal of Soil Science* 39: 493-504.

Bornemissza, G.F. and Williams, C.H. 1970. An effect of dung beetle activity on plant yield. *Pedobiologia* 10: 1-7.

Boswell, C.C. 1983. *An Assessment of Phosphorus and Sulphur Interactions on Aspects of the Sulphur Cycle in Pasture.* Ph.D. thesis, University of New England, Australia.

Boswell, C.C. 1985. Sulphur fertilizer strategies for the post-superphosphate era. *Proceedings of 20th Technical Conference, New Zealand Fertilizer Manufacturer Research Association,* pp 554-603.

Boswell, C.C. 1988. Sulphur fertilizer requirements. In *Towards the More Efficient Use of Soil and Fertilizer Sulphur.* (Eds. R.E. White and L.D. Currie) Occasional Report No. 2, Fertilizer and Lime Research Centre, Massey University, Palmerston North, pp 253-256.

Boswell, C.C. and Swanney, B. 1986. Alternative sulphur fertilizers in New Zealand. *Proceedings of the New Zealand Grassland Association* 47: 233-242.

Boswell, C.C. and Swanney, B. 1988. The effect of particle size, rate and frequency of application of elemental sulphur on the release of sulphate to lucerne in the upper Waitaki basin. In *Towards the More Efficient Use of Soil and Fertilizer Sulphur.* (Eds. R.E. White and L.D. Currie) Occasional Report No. 2, Fertilizer and Lime Research Centre, Massey University, Palmerston North, pp 159-168.

Boswell, C.C. and Swanney, B.S. 1991. Comparison of the long-term effects of different sulphur fertilizers on legume pasture production on a topo-sequence of North Otago soils, New Zealand. *New Zealand Journal of Agricultural Research* 34: 95-104.

Boswell, C.C., Owers, W.R. and Swanney, B. 1988a. Sulphur/sodium bentonite mixtures as fertilizers 1. the effect of S/Na-betonite ratio on the rate of dispersion and particle size distribution of elemental sulphur dispersed from laboratory-produced prills. *Fertilizer Research* 15: 13-31.

Boswell, C.C., Swanney, B. and Owers, W.R. 1988b. Sulphur/sodium betonite prills as sulfur fertilizers. 2. Effect of sulfur-sodium bentonite ratio on the availability of sulfur to pasture plants in the field. *Fertilizer Research* 15: 33-46.

Bouma, D. 1967. Nutrient uptake and distribution in subterranean clover during recovery from nutritional stress 2. experiment with sulphur. *Australian Journal of Biological Science* 20: 613-621.

Bouma, D., Spencer, K. and Dowling, E.J. 1969. Assessment of the phosphorus and sulphur status of subterranean clover pastures 3. plant tests. *Australian Journal of Experimental Agriculture and Animal Husbandry* 9: 329-340.

Bouma, J. 1980. Field measurement of soil hydraulic properties characterizing water movement through swelling clay soils. *Journal of Hydrology* 45: 149-158.

Bremner, J.M. and Tabatabai, M.A. 1971. Use of automated combustion techniques for total carbon, total nitrogen and total sulfur analyses of soils. In *Instrumental Methods for Analysis of Soil and Plant Tissue.* Soil Science Society of America, Inc., Madison.

Bromfield, S.M. 1961. Sheep faeces in relation to the phosphorus cycle under pasture. *Australian Journal of Agricultural Research* 12: 111-123.

Bromfield, S.M. and Jone, O.L. 1970. The effect of sheep on recycling of phosphorous in hayed-off pasture. *Australian Journal of Agricultural Research* 21: 699-711.

Brown, K.A. 1982. Sulphur in the environment: a review. *Environment Pollution.* Series B. 3: 47-80.

Brown, K.A. 1985. Sulfur distribution and metabolism in waterlogged peat. *Soil Biology and Biochemistry* 17: 39-43.

Burns, R.G. 1978. *Soil Enzymes.* Academic Press, London, 386 p.

Camberato, J.J. and Kamprath, E.J. 1986. Solubility of adsorbed sulfate in coastal plain soils. *Soil Science* 142: 211-213.

Castellano, S.D. and Dick, R.P. 1988. Distribution of sulfur fractions in soil as influenced by management of organic residues. *Soil Science Society of America Journal* 25: 1403-1407.

Chaiwanakupt, S., Parkpain, P., Pongsakul, P. and Phetchawee, S. 1987. Sulphur status and requirements in Thailand. *Proceeding of the Symposium on Fertilizer Sulphur Requirements and Sources in Developing Countries of Asia and the Pacific*, Bangkok, pp 89-94.

Chao, T.T., Harward, M.E. and Chang, S.C. 1962a. Movevement of ^{35}S tagged sulfate through soil columns. *Soil Science Society of America Journal* 26: 27-32.

Chao, T.T., Harward, M.E. and Fang, S.C. 1962b. Soil constituents and properties in the adsorption of sulfate ions. *Soil Science* 94: 276-283.

Chapman, S.J. 1987a. Microbial sulphur in some Scottish soils. *Soil Biology and Biochemistry* 19: 301-305.

Chapman, S.J. 1987b. Partitioning of ryegrass residue sulphur between the soil microbial biomass, other soil sulphur pools and ryegrass (*Lolium perenne* L). *Biology and Fertility of Soils.* 5; 253-257.

Chapman, S.J. 1989. Oxidation of micronized elemental sulphur in soil. *Plant and Soil* 116: 69-76.

Chatupote, W. 1990. *An Investigation on Some Factors Influencing the Rate of Oxidation of Elemental Sulphur Fertilizer.* Ph.D. thesis, Massey University, New Zealand.

Chaudhry, I.A. and Cornfield, A.H. 1967a. Effect of moisture content during incubation of soil treated with organic material on changes in sulphate and sulphide levels. *Journal of the Science of Food and Agriculture* 18: 38-41.

Chaudhry, I.A. and Cornfield, A.H. 1967b. Effect of temperature on sulphate levels in aerobic and sulphide levels in anaerobic soil. *Journal of the Science of Food and Agriculture* 18: 82-84.

Clark, P.E. and Paul E.A. 1970. The microflora of grassland. *Advance in Agronomy* 22: 375-435.

Cooper, M. 1968. A comparison of five methods for determining the sulphur status of New Zealand Soils. 9th International Congress of Soil Science, Transaction. II: 263-271.

Cooper, M. and Hogg, D.E. 1966. Leaching losses of sulphate from superphosphate and gypsum applied on soil from Rhyolitic pumice. *New Zealand Journal of Agricultural Research* 9: 345-349.

Cornforth, I.S. 1981. Soil testing and fertilizer requirements. *Proceedings of Technical Conference of New Zealand Fertilizer Manufacturers Research Association* 18: 46-53.

Cornforth, I.S. and Sinclair, A.G. 1984. *Fertilizer and Lime Recommendations for Pasture and Crops in New Zealand.* 2nd revised edition, Ministry of Agriculture and Fisheries Bulletin New Zealand.

Cornforth, I.S. and Sinclair, A.G. 1986. The MAF fertilizer advisory service. *Proceedings of the New Zealand Grassland Association* 47: 197-202.

Cornforth, I.S., Smith, G.S. and Wheeler, D.M. 1983. Soil testing and plant analysis for sulphur. In *Sulphur in New Zealand Agriculture.* (Eds. P.E.H. Gregg and J.K.Syers) Occasional report No, 5, Massey University, Palmerston North, pp 49-60.

Coughenour, M.B. 1978. *Grassland Sulphur Cycle and Ecosystem Responses to Level of Sulphur Dioxide.* Ph.D. thesis, Colorado State University, Fort Collins, Colorado.

Coulter, J.D. 1973. Prediction of evapotranspiration from climatological data. *Proceedings of Soil and Plant Water Symposium,* Inf. Ser. No. 96, pp 38-45.

Cowie, J.D. 1978. *Soil and Agriculture of Kairanga, North Island, New Zealand.* New Zealand Department of Scientific and Industrial Research. Soil Bulletin No. 33.

Crouchley, G. and Sinclair, A.G. 1982. Response to sulphur and phosphorus fertilizers on improved pastures on some soils of Wairarapa. *New Zealand Journal of Experimental Agriculture* 10: 185-189.

Curll, M.L. 1982. The grass and clover content of pastures grazed by sheep. *Herbage Abstracts* 52: 403-411.

Curtin, D. and Syers, J.K. 1990. Mechanism of sulphate adsorption by two tropical soils. *Journal of Soil Science* 41: 295-304.

David, M.B. and Mitchell, M.J. 1987. Transformations of inorganic and organic sulfur: Importance of sulfate flux in Adirondack forest soil. *JAPCA, the International Journal of Air Pollution and control and Harzardous Waste Management.* 37; 39-44.

David, M.B., Mitchell, M.J. and Nakas, J.P. 1982. Organic and inorganic constituents of a forest soil and their relationship to microbial activity. *Soil Science Society of America Journal* 46: 847-852.

David, M.B., Mitchell, M.J. and Schnindler, S.C. 1984. Dynamics of organic and inorganic sulfur constituents in hardwood forest soils and their relationships to nutrient cycling. In *Forest Soils and Treatment Impacts.* (Ed. E.I. Stone) Proceedings North America Forest Soil Conference 6th Knoxville, Tennessee, pp 221-245.

David, M.B., Mitchell, M.J. and Scott, T.J. 1987. Importance of biological processes in the sulphur budget of northern hardwood ecosystem. *Biology and Fertility of Soils* 5: 258-264.

David, M.B., Schindler, S.C., Mitchell, M.J. and Strick, J.E. 1983. Importance of organic and inorganic sulfur to mineralization processes in forest soil. *Soil Biology and Biochemistry* 15: 671-677.

Davidson, J.L. and Milthorpe F.L. 1966a. Leaf growth in *Dactylis glomerata* following defoliation. *Annals of Botany* 30: 174-185.

Davidson, J.L. and Milthorpe, F.L. 1966b. The effect of defoliation on the carbon balance in *Dactylis glomerata. Annals of Botany* 30: 185-189.

Day, F.H. 1963. *The Chemical Elements in Nature.* George G. Harrolo & Co Ltd., London.

De Long, W.A. and Lowe, L.E. 1962. Carbon-bonded sulphur in soil. *Canadian Journal of Soil Science* 42: 223.

Dean, G.A. 1966. A simple colorimetric finish for the Johnson-Nishita micro-distillation of sulphur. *Analyst* 91: 530-532.

Destain, J.P., Guiot, J., Francios, E and Riga, A. 1989. Fertilizer nitrogen budgets for two dose of $NaNO_3$ dressings split-applied to winter wheat in microplot on a loam soil. *Plant and Soil* 117: 177-183.

Devaud, E. and McFarlance, J.D. 1980. The fate of radioactive sulphur applied in irrigated lucerne. *Australian Journal of Agricultural Research* 31: 887-897.

Dick, W.A. and Tabatabai, M.A. 1977. An alkaline oxidation method for determination of total Phosphorus in soils. *Soil Science Society of America Journal* 41: 511-514.

Dijkshoorn, W. and Van Wijk, A.I. 1967. The sulphur requirement of plants as evident by the sulphur-nitrogen ratio in the organic matter: a review of published data. *Plant and Soil* 26: 129-157.

Dijkshoorn, W. Lampe, J.E.M. and Van Burg, D.F.G. 1960. A method for diagnosing the sulphur nutrition status of herbage. *Plant and Soil* 13: 227-241.

Doak. B.W. 1952. Some chemical changes in the nitrogenous constituents of urine voided on pasture. *Journal of Agricultural Science, Cambridge* 42: 162-171.

Donald, A.D. and Leslie, R.T. 1969. Population studies on the effective stage of some nematode parasites of sheep. *Parasitology* 59: 141-157.

Doyle, P.T. and Moir, R.J. 1979. Sulfur and methionine metabolism in sheep 1. first approximation of sulfur pools in and sulfur flows from the recticulo-rumen. *Australian Journal Biological Science* 32: 51-63.

Duke, S.H. and Reisenauer, H.M. 1986. Roles and requirements of sulfur in plant nutrition. In *Sulphur in Agriculture.* (Ed. M.A. Tabatabai), Agronomy series number 27, American Society of Agronomy, pp 124-168.

During, C. 1984. *Fertilizers and Soils in New Zealand Farming.* 3rd edition. Goverment printer, Wellington New Zealand. 355 p.

During, C. and Cooper, M. 1974. Sulphate nutrition and movement in a soil with high sulphate sorption characteristics. *New Zealand Journal of Experimental Agriculture* 2: 45-51.

During, C. and Martin, D.J. 1968. Sulphate nutrition, movement and sorption, with special reference to a gley podzol, West coast, South island. *New Zealand Journal of Agricultural Science* 11: 665-676.

During, C. and McNaught, K.J. 1961. The effect of cow urine in growth of pasture and uptake of nutrients. *New Zealand Journal of Agricultural Research* 4: 591-595.

During, C. and Weeda, W.C. 1973. Some effect of cattle dung on soil properties, pasture production and nutrient uptake, 1. dung as a sources of phosphorus. *New Zealand Journal of Agricultural Research* 16: 423-430.

Eaton, F.M. 1966. Sulfur In *Diagnostic Criteria for Plant and Soils.* (Ed. H.D. Chapman), University of California Press, pp 444-475.

Ensminger, l.E. 1954. Some factor affecting the adsorption of sulfate by Alabama soils. *Soil Science Society of America Proceedings* 19: 259-264.

Ensminger, L.E. 1958. Sulfur in relation to soil fertility. Alabama Agricultural experimental Station, Bulletin 312. 19 p.

Evan, P.S. 1971. Root growth of *Lolium perrenne L.* 2. effect of defoliation and chloroplast pigments. *Botanical Gazette* 115: 225-234.

Faires, R., and Boswell, G.G.J. 1981. *Radioisotope Laboratory Techniques.* 4th edition, Butterworths.

Fitzgerald, J.W. 1976. Sulfate ester formation and hydrolysis: a potentially important yet often ignored aspect of the sulfur cycle of aerobic soils. *Bacteriological Reviews* 40: 698-721.

Fitzgerald, J.W 1978. Naturally occurring organo sulfur compounds in soils. In *Sulfur in the Environment, part II Ecological Impacts.* (Ed. J.O. Nriagu), Wiley, New York. pp 391-443.

Fitzgerald, J.W., Strickland, T.C. and Swank, T.W. 1982. Metabolic fate of inorganic sulfate in soil samples from undisturbed and managed forest ecosystems. *Soil Biology and Biochemistry* 14: 529-536.

Fitzgerald J.W., Ash, J.T. and Strickland, T.C. 1983. Formation of organic sulfur in forest soils: a biologically mediated process. *Canadian Journal of Forest Research* 13: 1077-1082.

Fox, R.L. 1976. Sulphur requirements of sugarcane. *Agronomy Journal* 68: 891-896.

Fox, R.L. 1980. Responses to sulphur by crops growing in highly weathered soils. *Sulphur in Agriculture* 4: 16-22.

Fox, R.L., Asghar, M. and Cable, W. 1983. Sulfate accretions in soils of tropics. In *Sulphur in South East Asian and South Pacific Agriculture.* (Ed. G.J. Blair), pp 39-53.

Fox, R.L., Kang, B.T. and Nangju, D. 1977. Sulfur requirements of cowpeas and implications for production in the tropics. *Agronomy Journal* 69: 201-205.

Fox, R.L., Olsen, R.A. and Rhoades, H.F. 1964. Evaluation of sulfur status of soils by plant and soil tests. *Soil Science Society of America Proceedings* 28: 243-246.

Franklin, R.E. 1971. Cation effects on chloride, sulfate and phosphate uptake by excised roots. *Soil Science* 112: 343-347.

Freney, J.R. 1958. Determination of water-soluble sulfate in soils. *Soil Science* 86: 241-244.

Freney, J.R. 1961. Some observations on the nature of organic sulphur compounds in soils. *Australian Journal of Agricultural Research* 12: 424-432.

Freney, J.R. 1967. Sulphur Containing Organics. In *Soil Biochemistry.* (Eds. A.D. McLaren and G.H. Peterson) Marcel Dekker Inc., New York.

Freney, J.R. 1986. Form and reactions of organic sulfur compounds in soils. In *Sulphur in Agriculture.* (Ed. M.A. Tabatabai), Agronomy series number 27, American Society of Agronomy, pp 207-232.

Freney, J.R. and Spencer, K. 1960. Soil sulphate changes in the presence and absence of growing plants. *Australian Journal of Agricultural Research* 11: 339-345.

Freney, J.R. and Stevenson, F.J. 1966. Organic sulfur transformations in soils. *Soil Science* 101: 307-316.

Freney, J.R. and Swaby, R.J. 1975. Sulphur transformation in soils. In *Sulphur in Australasian Agriculture*. (Ed. by K.D. McLachlan). Sydney University Press, pp 22-30.

Freney, J.R. and Williams C.H. 1983. The Sulphur Cycle in Soil. In *Global Biogeochemical Sulphur Cycle*. (Eds. M.V. Ivanov and J.R. Freney) John Wiley & Sons, pp 129-201.

Freney, J.R., Melville, G.E. and Williams, C.H. 1969. Extraction, chemical nature and properties of soil organic sulphur. *Journal of Science of Food and Agriculture* 20: 440-445.

Freney, J.R., Melville, G.E. and Williams C.H. 1970. The determination of carbon-bonded sulphur in soil. *Soil Science* 109: 310-318.

Freney, J.R., Melville, G.E. and Williams, C.H. 1971. Organic sulphur fractions labelled by addition of ^{35}S-sulphate to soil. *Soil Biology and Biochemistry* 3: 133-141.

Freney, J.R., Melville, G.E. and Williams, C.H. 1975. Soil organic matter fractions as sources of plant-available sulphur. *Soil Biology and Biochemistry* 4: 227-221.

Freney, J.R., Stevenson, F.J. and Beaver, A.H. 1972. Sulphur containing amino-acids in soil hydrolysates. *Soil Science* 114: 486-476.

Fuller, R.D., David, M.B. and Driscoll, C.T. 1985. Sulfate adsorption relationships in forest Spodosols of Northeastern USA. *Soil Science Society of America Journal* 49: 1034-1040.

Garwood, E.A. 1967. Seasonal variation in appearance and growth of grass roots. *Journal of the British Grassland Society* 23: 117-128.

Ghani, A., McLaren, R.G. and Swift, R.S. 1988. The incorporation and re-mineralization of ^{35}S in soil organic sulphur fractions. In *Towards the More Efficient Use of Soil and Fertilizer Sulphur*. (Eds. R.E. White and L.D. Currie) Occasional Report No. 2, Fertilizer and Lime Research Centre, Massey University, Palmerston North, pp 32-46.

Ghani, A., McLaren, R.G. and Swift, R.S. 1990. Seasonal fluctuations of sulphate and soil microbial biomass-S in the surface of a Wakanui soil. *New Zealand Journal of Agricultural Research* 33: 467-472.

Ghani, A., McLaren, R.G. and Swift, R.S. 1991. Sulphur mineralization in some New Zealand soils. *Biology and Fertility of Soils* 11: 58-74.

Gillingham, A.G. 1978. *Phosphorus Cycling in Grazed, Steep Hill Country*. Ph.D. thesis, Massey University, New Zealand.

Gillingham, A.G. 1980. Phosphorus uptake and return in grazed, steep-hill country 1. Pasture production dung and litter accumulation. *New Zealand Journal of Agricultural Research* 23: 313-321.

Gillingham, A.G. and During, C. 1973. Pasture production and transfer of fertility within long-established hill Pasture. *New Zealand Journal of Experimental Agriculture* 1: 227-232.

Gillingham, A.G., Syers, J.K., and Gregg, P.E.H. 1980. Phosphorus uptake and return in grazed, steep-hill country 1. above ground components of the phosphorus cycle. *New Zealand Journal of Agricultural Research* 23: 323-330.

Gillman, G.P. 1973. Studies on some deep sandy soils in Cape York Peninsula, North Queensland 3. losses of applied phosphorus and sulphur. *Australian Journal of Experimental Agriculture and Animal Husbandry* 13: 418-422.

Goedewaagen, M.A.J. and Schuurman, J.S. 1950. Root production by agricultural crops on arable land and grassland as source of organic matter in soil. Transaction 4th international Congress of Soil Science 2: 28-31.

Goh, K.M. 1988. Sulphur transformation in field soils - a review summary. In *Towards the More Efficient Use of Soil and Fertilizer Sulphur.* (Eds. R.E. White and L.D. Currie) Occasional Report No. 2, Fertilizer and Lime Research Centre, Massey University, Palmerston North, pp 78-94.

Goh, K.M. and Gregg, P.E.H. 1980. Re-utilization by perennial ryegrass (*Lolium perenn* L.) of labelled fertilizer sulphur incorporated in field-growth tops and roots of pasture plants added to soils. *Fertilizer Research* 1: 73-85.

Goh, K.M. and Gregg, P.E.H. 1982a. Field study on the fate of radioactive sulphur fertilizer applied to pastures. *Fertilizer Research* 3: 337-351.

Goh, K.M. and Gregg, P.E.H. 1982b. Field studies with radioactive sulphur-labelled gypsum fertilizer III. Sulphur pools and their significance. *New Zealand Journal of Agricultural Research* 25: 135-139.

Goh, K.M. and Nguyen, M.L. 1990. Effect of grazing animals on the plant availability of sulphur fertilizers in grazed pastures. *Proceeding of New Zealand Grassland Association.* 52:181-185.

Goh, K.M. and Tsuji, T. 1979. Changes in soil sulphur during incubation with and without added sulphur. *New Zealand Journal of Agricultural Research* 22: 585-594.

Goodrich, R.D. and Garrett, J.E. 1986. Sulfur in livestock nutrition. In *Sulphur in Agriculture.* (Ed. M.A. Tabatabai), Agronomy series number 27, American Society of Agronomy, pp 617-634.

Grayston, S.J. and Germida, J.J. 1990. Influence of crop rhizospheres on populations and activity of heterotropic sulphur-oxidizing microorganisms. *Soil Biology and Biochemistry* 22: 457-463.

Gregg, P.E.H. 1976. *Field Investigations into the Fate of Fertilizer Sulphur Added to Pasture-Soil Systems.* Ph.D. thesis, University of Canterbury, New Zealand.

Gregg, P.E.H. and Goh, K.M. 1978. Field studies with radioactive sulphur-labelled gypsum fertilizer I. Soil factors affecting the movement of fertilizer sulphur. *New Zealand Journal of Agricultural Research* 21: 593-601.

Gregg, P.E.H. and Goh, K.M. 1979. Field studies with radioactive sulphur-labelled gypsum fertilizer II. Climatic and management factors affecting the movement of fertilizer sulphur in soils. *New Zealand Journal of Agricultural Research* 22: 425-429.

Gregg, P.E.H. and Goh, K.M. 1982. Field studies with radioactive sulphur-labelled gypsum fertilizer IV. recovery by pasture and soil component. *New Zealand Journal of Agricultural Research* 25: 345-353.

Gregg, P.E.H., Goh, K.M. and Bash, D.W. 1977. Isotope studies on the uptake of sulphur by plants 2. Uptake from various soil depths at several field sites. *New Zealand Journal of Agricultural Research* 20: 299-233.

Halstead, R.L. and Rennie, P.J. 1977. The effects of sulphur on soils in Canada. In *Sulphur and Its Organic Derivatives in Canadian Environment.* Environmental Secretariat, National Research Council, Bubl. NRCC, 15015, Ottawa.

Gregory, P.J. 1988. Growth and function of plant roots. In *Russell's Soil Condition and Plant Growth* (Ed. A. Wild), John Wiley & Sons, New York.

Harada, T. and Spencer B. 1960. Choline sulphate in fungi. *Journal of General Microbiology* 22: 520-527.

Harward, M.E. and Reisenauer, H.M. 1966. Reactions and movement of inorganic soil sulfur. *Soil Science* 101: 326-335.

Harward, M.E., Chao, T.T. and Fang, S.C. 1962. The sulfur status and sulfur supplying power of Oregon soils. *Agronomy Journal* 54: 101-106.

Hasan, S.M., Fox, R.L. and Boyd, C.C. 1970. Solubility and availability of sorbed sulfate in Hawaiian soils. *Soil Science Society of America Proceedings* 34: 897-901.

Hayes, M.H.B. and Swift, R.S. 1978. The chemistry of soil organic matter colloids. In *Chemistry of Soil Constituents.* (Eds. D.J. Greenland and M.H.B. Hayes) John Wiley & Sons Ltd., New York, pp 179-320.

Hayman, J.M. and Stocker, R.V. 1982. Soil Water extraction patterns under pasture and lucerne on two soil types in Mid Canterbury. *Proceedings of Agronomy Society of New Zealand* 12: 61-63.

Haynes, R.J. and Williams, P.H. 1991. Effect of grazing animals on the nutrient status of pasture soils. In *Soil and Plant Testing for Nutrient Deficiencies and Toxicities* (Eds. R.E. White and L.D. Currie) Occasional Report No. 5, Fertilizer and Lime Research Centre, Massey University, Palmerston North, pp 224-236.

Hedley, M.J. and Stewart, J.W.B. 1982. Method to measure microbial phosphate in soils. Soil Biology and Biochemistry 14: 377-385.

Hedley, M.J., Tillman, R.W. and Roger, P. 1990. The environment: nutrient losses from New Zealand agriculture. In *Proceedings of the New Zealand Fertilizer Manufacturers' Research Association Conference, The Challenge for Fertilizer Research in 1990*, pp 62-91.

Heng, L.K. 1991. *Measurement and Modelling of Chloride and Sulphate Leaching from a Mole-Drained Soil.* Ph.D. thesis, Massey University, New Zealand.

Heng, L.K., White, R.E., Bolan, N.S. and Scotter, D.R. 1991. Leaching losses of major nutrients from a mole-drained soil under pasture. *New Zealand Journal of Agricultural Research* 34: 325-334.

Herriott, J.B.O. and Well D.A. 1963. The grazing animal and sward productivity. *Journal of Agricultural Science, Cambridge* 61: 89-99.

Hesse, P.R. 1957. The effect of colloidal organic matter on the precipitation of Barium sulphate and modified method for determining soluble sulphate in soils. *Analyst* 82: 710-712.

Higgins, D.J. 1987. Some recent developments in sulphur fertilizer technology. *Proceeding of the Symposium on Fertilizer Sulphur Requirements and Sources in Developing Countries of Asia and the Pacific*, Bangkok, pp 112-122.

Hilder, E.J. 1964. The distribution of plant nutrients by sheep at pasture. *Proceeding of Australian Society of Animal Production* 5: 241-248.

Hilder, E.J. 1966. Distribution of excreta by sheep at pasture. Proceedings of the 10th International Grassland Congress, Helsinki. pp 977-981.

Hingston, F.J. 1959. The loss of applied phosphorus and sulphur from soils under pasture in W.A. *The Journal of the Australian Institute of Agricultural Science* 25: 209-213.

Hingston, F.J., Atkinson, R.J., Posner, A.M. and Quirk, J.P. 1967. Specific adsorption of anions. *Nature* 215: 1459-1461.

Hoeft, R.G., Walsh, L.M. and Keeney, D.R. 1973. Evaluation of various extractants for available sulfur. *Soil Science Society of America Proceedings* 37: 401-404.

Hogg, D.E. 1965. Studies on leaching losses of sulphur from pumice soils, yellow brown loams and sands. *New Zealand Grassland Association* 27: 123-128.

Hogg, D.E. and Toxopeus, M.R.J. 1966. Studies on the retention of sulphur by yellow-brown pumice soils. *New Zealand Journal of Agricultural Research* 9: 93-97.

Hoque, S., Heath, S.B. and Killham, K. 1987. Evaluation of method to asses adequacy of potential soil S supply to crops. *Plant and Soil* 101: 3-8.

Hue, N.V., Adam, F. and Evan, C.V. 1984. Plant available sulphur as measured by soil solution sulphate and phosphate extractable sulphate in Ultisol. *Agronomy Journal* 76: 726-730.

I.A.E.A. 1976. Tracer manual on crops and soils. Technical report No. 171. International Atomic Energy Agency, Vienna.

Jackman, R.H. 1964a. Accumulation of organic matter in some New Zealand soils under permanent pasture II. rate of mineralization of organic matter and the supply of available nutrients. *New Zealand Journal of Agricultural Research* 7: 472-479.

Jackman, R.H. 1964b. Accumulation of organic matter in some New Zealand soils under permanent pasture I. pattern of change of organic carbon, nitrogen, sulphur and phosphorus. *New Zealand Journal of Agricultural Research* 7: 445-471.

Janzen, H.H. and Bettany, J.R. 1987a. Measurement of sulfur oxidation in soil. *Soil Science* 143: 444-452.

Janzen, H.H. and Bettany, J.R. 1987b. Effect of temperature and water potential on sulfur oxidation in soil. *Soil Science* 144: 81-89.

Janzen, H.H. and Bettany, J.R. 1987c. Oxidation of elemental sulfur under field conditions in central Saskatchewan. *Canadian Journal of Soil Science* 67: 609-618.

Jenkinson, D.S. 1976. The effect of biocidal treatments on metabolism in soils 4. the decomposition of fumigated organisms in soil. *Soil Biology and Biochemistry* 8: 167-177.

Jenkinson, D.S., Powlson, D.S. and Weddweburn, R.W.W. 1976. The effect of biocidal on metabolism in soil 3. the relationship between soil bio-volume measured by optical microscopy, the flush of decomposition caused by fumigation. *Soil Biology and Biochemistry* 8: 189-202.

Jensen, M.E. 1973. *Consumptive Use of Water and Irrigation Water Requirements.* American Society of Civil Engineers, New York.

Johnson, C.M. and Nishita, H. 1952. Micro-estimation of sulphur in plant materials, soils and irrigation waters. *Analytical Chemistry* 24: 736-742.

Johnson, D.W. and Cole, D.W. 1977. Sulfate mobility in an out-wash soil in western Washington. *Water, Air and Soil Pollution* 7: 489-495.

Johnson, D.W. and Henderson, G.S. 1979. Sulfate adsorption and sulfur fractions in a highly weathered soil under a mixed deciduous forest. *Soil Science* 128: 34-40.

Johnson, D.W. and Todd, D.E. 1983. Relationships among iron, aluminium, carbon and sulfate in variety of forest soils. *Soil Science Society of America Journal* 47: 792-800.

Johnson, D.W., Hornbeck, J.W., Kelly, J.M., Swank, W.T. and Todd, D.E. 1979. Regional pattern of soil sulfate accumulation: relevance to ecosystem sulfur budgets. In *Proceedings; Atmospheric Sulphur Deposition Environmental Impact and Health Effects.* (Ed. D.S. Shriner, C.R. Lichmond and S.E. Lindberg), Ann Arbor Science Publishing Inc., the Butterworth Group, pp 507-520.

Jones, J.B. and Isaac, R.A. 1972. Determination of sulfur in plant material using a Leco sulfur analyzer. *Agricultural and Food Chemistry* 20: 1292-1294.

Jones, L.H.P., Cowling, D.W. and Lockyer, D.R. 1972a. Plant available and extractable sulphur in some soils of England and Wales. *Soil Science* 114: 104-114.

Jones, M.B. 1964. Effect of Applied sulphur I. uptake of various California dryland pasture species. *Agronomy Journal* 56: 235-237.

Jones, M.B. 1975. Plant assays for the recognition of sulphur deficiency. In *Sulphur in Australasian Agriculture* (Ed. K.D. McLachlan). Sydney University Press, pp 175-182.

Jones, M.B. 1986. Sulfur availability indexes. In *Sulphur in Agriculture.* (Ed. M.A. Tabatabai), Agronomy series number 27, American Society of Agronomy, pp 549-566.

Jones, M.B., Martin, W.E. and Ruckman, J.E. 1970. Effectiveness of various sulfur sources applied to annual type grasslands of California. Proceedings of 9th International Grassland Congress, pp 373-376.

Jones, M.B., Martin, W.E. and Williams, W.A. 1968. Behavior of sulfate sulfur and elemental sulfur in three California soils in lysimeters. *Soil Science Society of America Proceedings* 32: 535-540.

Jones, M.B., Oh J.H. and Ruckman, J.E. 1970. Effect of phosphorus and sulphur fertilization on the nutritive value of subterranean clover. In *Proceedings of 32rd Conference on New Zealand Grassland Association*, pp 69-76.

Jones, M.B., Rickman, J.E. and Lawler, P.W. 1972b. Critical levels of sulphur in bur clover. *Agronomy Journal* 64: 55-58.

Jones, S.L. and Smith, I.K. 1981. Sulfate transport in cultured tobacco cells- effect of calcium and sulfate concentration. *Plant Physiology* 67: 445-448.

Kamprath, E.J., Nelson, W.L. and Fitts, J.W. 1956. The effect of pH, sulfate and phosphate concentrations on the adsorption of sulfate by soils. *Soil Science Society of America Proceedings* 21: 463-466.

Kane, A. 1983. How I handle my fertilizer program. Proceedings of the 1983 hill and high country. Centre for Resources Management, Lincoln, New Zealand, pp 45-48.

Kenendy, A.P. and Till, A.R. 1981a. The distribution in soil and plant of ^{35}S from sheep excreta. *Australian Journal of Agricultural Research* 32: 339-351.

Kennedy, A.P., and Till, A.R. 1981b. Effect of rate of application of gypsum and rainfall on the movement of ^{35}S in soil and plant. *The Journal of Australian Institute of Agricultural Science.* 47: 110-113.

Kennedy, P.M. 1974. Utilization and excretion of sulphur in cattle fed on tropical roughages. *Australian Journal of Agricultural Research* 25: 1018-1022.

King, K.L. and Hutchison, K.J. 1976. The effects of sheep stocking intensity on the abundance and distribution of meso-fauna in pasture. *Journal of Applied Ecology* 13: 41-45.

Kirk, G.J.D. and Nye, P.H. 1986. A simple model for predicting the rate of dissolution of sparlingly soluble calcium phosphates in soil, 2. appliction of the model. *Journal of Soil Sceince* 37: 541-554.

Kittams, H.A., and Attoe, O.J. 1965. Availability of phosphorus in rock phosphate-sulphur fusions. *Agronomy Journal* 57: 331-334.

Kononova, M.M. 1966. *Soil Organic Matter.* Pergamon Press, Oxford.

Konopka, A.E., Miller, R.H., Sommers, L.E. 1986. Microbiology of the sulfur cycle. In *Sulphur in Agriculture.* (Ed. M.A. Tabatabai), Agronomy series number 27, American Society of Agronomy, pp 23-56.

Korentajer, L., Byrnes, B.H. and Hellums, D.T. 1984. Leaching losses and plant recovery from various sulfur fertilizers. *Soil Science Society of America Journal* 48: 671-676.

Kowalenko, C.G. and Low. L.E. 1975. Evaluation of several extraction methods and of a closed incubation method for soil sulfur mineralization. *Canadian Journal of Soil Science* 55: 1-8.

Kowlenko, C.G. 1978. Organic nitrogen, phosphorus and sulphur. In *Soil Organic Matter.* (Eds. M. Schnitzer and S.U. Khan) Elsevier Scientific Publishing Co., New York, pp 95-136.

Kurmarohita, K. 1973. *Distribution, Nature and Availability of Sulphur in Soil of Thailand.* Ph.D. thesis, University of Illinois.

Lambert, M.G., Clark, D.A., Costall, D.A. and Budding, P.J. 1988. Phosphorus and sulphur accumulation after long-term superphosphate application to grazed hill country. In *Towards the More Efficient Use of Soil and Fertilizer Sulphur.* (Eds. R.E. White and L.D. Currie) Occasional Report No. 2, Fertilizer and Lime Research Centre, Massey University, Palmerston North, pp 95-100.

Landers, D.H., David, M.B. and Mitchell, M.J. 1983. Analysis of organic and inorganic sulfur constituents in sediments, soils and water. *International Journal of Environment, Analytical Chemistry* 14: 245-256.

Langlands, J.P., Sutherland, H.A.M. and Playne, M.J. 1973. Sulfur as nutrient for merino sheep 2. utilization of sulfur in forage diets. *British Journal of Nutrition* 30: 537-543.

Lantinga, E.A., Keuming, J.A., Groenwold, J. and Deeman, P.J.A.G. 1987. Distribution of extreta nitrogen by grazing cattle and its effect on sward quality, herbage production and utilization. In *Animal Mannure on Grassland and Fodder Crops.* (Eds. H.G. Meer), Martinus Nijhoff Publishers.

Laskin, A.I. and Lechevalier, H.A. 1973. Handbook of Microbiology vol 2. Microbial composition CRC press, Cleveland. 1060 p.

Ledgard, S.F. and Upsdell, M.P. 1991. Sulphur inputs from rainfall throughout New Zealand. *New Zealand Journal of Agricultural Research* 34: 105-111.

Ledgard, S.F., Sporsen, M.S., Brier G.J. and Saunders, W.M.H. 1991. Temporal changes in various soil and plant sulphur analyses in relation to the development of pasture response to sulphur fertilizer. In *Soil and Plant Testing for Nutrient Deficiencies and Toxicities* (Eds. R.E. White and L.D. Currie) occasional Report No. 5. Fertilizer and Lime Research centre, Massey University, Palmerston North, pp 115-121.

Lee, A. 1983. Sulphur oxidation in soils. In *Sulphur in New Zealand Agriculture.* (Eds. P.E.H. Gregg and J.K.Syers) Occasional report No, 5, Massey University, Palmerston North, pp 29-38.

Lee, A. and Boswell, C.C. 1988. Laboratory and field measurements of elemental sulphur oxidation in some New Zealand soils. In *Towards the More Efficient Use of Soil and Fertilizer Sulphur.* (Eds. R.E. White and L.D. Currie) Occasional Report No. 2, Fertilizer and Lime Research Centre, Massey University, Palmerston North, pp 101-108.

Lee, A., Boswell, C.C. and Watkinson, J.H. 1988. Effect of particle size on the oxidation of elemental sulphur, *Thiobacilli* numbers, soil sulphate, and its availability to pasture. *New Zealand Journal of Agricultural Research* 31: 179-186.

Lee, A., Watkinson, J.H. and Lauren, D.R. 1988. Factors affecting oxidation rate of elemental sulphur in soil under a ryegrass dominant sward. *Soil Biology and Biochemistry* 20: 809-816.

Lee, A., Watkinson, J.H. and Lauren, D.R. 1988. Factor affecting oxidation rates of elemental sulphur in a soil under ryegrass dominant sward. *Soil Biology and Biochemistry* 20: 809-816.

Lee, A., Watkinson, J.H., Orbell, G. and Bagyaraj, J. and Lauren, D.R. 1987. Factors influencing dissolution of phosphate rock and oxidation of elemental sulphur in some New Zealand soils. *New Zealand Journal of Agricultural Research* 30: 373-385.

Lee, R. and Speir, T.W. 1979. Sulphur uptake by ryegrass and its relationships to inorganic and organic sulphur levels and sulphatase activity in soil. *Plant and Soil* 53: 407-425.

Lee, R., Blakemore, E.J., Gibson, E.J. and Daly, B.K. 1981. Effect of extraction time and charcoal treatment on the adsorbed sulphate values of several New Zealand topsoils. *Communication in Soil Science and Plant Analysis* 12: 1195-1206.

Lee, R., Blakemore, L.C., Daly, B.K., Gibson, E.J., Speir, T.W. and Orchard, V.A. 1985. Sulphur supply to ryegrass during a pot trial and correlations with soil biological activity: the influence of two difference methods of determining the adsorbed sulphate status of soils. *Communication in Soil Science and Plant Analysis* 16: 97-117.

Lehninger, A.L. 1982. *Principles of Biochemistry.* Worth Publishers Inc., New York. 1011 p.

Lewis, D.C. and Quirk, J.P. 1967. Phosphate diffusion in soil and uptake by plants; 3. ^{31}P movement and uptake by plants as indicated by ^{32}P autoradiography. *Plant and Soil* 26: 445-453.

Lewis, D.C., Clarke, A.L. and Hall, W.B. 1987. Accumulation of nutrients and changes in soil properties of sandy soil under fertilized pasture in South-eastern South Australia 2. Total sulfur and nitrogen, organic carbon and pH. *Australia Journal of Agricultural Research* 25: 203-210.

Li, P and Caldwell, A.C. 1966. The oxidation of elemental sulfur in soil. *Soil Science Society of America Proceedings* 30: 370-372.

Lotero, J., Woodhouse, W.W. jr. and Petersen, R.G. 1966. Local Effect on fertility of urine voided by grazing cattle. *Agronomy Journal* 58: 262-265.

Lowe, L.E. 1964. An approach to the study of the sulphur status of soils and its application to selected Quebec soils. *Canadian Journal of Soil Science* 44: 176-179.

Lowe, L.E. 1965. Sulphur fractions of selected Alberta soil profiles of the Chernozem and Podzolic order. *Canadian Journal of Soil Science* 45: 297-303.

Lowe, L.E. 1969a. Forms of sulfur in soils. Report of Meeting of Western Section, National Soil Fertility Committee, Edmonton, Alberta, pp 72-80.

Lowe, L.E. 1969b. Distribution and properties of organic fraction in selected Alberta soils. *Canadian Journal of Soil Science* 49: 129-141.

Lowe, L.E. and De long, W.A. 1963. Carbon bonded sulphur in selected Quebec soils. *Canadian Journal of Soil Science* 43: 151-155.

Ludecke, T.E. 1965. Further aspects of sulphur nutrition of legumes. *Proceedings of New Zealand Grassland Association* 27: 129-138.

MacDiarmid, B.N. and Watkin, B.R. 1971. The cattle dung patch 1. Effect of dung patch on yield and botanical composition of surrounding and underlining pasture. *Journal British Grassland Society* 26: 239-245.

MacDiarmid, B.N. and Watkin, B.R. 1972a. The cattle dung patch 2. The effect of dung patch on the chemical status of soil, and ammonia nitrogen losses from the patch. *Journal of British Grassland Society* 27: 43-48.

MacDiarmid, B.N. and Watkin, B.R. 1972b. The cattle dung patch 3. Distribution and rate of decay of dung patches and their influence on grazing behavior. *Journal of British Grassland Society* 27: 48-54.

MacFadyen, A. 1978. The role of fauna in decomposition processes in grasslands In *Proceedings of the Symposium of Grassland Fauna.* Scientific Proceedings of the Royal Dublin Society, Series A. 6(11) :197-206.

MacLusky, D.S. 1960. Some estimation of the area of pasture foule by excretion of dairy cows. *Journal of The British Grassland Society* 15: 181-188.

Manzel, R.G. and Smith, S.J. 1984. Soil fertility and plant nutrition. In *Isotope and Radiation in Agricultural Sciences.* (Eds. M.F. L'annunziata and J.O. Legg), Academic Press. 292 p.

Martin, J.K. 1985. A single technique for field studies of distribution of ^{32}P-labelled phosphate between plant and soil pools. *Plant and soil* 86: 415-423.

Martin, R.J. 1990. Measurement of water use and pasture growth on Templeton silt loam. *New Zealand Journal of Agricultural Research* 33: 343-349.

Martin-pervel, P. and Gagnard, J. 1986. Plant analysis and a guide to nutrient requirements for temperate and tropical crops. Lavoiser, Paris.

May, P.F., Till, A.R. and Downes, A.M. 1968. Nutrient cycling in grazed pastures 1. a preliminary investigation of the used of ^{35}S gypsum. *Australian Journal of Agricultural Research.* 19: 531-543.

May, P.F., Till, A.R. and Cumming, M.J. 1972. System analysis of ^{35}S sulpur kinetics in pastures grazed by sheep. *Journal of Applied Ecology* 9: 25-49.

May, P.F., Till, A.R. and Cumming, M.J. 1973. System analysis of the application methods on the entry of sulphur into pastures grazed by pasture. *Journal of Applied Ecology.* 10: 607-626.

Maynard, D.G., Stewart, J.W.B. and Bettany, J.R 1983a. Sulphur and nitrogen mineralization in soil compared using two incubation techniques. *Soil Biology and Biochemistry* 15: 251-256.

Maynard, D.G., Stewart, J.W.B. and Bettany, J.R. 1983b. Use of plant analysis to predict sulfur deficiency in rapeseed (*Brassica napas* and *B. campestris*). *Canadian Journal of Soil science* 63: 387-391.

Maynard, D.G., Stewart, J.W.B. and Bettany, J.R. 1985. The effects of plants on soil sulfur transformations. *Soil Biology and Biochemistry* 127: 127-134.

McAneney, K.J. and Judd, H.J. 1983. Pasture production and water use measurements in Waikato. *New Zealand Journal of Agricultural Research* 26: 7-13.

McAuliffe, C. and Peech, M. 1949. Utilization of plant of phosphorus in farm manure, 1.Labelling of phosphorus in sheep with ^{32}P. *Soil Science* 68: 179-184.

McAuliffe, C. and Peech, M. 1949. Utilization of plant of phosphorus in farm manure, 1. Availability to plants of organic and inorganic forms of phosphorus in sheep manure. *Soil Science* 68: 185-195.

McCaskill, M.R. and Blair, G.J. 1987. Particle size and soil texture effects on elemental sulfur oxidation. *Agronomy Journal* 79: 1079-1083.

McCaskill, M.R. and Blair, G.J. 1988. Development of a simulation model of sulphur cycling in grazed pastures. *Biogeochemistry* 5: 165-181.

McCaskill, M.R. and Blair, G.J. 1989. A model for the release of sulphur from elemental S and superphosphate. *Fertilizer Research* 19: 77-84.

McCaskill, M.R. and Blair, G.J. 1990. A model of S, P and N uptake by a perennial pature, calibration and predictions. *Fertilizer Research.* 22: 173-179.

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

McLachlan, K.D. and Marco, D.G. 1971. Current and residual effects of calcium sulphate applications on sulphur deficient basaltic soil. *Australia Journal of Agriculture and Animal Husbandry* 11: 64-70.

McLachlan, K.D. and De Marco, D.G. 1975. Changes in soil sulphur fractions with fertilizer additions and cropping treatment. *Australian Journal of Soil Research* 13: 169-176.

McLaren, R.G. and Swift, R.S. 1977. Changes in soil organic matter fractions due to the long term cultivation of soil. *Journal of Soil Science* 28: 445-453.

McLaren, R.G., Ghani, A. and Swift, R.S. 1988. Studies on sulphur mineralization from soils using open incubation. In *Towards the More Efficient Use of Soil and Fertilizer Sulphur.* (Eds. R.E. White and L.D. Currie) Occasional Report No. 2, Fertilizer and Lime Research Centre, Massey University, Palmerston North, pp 66-75.

McLaren, R.G., Keer, J.I. and Swift, R.S. 1985. Sulphur transformations in soils using sulphur-35 labelling. *Soil Biology and Biochemistry* 17: 73-79.

McLay, C.A.D., Cameron, K.C. and McLaren, R.G. 1988. Sulphate leaching in undisturbed soil monoliths and repacked soil columns. In *Towards the More Efficient Use of Soil and Fertilizer Sulphur* (Eds. R.E. White and L.D. Currie) Occasional Report No. 2, Fertilizer and Lime Research Centre, Massey University, Palmerston North, pp 129-136.

McLaughlin, B. 1983. *Taupaka Soil Survey-an exercise in classification and detail mapping in New Zealand hill country.* M. Phil. thesis, Massey University, New Zealand.

McNaught, K.J. 1970. Diagnosis of mineral deficiencies in grass-legumes pasture by plant analysis. *Proceedings of 11th International Grassland Congress,* pp 334-338.

McNaught, K.J. and Christoffels, P.J.E. 1961. Effect of sulphur deficiency on sulphur and nitrogen levels in pastures and lucrene. *New Zealand Journal Agricultural Research* 4: 177-196.

McNaughton, K.J., Clothier, B.E. and Kerr, J.P. 1979. Evaporation from land surfaces. In *Physical Hydrology- A New Zealand Experience.* (Eds., D.L. Murray and L.D Ackroyd) New Zealand Hydrological Society, Wellington, pp 97-119.

Mengel, K. and Kirkby, E.A. 1978. *Principles of Plant Nutrition.* International Potash Institute, Berne, Switzerland.

Metson, A.J. 1969. Sulphur status of New Zealand soils. *Proceedings of Technical Conference of New Zealand Fertilizer Manufacturers Association* 12: 73-84.

Metson, A.J. 1973. *Plant Analysis as a Guide to Sulphur Status of Forage Grasses and Legumes.* The Sulphur Institute. 24 p.

Metson, A.J. 1979a. Sulphur in New Zealand soils 1. A review of sulphur in soils with particular reference to adsorbed sulphate-sulphur. *New Zealand Journal of Agricultural Research* 22: 95-114.

Metson, A.J. 1979b. Sulphur in New Zealand Soils 3. Sulphur distribution in soils derived from parent materials of predominantly sedimentary origin. New Zealand Soil Bureau Scientific Report No. 41: 42 p.

Metson, A.J. 1979c. Sulphur in New Zealand Soils 4. Sulphur distribution in soils derived from parent materials of predominantly volcanic origin. New Zealand Soil Bureau Scientific Report No. 42: 37 p.

Metson, A.J. and Blakemore, L.C. 1978. Sulphate retention by New Zealand soils in relation to the competitive effect of phosphate. *New Zealand Journal of Agricultural Research* 21: 243-253.

Meyer, B. 1977. *Sulfur, Energy and Environment.* Elsevier Scientific Publishing Company.

Microsoft Inc. 1987. Microsoft QuicBASIC 4.0. Microsoft Inc.

Middleton, K.R. and Toxopeus, M.K.J. 1973. Diagnosis and measurement of multiple soil deficiencies by subtractive technique. *Plant and Soil* 38: 219-226.

Minitab Inc. 1989. Minitab Reference Manual. Minitab Inc.

Morton, J.D. and Baird, D.B. 1990. Spatial distribution of dung patches under sheep grazing. *New Zealand Journal of Agricultural Research* 33: 285-294.

Moser, U.S. and Olson, R.V. 1953. Sulphur oxidation in four soils influenced by soil moisture tension and sulphur bacteria. *Soil Science* 76: 251-257.

Muller, F.B. and McSweeney, G. 1974. Effect of granulation of superphosphates on movement of phosphorus and sulphur in two soils. *New Zealand Journal of Agricultural Research* 2: 53-62.

Murphy, J. and Riley, J.P. 1962. Modified single solution method for the determination of phosphate in natural waters. *Analytica Chemica Acta* 27: 31-36.

Murphy, M.D. 1990. Fifteen year of sulphur research in Ireland. *Sulphur in Agriculture* 14: 10-15.

Murphy, M.D. and Borgan, J.M. 1988. Sulphur deficiency in herbage in Ireland 1. cause and extent. *Irish Journal of Agricultural Research* 27: 83-90.

Nearpass, D.C., Fried, M. and Kilmer, V.J. 1961. Greenhouse measurement of available sulfur using radioactive sulfur. *Soil Science Society of America Proceedings* 25: 287-289.

Neptune, A.M., Tabatabai, M.A. and Hanway, J.J. 1975. Sulphur fractions and carbon-nitrogen-phosphorus-sulphur relationships in some Brazilian and Iowa soils. *Soil Science Society of America Proceedings* 39: 51-55.

Nguyen, M.L. 1982. *An Evaluation of S Topdressing Strategies in Eastland Pasture.* M. Agr. Thesis, Massey University. New Zealand, New Zealand.

Nguyen, M.L. 1988. Direct determination of elemental sulphur in soils and the application of this method in measuring oxidation potentials of elemental sulphur fertilizers. In *Towards the More Efficient Use of Soil and Fertilizer Sulphur.* (Eds. R.E. White and L.D. Currie) Occasional Report No. 2, Fertilizer and Lime Research Centre, Massey University, Palmerston North, pp 9-25.

Nguyen, M.L. and Goh, K.M. 1990. Accumulation of soil sulphur fractions in grazed pasture receiving long-term superphosphate application. *New Zealand Journal of Agricultural Research* 33: 111-128.

Nguyen, M.L. and Goh, K.M. 1991. Applicability of the MAF soil sulphate quick test and the MAF sulphur model in predicting short-term sulphur requirement of grazed pastures in Canterbury. In *Soil and Plant Testing for Nutrient Deficiencies and Toxicities.* (Eds. R.E. White and L.D. Currie) Occasional Report No. 5, Fertilizer and Lime Research Centre, Massey University, Palmerston North, pp 280-294.

Nguyen, M.L. and Rickard, D.S. 1988. Soil organic sulphur and its contribution to pasture growth in the absence of superphosphate application. In *Towards the More Efficient Use of Soil and Fertilizer Sulphur*. (Eds. R.E. White and L.D. Currie) Occasional Report No. 2, Fertilizer and Lime Research Centre, Massey University, Palmerston North, pp 292-297.

Nguyen, M.L., Tillman, R.W. and Gregg, P.E.H. 1989a. Evaluation of sulphur status of East Coast pasture of North Island, New Zealand 1. soil and herbage survey. *New Zealand Journal of Agricultural Research* 32: 263-271.

Nguyen, M.L., Tillman, R.W. and Gregg, P.E.H. 1989b. Evaluation of sulphur status of East Coast pasture of the North Island, New Zealand 2. field trials. *New Zealand Journal of Agricultural Research* 32: 273-280.

Nguyen, M.L., Rickard, D.S. and McBride, S.D. 1989. Pasture production and changes in phosphorus and sulphur status in irrigated pastures receiving long-term application of supherphosphate. *New Zealand Journal of Agricultural Research* 32: 245-262.

Nunn, R.J. and Dee, T.P. 1954. Superphosphate production: the influence of various factors on speed of reaction and the composition of the product. *Journal of Science and Food and Agriculture* 5: 257-265.

Nye, P.H. and Tinker, P.B.H. 1977. Solute movement in the soil-root system. In *Studies in Ecology* No. 4. Scientific Publications. 342 p.

Oertli, J.J. 1990. Limitation to the diagnostic information obtained from soil analysis. *Fertilizer Research* 26: 189-196.

Olsen, S.R., Cole, C.V. Watanabe, F.S. and Dean, L.A. 1954. Estimation of available phosphorus in soil by extraction with sodium bicarbonate. United States Department of Agriculture, Circular 937.

Parfitt, R.L. and Smart, R.C. 1978. The mechanism of sulfate adsorption on iron oxides. *Soil Science Society of America Journal* 42: 48-50.

Payne, D. 1988. The behaviour of water in soil. In *Russell's Soil Conditions and Plant Growth* (Ed. A. Wild), John Wiley & sons, New York.

Perrott, K.W. and Surathchandra, S.U. 1987. Nutrient and organic matter levels in a range of New Zealand soils under established pasture. *New Zealand Journal of Agricultural Research* 30: 249-259.

Petersen, R.G., Lucal, H.L. and Woodhouse, W.W. 1956. Distribution of excreta by freely grazing cattle ant its effect on pasture fertility. *Agronomy Journal* 48: 440-444.

Peverill, K.I. and Douglas, L.A. 1976. Use of undisturbed cores of surface soil for investigating leaching losses of sulphur and phosphorus. *Geoderma.* 193-199.

Peverill, K.I., Briner, G.P. and Douglas, L.A. 1975. Changes in extractable sulphur and potassium levels in soil due to oven drying and storage. *Australian Journal of Soil Research* 13: 67-75.

Peverill, K.I., Douglus, L.A. and Greenhill, N.B. 1977. Leaching losses of applied P and S from undisturbed cores of some Australian surface soils. *Geoderma* 19: 91-96.

Pirela, H.J. and Tabatabai, M.A. 1988. Sulfur mineralization rates and potential of soils. *Biology and Fertility of Soils* 6: 26-32.

Pollok, J.A. 1975. *A Compartive Study of Certain New Zealand and German Soil Formed From Loess.* Ph.D. thesis, Friedrich-Wihelms Universitat, Bonn.

Pollok, J.A and McLaughlin, B. 1986. The Soils of Taupaka Farm. Massey University, Taupaka Farm Series. Publication No. 3.

Postgate, J.R. 1968. Inorganic Sulphur Chemistry. In *The Sulphur Cycle.* (Ed. G. Nickless) Elsevire Scientific Publishing Co., New York, pp 259-272.

Priestly, C.H.B. and Taylor, R.J. 1972. On assessment of surface heat flux and evaporation using a large-scale parameters. *Monthly Weather Review* 100: 81-92.

Probert, M.E. 1976. Studies of 'available' and isotopically exchangeable sulphur in some North Queensland soils. *Plant and Soil* 45: 461-475.

Probert, M.E. and Jones, K.R
. 1977. The use of soil analysis for predicting the response to sulphur of pasture legumes in the Australian tropics. *Australian Journal of Agricultural Research* 15: 137-146.

Rajan, S.S.S. 1978. Sulfate adsorbed on hydrous alumina, ligannd displaced , and changes in surface charge. *Soil Science Society of America Journal* 42: 39-44.

Randall, P. and Sakai, H. 1983. Sulfur analytical methods for soils, plants and animal samples. In *Sulphur in South East Asian and South Pacific Agriculture.* (Ed. G.J. Blair), pp 255-269.

Raymant, G.E. 1983. Prediction of response to sulphur by established sirato/grass pasture in Southeast Queensland. *Australian Journal of Experimental Agriculture and Animal Husbandry* 23: 280-287.

Reisenauer, H.M. 1975. Soil assays for the recognition of sulphur deficiency. In *Sulphur in Australasian Agriculture.* (Ed. by K.D. McLachlan). Sydney University Press, pp 182-187.

Reisenauer, H.M., Walsh, L.M. and Hoeft, R.G. 1973. Testing soil for sulfur, boron, molybdenum and chlorine. In *Soil Testing and Plant Analysis.* (Eds. L.M. Walsh and J.D. Beaton). Soil Science Society of America Inc., pp 173-200.

Rennenberg, H. 1984. The fate of excess sulpuur in higher plants. *Annual Review of Plant Physiology* 35: 121-153.

Reuter, D.J. and Robinson, J.B. 1986. *Plant Analysis: an interpretation and manual.* Itaka Press, Melbourne, Australia.

Rhue, R.D. and Kamprath, E.J. 1973. Leaching losses of sulfur during winter months when applied as gypsum, elemental S or prilled S. *Agronomy Journal* 65: 603-605.

Richard, I.R. and Wolton, K.M. 1976. The spatial distribution of excreta under intensive cattle grazing. *Journal of British Grassland Society* 31:89-92.

Rickard, D.S., McBride, S.D. and Fitzgerald, P.D. 1986. The effect of soil moisture on pasture yield. *New Zealand Journal of Agricultural Science* 20: 7-12.

Risk, W.H. and Boswell, C.C. 1988. Sulphate sulphur in the subsoil of Southland and Otago soils. In *Towards the More Efficient Use of Soil and Fertilizer Sulphur.* (Eds. R.E. White and L.D. Currie) Occasional Report No. 2, Fertilizer and Lime Research Centre, Massey University, Palmerston North, pp 118-128.

Rogers, D.A. 1985. The importance of the form of sulphur and the sulphur to phosphorus ratio in fertilizers. *Proceedings 20th Technical Conference of New Zealand Fertilizer Manufacturers' Research Association,* pp 573-583.

Rorison, I.H. 1973. The effect of soil acidity on nutrient uptake and physiology of plants. In *Acid Sulphate Soils.* (Ed. H. Dost) Wageningen, International Institute of Land Reclamation and Improvement 1: 223-254".

Rothbaum, H.P., Sewell, J.R. and Evason, W. 1980. Water-degradable sulphur/anhydrite pellets for fertilizer use. *New Zealand Journal of Science* 23: 371-382.

Rowarth, J.S. 1987. *Phosphorus Cycling in Grazed Hill-Country Pasture.* Ph.D. thesis, Massey University, New Zealand.

Rowarth, J.S., Gillingham, A.G. and Syers, J.K. 1988. Effects of season and fertilizer rate on phosphate concentrations in pasture and sheep faeces. *New Zealand Journal of Agricultural Research* 31: 187-193.

Rowarth, J.S., Gillingham, A.G., Tillman, R.W. and Syers, J.K. 1985. Release of phosphorus from sheep faeces on graze hill country pasture. *New Zealand Journal of Agricultural Research* 28: 497-504.

Rowarth, J.S., Tillman, R.W., and Gillingham, A.G. 1990. comparison of release of phosphorus from monocalcium phosphate and sheep faeces. *New Zealand Journal of Agricultural Research* 33: 473-477.

Roy, A.B. and Trudinger, P.A. 1970. *The Biochemistry of Inorganic Compounds of Sulphur.* Cambridge University Press, Cambridge 400 p.

Sachdev, M.S. and Chhabra, P. 1974. Transformation of ^{35}S labelled sulphate in aerobic and flooded soil conditions. *Plant and Soil* 41: 335-341.

Saggar, S. 1980. *Studies of Sulfur in Relationships to Carbon and Nitrogen in Soil Organic Matter.* Ph.D. thesis, University of Saskatchewan.

Saggar, S and Hedley, M.J. 1989. Some recent development and areas for future research into soil and fertilizer in New Zealand. *SCOPE Workshop on Sulphur Cycle in the Wetland and Terrestrial Ecosystems.* Peterborough, Canada.

Saggar, S., Bettany, J.R. and Stewart, J.W.B. 1981a. Measurement of microbial sulfur in soil. *Soil Biology and Biochemistry* 13: 493-498.

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

Saggar, S., Mackay, A.D., Hedley, M.J., Lambert, M.G. and Clark, D.A. 1990a. A nutrient transfer model to explain the fate of phosphorus and sulphur in grazed hill-country pasture. *Agriculture, Ecosystem and Environment* 30: 295-315.

Saggar, S., Hedley, M.J., Gillingham, A.G., Rowarth, J.S., Richardson, S., Bolan, N.S. and Gregg, P.E.H. 1990b. Predicting the fate of fertilizer sulphur in grazed hill country pasture by modelling the transfer and accumulation of soil phosphorus. *New Zealand Journal of Agricultural Research* 33: 129-138.

Saggar, S., Hedley, M.J., White, R.E., Gregg, P.E.H., Perrott, K.W. and Cornforth, I.S. 1991. A glasshouse assessment of the ability of Olsen and resin P extractions to predict the relative yield of ryegrass in soils fertilized with phosphate rock. In *Soil and Plant Testing for Nutrient Deficiencies and Toxicities.* (Eds. R.E. White and L.D. Currie) Occasional Report No. 5, Fertilizer and Lime Research Centre, Massey University, Palmerston North, pp 149-150.

Sakadevan, K. 1991. *Factors Influencing the Transformation and Fate of Sulphur and Nitrogen in Grazed Hill Country Pasture.* Ph.D. thesis, Massey University, New Zealand.

SAS. 1985. *SAS User's Guide: Statistics.* Version 5, SAS Institute Inc. 956 p.

Saunders, W.M.H. 1965. Phosphate retention by New Zealand soils and its relationships to free sesquioxides, organic matter, and other soil properties. *New Zealand Journal of Agricultural Research* 8: 30-57.

Saunders, W.M.H. and Cooper, D.M. 1975. Evaluation of soil and plant analysis to determine sulphur status of New Zealand soils. In *Sulphur in Australasian Agriculture* (Ed. by K.D. McLachlan). Sydney University Press, pp 188-195.

Saunders, W.M.H. and Hogg, D.E. 1971. Significance of phosphate and sulphate retention. Technical report 1971/1 Ruakura Soil Research Station. pp 1-9.

Saunders, W.M.H., Cooper, D.M. and Sinclair, A.G. 1981. Soil and fertilizer- Sulphur: use and interpretation of sulphur in soil tests for pastures. Aglink, FPP 649, Ministry of Agriculture and Fisheries, Wellington, New Zealand, pp 1-2.

Saunders, W.M.H., Cooper, D.M. and Gravett, I.M. 1988. Response to sulphur and comparison of soil tests for predicting sulphur status of soils for grazed clover-grass pastures in New Zealand. *New Zealand Journal of Agricultural Research* 31: 195-203.

Scharpenseel, W.H. and Krausse, R. 1963. Radiochromatographic investigations on the cycle of sulfate and the S-amino acid cysteine and methionine. Z. Pflanzenernahr, Dung Bodenk 101: 11-23.

Schindler, S.C. and Mitchell, M.J. 1987. Dynamics of ^{35}S in horizons and leachates from a Hardwood forest Spodosol. *Soil Biology and Biochemistry* 19: 531- 538.

Schindler, S.C., Mitchell, M.J., Scott T.J., Fuller, R.d. and Driscoll, C.T. 1986. Incorporation of ^{35}S sulfate into inorganic and inorganic constituents of two forest soils. *Soil Science Society of American Journal* 50: 457-462.

Schnug, E. 1990. Sulphur nutrition and quality of vegetables. *Sulphur in Agriculture* 14: 3-7.

Scott, N.M. 1981. Evaluation of sulphur status of soil by plant and soil tests. *Journal of the Science of Food and Agriculture* 32: 173-191.

Scott, N.M. 1985. Sulphur in soils and plant. In *Soil Organic Matter and Biological Activity.* Development in plant and soil sciences 16: 379-402, (Eds. D Vaughan and R.E. Malcolm) Martinus Nijhoff/Dr W Junk Publishers, pp 379-402.

Scotter, D.R., Clother, B.E. and Turner, M.A. 1979. The soil water balance in Fragiaqualf and its effect on pasture growth in central New Zealand. *Australian Journal of Soil Research* 17: 455-465.

Searle, P.L. 1979. Measurement of adsorbed sulphate in soils : effects of varying soil:extractant ratios and method of measurement. *New Zealand Journal of Agricultural Research* 22: 287-290.

Searle, P.L. 1982. Sulphate retention studies of three Yellow-brown loams. *Soil of New Zealand, Part 6*, Department of Scientific and Industrial Research, pp 68-75.

Searle, P.l. and Sparling, G.P. 1987. The determination of air-drying and storage conditions on the amounts of sulphate and phosphate extracted from a range of new Zealand topsoils. *Communication in Soil Science and Plant Analysis* 18: 725-734.

Searle, P.L., and Speir, T.W. 1988. An anion exchange membrane method for extracting soil sulphate and a research application. In *Towards the More Efficient Use of Soil and Fertilizer Sulphur.* (Eds. R.E. White and L.D. Currie) Occasional Report No. 2, Fertilizer and Lime Research Centre, Massey University, Palmerston North, pp 47-53.

Sears. P.D. 1950. Soil fertility and pasture growth. *Journal of British Grassland Society.* 5: 267-280.

Sears, P.D. and Newbold, R.P. 1942. The effect of sheep droppings on yield and botanical composition, and chemical composition of pasture 1. establishment of the trial, technique of measurement and result for 1940-41 season. *New Zealand Journal of Agricultural Science and Technology* A:24: 36-61.

Sears, P.D. and Goodall, V.C. 1948. The effect of sheep droppings on yield, botanical composition and chemical composition of pasture 2. results for the years 1942-1944 and final summary of the trial. *The New Zealand Journal of Science and Technology* 30: 231-250.

Shamoot, S., McDonald, L. and Batholomew, W.W. 1968. Rhizo-deposition of organic debris in soil. *Soil Science Society of America Proceedings* 32: 817-820.

Sheard, R.W., Jackman, R.H. and Butler, G.W. 1978. Utilization by white clover and ryegrass so sulphur from ^{35}S labelled gypsum. *Communication in Soil Science and Plant Analysis* 9: 935-954.

Shedley, C.D. 1982. *An Evaluation of Elemental Sulphur as a Pasture Fertilizer.* Ph.D. thesis, University of New England, Australia.

Shedley, C.D. and Till, A.R. and Blair, G.J. 1979. A radiotracer technique for studying of nutrient release from different fertilizer materials and its uptake by plants. *Communication in Soil Science and Plant Analysis* 10: 737-745.

Shipley, R.A. and Clark, R.E. 1972. *Tracer Method for in vivo Kinetics: theory and applications.* Academic Press Inc. 330 p.

Shock, C.C., Jones, M.B., Williams, W.A. and Center, C.M. 1983. Effect of sulfur fertilization on three annual range species 1. Laughlin soil experiment. *Agronomy Journal* 75: 515-520.

Siems, H.B. 1953. Chemistry and manufacture of superphosphates and phosphoric acid. In *Fertilizer Technology and Resource.* (Ed. K.D. Jacob), pp 167-204.

Sinclair, A.G. 1982. Soil and fertilizer sulphur: sulphate leaching index. AgLink, Ministry of Agriculture and Fishery, New Zealand.

Sinclair, A.G. 1983. Problems in modelling sulphur requirements in New Zealand. In *Sulphur in New Zealand Agriculture.* (Eds. P.E.H. Gregg and J.K.Syers) Occasional report No, 5, Massey University, Palmerston North, pp 106-117.

Sinclair, A.G. and Boswell, C.C. 1983. The agronomic properties of sulphur fertilizers. *Proceedings of the 19th Technical Conference of the New Zealand Fertilizer Manufacturer's Research Association,* pp 477-389.

Sinclair, A.G. and Cornforth, I.S. 1981. Superchoice: a model of calculating superphosphate requirement in grazed pastures. Proceedings Lincoln College Farmer Conference, pp 57-65.

Sinclair, A.G. and Enright, D.P. 1983. Effectiveness of elemental sulphur fertilizers. In *Sulphur in New Zealand Agriculture.* (Eds. P.E.H. Gregg and J.K.Syers) Occasional report No, 5, Massey University, Palmerston North, pp 164-175.

Sinclair, A.G. and Jones, M.B. 1991. Use of the diagnosis and recommendation integrated system (DRIS) on New Zealand clover-based pastures. In *Soil and Plant Testing for Nutrient Deficiencies and Toxicities* (Eds. R.E. White and L.D. Currie) Occasional Report No. 5, Fertilizer and Lime Research Centre, Massey University, Palmerston North, pp 304-317.

Sinclair, A.G. and Saunders, W.H.M. 1982. Sulphur fertilizer in New Zealand. *Sulphur in Agriculture* 6: 15-22.

Sinclair, A.G. and Saunders, W.H.M. 1984. Sulphur. In *Fertilizer and Lime Recommendations for Pastures and Crops in New Zealand* (Eds. I.S. Cornforth and A.G. Sinclair) New Zealand Ministry of Agriculture and Fisheries, Wellington, pp 15-17.

Sinclair, A.G., Boswell, C.C., Cornforth, I.S., Lee, A., Morgan, C., Morton, J., Nguyen, M.L., Saunders, W.H.M., Shannon, P., Smith R.G. and Whelan, G. 1985. Agronomic requirements of sulphur in New Zealand Pastures. *Proceeding of the 20th Technical Conference of the New Zealand Fertilizer Manufacturer's Research Association,* pp 538-572.

Skjelbreid, E. and Nissen, P. 1980. Effect of Calcium on sulphate uptake by barley roots. *Physiology and Plant* 49: 383-387.

Skrijka, P. 1987. Investigation of the fertilizer value of sheep excrements left of pasture. In *Animal Manure on Grassland and Fodder Crops.* (Ed. H.G. v.d. Meer), Martinus Nijhoff Publishers, Netherlands, pp 325-327.

Smith, C.M. 1976. *Occurrence of Sulphur Deficiency in some Manawatu Soils.* Diss. Dip. of Agri Sci. Massey University, New Zealand.

Smith, C.M. 1979. *Leaching and Runoff Losses of Sulphur and Potassium from a Tokomaru Soil.* M. Agr. Thesis, Massey University, New Zealand.

Smith, C.M., Gregg, P.E.H. and Tillman, R.W. 1983. Drainage losses of sulphur from a yellow-grey earth soil. *New Zealand Journal of Agricultural Research* 26: 363-371.

Smith, F. and Siregar, M.E. 1983. Sulfur requirements of tropical forages. In *Sulphur in South East Asian and South Pacific Agriculture.* (Ed.3 G.J. Blair), pp 76-86.

Smith, R.G. and McDougall, D.B. 1988. Evaluation of sulphur/sodium bentonite prill as sulphur fertilizers in the Manawatu. In *Towards the More Efficient Use of Soil and Fertilizer Sulphur.* (Eds. R.E. White and L.D. Currie) Occasional Report No. 2, Fertilizer and Lime Research Centre, Massey University, Palmerston North pp 169-178.

Sorn-srivichai, P. 1980. *Mineralization of Soil Organic Sulphur in the Tokomaru Silt Loam.* M.Agr.Sci. thesis, Massey University, New Zealand.

Sorn-srivichai, P 1985. *An Evaluation of Water Extraction as Soil Testing Procedure for Phosphate.* Ph.D. thesis, Massey University, New Zealand.

Sparling, G.P. 1985. The soil biomass. In *Soil Organic Matter and Biological Activity.* Development in plant and soil sciences (Eds. D Vaughan and R.E. Malcolm), Martinus Nijhoff/Dr W Junk Publishers 16: 223-263

Sparling, G.P., Whale, K.N. and Ramsay, A.J. 1985. Quantifying the contribution from soil microbial biomass to extractable P level from fresh and air-dried soils. *Australian Journal of Soil Research* 23: 613-621.

Speir, G.A. and McGill, W.B. 1979. Effect of phosphorus addition and energy supply on acid phosphatase production and activity in soils. *Soil Biology and Biochemistry* 11: 3-8.

Speir, T.W. 1977. Studies on climo-sequence of soils in Tussock grasslands 2. urease, phosphatase, and sulphatase activities with other properties including plant available sulphur. *New Zealand Journal of Science* 20: 159-166.

Spencer, K. 1975. Sulphur requirements of plants. In *Sulphur in Australasian Agriculture.* (Ed. by K.D. McLachlan). Sydney University Press, pp 98-108.

Spencer, K. and Freney, J.R. 1960. Comparison of several procedures for estimating sulphur status of soils. *Australian Journal of Agricultural Research* 2: 948-959.

Spencer, K. and Glendinning, J.S. 1980. Critical soil test values for predicting the phosphorus and sulfur status of sub-humid temperate pastures. *Australian Journal of Soil Research* 18: 435-445.

Spencer, B. and Harada, T. 1960. The role of choline sulphate in the sulphur metabolism of fungi. *Biochemistry Journal* 77: 305-315.

Spencer, K., Jones, M.B. and Freney, J.R. 1977a. Diagnostic indices for sulphur status of subterranean clover. *Australian Journal of Agricultural Research* 28: 401-412.

Spencer, K., Freney, J.R. and Jones, M.B. 1977b. Diagnosis of sulphur deficiency in subterranean clover. *Sulphur in Agriculture* 1: 12-17.

Stevenson, F.J. 1986. *Cycle of Soil.* John Wiley & Sons, New York.

Stewart, B.A., Porter, L.K. and Viets, F.G. Jr. 1966a. Sulfur requirements for decomposition of cellulose and glucose in soil. *Soil Science Society of America Proceedings* 30: 355-358.

Stewart, B.A., Porter, L.K. and Viets, F.G. Jr. 1966b. Effect of sulfur containing content of straw on rate of decomposition and plant growth. *Soil Science Society of America Proceedings* 30: 453-456.

Strick, J.E. and Nakas, J.P. 1984. Calibration of microbial sulfur technique for use inn forest soils. *Soil Biology and Biochemistry* 16: 289-291.

Strickland, T.C. and Fitzgerald, J.W. 1985. Incorporation of sulphate-sulphur into organic matter extracts of litter and soil: involvement of ATP sulphurylase. *Soil Biology and Biochemistry* 17: 779-784.

Strickland, T.C. and Fitzgerald, J.W. 1987. Bacterial production of organic sulphur in a forest litter extract. *Soil Biology and Biochemistry* 19: 771-774.

Strickland, T.C., Fitzgerald, J.W., Ash, J.T. and Swank, W.T. 1987. Organic sulfur transformations and sulfur pool sizes in soil and litter from a southern Aplachian hardwood forest. *Soil Science* 143: 453-458.

Stuanes, A.O. 1985. Yearly and seasonal variation in extractable soil sulphate at two locations in Norways. *Acta Agriculture Scandinavian* 35: 33-40.

Stumm, W. and Morgan, J.J. 1970. *Aquatic Chemistry.* John Wiley & Sons Inc., New York.

Swank, W.T. and Fitzgerald, J.W. 1984. Microbial transformation of sulfate in forest soils. *Science* 223: 182-184.

Swanney, B., Boswell, C.C., Enwright, P.D. and Sinclair, A.G. 1988. A comparative field evaluation of some potential sulfur fertilizer materials. *New Zealand Journal of Agricultural Research* 16: 183-192,.

Swift, R.S. 1977. Mineralization of nitrogen and sulphur from soil organic matter. In *Soil Organic Studies.* Proceedings; symposium IAEA Viena, pp 275-281.

Swift, R.S. 1983. Mineralization and immobilization of sulphur in soils. In *Sulphur in New Zealand Agriculture.* (Eds. P.E.H. Gregg and J.K.Syers) Occasional report No, 5, Massey University, Palmerston North, pp 18-28.

Swift, R.S., McLaren, R.G. and Keer, J.I. 1988. Some method for the extraction and fractionation of soil organic sulphur. In *Towards the More Efficient Use of Soil and Fertilizer Sulphur.* (Eds. R.E. White and L.D. Currie) Occasional Report No. 2, Fertilizer and Lime Research Centre, Massey University, Palmerston North, pp 55-65.

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

Syers, J.K., Curtin, D. 1987. Soil and Fertilizer Fulphur in UK Agriculture. Paper read before The Fertilizer Society of London on 10 December 1987.

Tabatabai, M.A. 1982. Sulfur. In *Methods of Soil Analysis, Part 2, Chemical and Microbiological Properties.* (Ed. A.L. Page), Soil Science Society of America Inc.

Tabatabai, M.A. 1987. Physicochemical fate of sulfate in soils. *JAPA the International Journal of Air Pollution and control and Harzardous Waste Management* 37: 34-39.

Tabatabai, M.A. and Al-khafaji, A.A. 1980. Comparison of nitrogen and sulfur mineralization in soils. *Soil Science Society of America Journal* 44: 1000-1006.

Tabatabai, M.A. and Bremner, J.M. 1970a. An alkaline method for the determination of total sulfur in soils. *Soil Science Society of America Proceedings* 34: 62-65.

Tabatabai, M.A. and Bremner, J.M. 1970b. Factors affecting soil arysulfatase activities. *Soil Science Society of America Proceedings* 34: 427-429.

Tabatabai, M.A. and Bremner, J.M. 1972a. Forms of sulfur, and carbon, nitrogen and sulfur relationships, in Iowa soils. *Soil Science* 114: 380-386.

Tabatabai, M.A. and Bremner, J.M. 1972b. Distribution of total and available sulfur in selected soils profiles. *Agronomy Journal* 64: 40-44.

Takebe, I. 1960. Choline sulphate as major soluble component of condiospores of *Aspergillus niger. Journal of General Applied Microbiology* 6: 83-89.

Tallis, G.H. and Donald, A.D. 1964. Model for the distribution on pasture of infective larvae of the gastrointestinal nematode parasites of sheep. *Australian Journal of Biological Science* 17: 504-513.

Taylor, J.A. 1980. *Marino Sheep and the Interpaddock Pattern of Herbaceous Species on the Northern Tablelands of New South Wales, Australia.* Ph.D. thesis, University of New England.

Thorrold, B.S., O'conner, K.F. and White, J.G.H. 1985. Management influences on sheep behaviour, dung distribution and soil phosphate. *Proceedings of the New Zealand Grassland Association* 46: 127-134.

Till, A.R. 1975. Sulphur cycle in grazed pasture. In *Sulphur in Australasian Agriculture.* (Ed. by K.D. McLachlan). Sydney University Press, pp 68-76.

Till, A.R. 1979. Nutrient cycling. In *Grassland Ecosystems of the World: Analysis of Grassland and Their Uses.* (Ed., R.T. Coupland), Cambridge University Press, London, pp 277-285.

Till, A.R. and Blair, G.J. 1978. The utilization by grass of sulphur and phosphorus from clover litter. *Australian Journal of Agricultural Research* 29: 235-242.

Till, A.R. and May, P.F. 1970a. Nutrients cycling in grazed pastures 2. Further observation with (35-S) gypsum. *Australian Journal of Agricultural Research* 21: 253-260.

Till, A.R., and May, P.F. 1970b. Nutrient cycling in grazed pastures 3. Studies on labelling of the grazed pasture system. *Australian Journal of Agricultural Research* 21: 455-463.

Till, A.R. and McCabe, T.P. 1976. Sulphur leaching and lysimeter characterization. *Soil Science* 121: 44-47.

Till, A.R., and May, P.F. 1971. Nutrient cycling in grazed pastures 4. the fate of sulphur-35 following its application to small area in grazed pasture. *Australian Journal of Agricultural Research* 22: 391-400.

Till, A.R., Blair, G.J. and Dalal, R.C. 1982. Isotopic studies of the recycling of carbon, nitrogen, sulphur and phosphorus from plant material. In *The Cycling of Carbon, Nitrogen Sulphur and Phosphorus in Terrestrial and Aquatic Ecosystems.* (Ed. I.E. Galbally and J.R. Freney) Australian Academic of Science, pp 51-59.

Till, A.R., Saunders, W.H.M., Sinclair, A.G. 1987. Plant nutrient requirements; Oceania. In *World Regional Plant Requirements Plant Nutrient Sulphur, Trade, Promotion and Marketing. Sulphur 87. Proceedings*, Houston, Texas pp 102-115.

Tisdale, S.L. 1977. Sulphur in Forage Quality and Ruminant Nutrition. Technilcal Bulletin No. 22 The Sulphur Institute.

Tisdale, S.L., Reneau Jr., R.B. and Platou, J.S. 1986. Atlas of sulfur deficiencies. In *Sulphur in Agriculture.* (Ed. M.A. Tabatabai), Agronomy series number 27, American Society of Agronomy, pp 295-322.

Toxopeus, M.R.J. 1970. Sulphur deficiency in grassland on young soils from volcanic ash and its possible prediction by laboratory tests. *Proceeding of 11th International Grassland Congress,* pp 346-346.

Toxopeus, M.R.J. and Gordon, R.B. 1971. Rational fertilizer practice on pumice soils. *Proceedings of New Zealand Grassland Association.* 33: 35-41.

Troughton, A. 1957. The underground organ of herbage grasses. Bulletin 44. 163 p.

Trudinger, P.A. 1979. The biological of sulphur cycle. In *Biogeochemical Cyling of Mineral Forming Elements.* (Eds. P.A. Trudinger and D.J. Swaine) Elsevier, Amsterdam, pp 293-313.

Tsuji, T. and Goh, K.M. 1979. Evaluation of soil sulphur fractions as sources of plant-available sulphur using radioactive sulphur. *New Zealand Journal of Agricultural Research* 22: 595-602.

Van Praag, H.J. 1973. Cycle interne de I' azote et du soufre dans horizons humiferes forestiers des sols bruns acides III cycle interne du soufre. *Plant and Soil* 39: 61-69.

Vaughn, C.E. 1987. Sulphur soil test on Northern California subclover-annual grass pastures surface soils. *Soil Science* 143: 184-191.

Vitolin, M.I. and Swaby, R.J. 1969. Activity of sulphur-oxidizing microorganism in some Australian soils. *Australian Journal of Soil Research* 7: 171-183.

Vose, P.B. 1980. *Introduction to Nuclear Techniques in Agronomy and Plant Bilogy.* Pergamon Press. 391 p.

Wainwright, M. 1978. *Microbial Sulphur Oxidation in Soil.* Science Progress, Oxford, 65: 459-475.

Wainwright, M. 1984. Sulphur Oxidation in soils. *Advance in Agronomy.* 37: 350-392.

Wainwright, M., Nevel, W. and Grayston, S.J. 1986. Effect of organic matter on sulphur oxidation in soil and influence of sulphur oxidation in soil nitrification. *Plant and Soil* 96: 369-376.

Walker, T.W. 1957. The sulphur cycle in grassland soils. *Journal of British Grassland Society* 12: 10-18.

Walker, T.W. 1968. Changes induced in the soil by pastoral farming. In *Soil of New Zealand.* part 1, Soil Bureau Bulletin 26(1), New Zealand Department of Scientific and Industrial Research, pp 104-109.

Walker, T.W. and Adams, A.F.R. 1958a. Studies on soil organic matter I. influence of phosphorus content of parent materials on accumulations of carbon, nitrogen, sulphur and organic phosphorus in grassland soils. *Soil Science* 85: 307-318.

Walker, T.W. and Adams, A.F.R. 1959. Studies on soil organic matter 2. influence of increased leaching at various stages of weathering on level of carbon, nitrogen, sulphur, and organic and total phosphorus. *Soil Science* 87: 3-10.

Walker, T.W. and Adams, A.F.R. 1958b. Competition for sulphur in a grass-clover association. *Plant and Soil* 9: 353-366.

Walker, T.W. and Gregg, P.E.H. 1975. The occurrence of sulphur deficiency in New Zealand. In *Sulphur in Australasian Agriculture.* (Ed. by K.D. McLachlan). Sydney University Press, pp 145-154.

Walker, T.W., Thupa, B.K. and Adams, A.F.R. 1959. Studies on soil organic matter 3. accumulation of carbon, nitrogen and sulphur organic and total phosphorus in improved grassland soil. *Soil Science* 87: 135-139.

Watkin, B.R. 1954. The animal factor and level of nitrogen. *Journal of British Grassland Society* 30: 35-40.

Watkinson, J.H. 1988. Rate equation for the oxidation of elemental sulphur in soil. In *Towards the More Efficient Use of Soil and Fertilizer Sulphur.* (Eds. R.E. White and L.D. Currie) Occasional Report No. 2, Fertilizer and Lime Research Centre, Massey University, Palmerston North, pp 26-31.

Watkinson, J.H. 1989. Measurement of the oxidation rate of elemental sulphur in soil. *Australian Journal of Soil Research* 27: 365-375.

Watkinson, J.H. and Perrott, K.W. 1990. A new soil test for sulphate and mineralized organic sulphur. *Proceeding of the new Zealand Fertlizer Manufacturer'S Research Association Conference, 1990 'the Challenges for Fertilizer research in 1990'*, pp 188-201.

Watkinson, J.H., Lee, A. and Lauren, D.R. 1987. Measurement of elemental sulfur in soil and sediments: Field sampling, sample storage, pretreatment, extraction and analysis by High-performance liquid chromatography. *Australian Journal of Soil Research* 25: 167-178.

Watkinson, J.H., Perrott, K.W. and Thorrold, B.S. 1991. Relationship between the MAF pasture development index of soil and extractable organic sulphur. In *Soil and Plant Testing for Nutrient Deficiencies and Toxicities* (Eds. R.E. White and L.D. Currie) Occasional Report No. 5, Fertilizer and Lime Research Centre, Massey University, Palmerston North, pp 66-71.

Watson, E.R. and Lapins, P. 1969. Loss of nitrogen from urine on soils from South-western Australia. *Australian Journal of Experimental Agriculture and Animal Husbandry* 9: 85-91.

Weeda, W.C. 1967. The effect of cattle dung patches on pasture growth, botanical composition and pasture utilization. *New Zealand Journal of Agricultural Research* 10: 150-159.

Weir, R.G. 1975. The oxidation of elemental sulphur and sulphides in soils. In *Sulphur in Australasian Agriculture.* (Ed. by K.D. McLachlan). Sydney University Press, pp 40-49.

Westerman, D.T. 1974. Indexes of sulfur deficiency in alfalfa. 1. extractable soil $SO_4^{=}$-S. *Agronomy Journal* 66: 578-581.

Whitaker, R.H. 1970. Communities and ecosystems. In *Current Concepts in Biology Series.* The Macmillan Co., Collier-Macmillan Ltd., London.

White, J.G. 1959. Mineralization of nitrogen and sulfur in sulfur deficient soil. *New Zealand Journal of Agricultural Research* 2: 255-258.

Widdowson, J.P. 1970. *Available of Sulphur in Iowa Soils.* Ph.D. thesis, Iowa State University.

Wilkinson, S.R. and Lowrey, R.W. 1973. Cycling of mineral nutrient in pasture ecosystem. In *Chemistry and Biochemistry of Herbage.* (Eds. G.W. Buttler and R.W. Bailey) 2: 247-315.

Williams, C.H. 1967. Some factors affecting the mineralization of organic sulphur in soils. *Plant and Soil* 26: 205-223.

Williams, C.H. 1968. Seasonal fluctuations in mineral sulphur under subterranean clover pasture in Southern New South Wales. *Australian Journal of Soil Research* 6: 131-139.

Williams, C.H. 1971a. Reaction of surface-applied superphosphate with soil, 1. the fertilizer solution and its initial reaction with soil. *Australian Journal of Soil Research* 9: 83-94.

Williams, C.H. 1971b. Reaction of surface-applied superphosphate with soil, 2. movement of the phosphate and sulphur into the soil. *Australian Journal of Soil Research* 9: 95-106.

Williams, C.H. 1974. The chemical nature of sulphur in some New South Wales soils. In *Handbook on Sulphur in Australian Agriculture.* (K.D. McLachlan), CSIRO, Melbourne.

Williams, C.H. 1975. The chemical nature of sulphur compounds in soils. In *Sulphur in Australasian Agriculture.* (Ed. K.D. McLachlan). Sydney University Press, Sydney, pp 21-30.

Williams, C.H. and Steinberges, A. 1958. Sulphur and phosphorus in some Eastern Australian soils. *Australian Journal of Agricultural Research* 9: 483-491.

Williams, C.H. and Steinbergs, A. 1959. Soil sulphur fractions as chemical indices of available sulphur in some Australian soils. *Australian Journal of Agricultural Research* 10: 340-352.

Williams, C.H. and Steinbergs, A. 1962. The evaluation of plant-available sulphur in soils 1. The chemical nature of sulphate in some Australian soils. *Plant and Soil* 17: 279-294.

Williams, C.H. and Steinbergs, A. 1964. The evaluation of plant-available sulphur in soil II. the availability of adsorbed and soluble sulphates. *Plant and Soil* 21: 50-62.

Williams, P.H. 1988. *The Fate of Potassium in Grazed Dairy Pastures.* Ph.D. thesis, Massey University, New Zealand.

Williams, P.H. and Morton, J.D. 1985. Pasture responses to superphosphate and elemental sulphur on soils of West Coast, South Island. *New Zealand Journal of Experimental Agriculture* 13: 369-374.

Williams, P.H., Gregg, P.E.H. and Hedley, M.J. 1990a. Fate of potassium in diary cow urine applied to intact cores. *New Zealand Journal of Agricultural Research* 33: 151-158.

Williams, P.H., Hedley, M.J.H. and Horne, D.J. 1988. The effect of drainage on some aspects of nutrient loss from silt loam under dairying. Proceedings of the 4th National Drainage Seminar, New Zealand.

Williams, P.H., Hedley, M.J. and Gregg, P.E.H. 1989. Uptake of potassium and nitrogen by pasture from urine-affected soil. *New Zealand Journal of Agricultural Research* 32: 415-421.

Williams, P.H., Hedley, M.J. and Gregg, P.E.H. 1990b. The effect of preferential flow of dairy cow urine and simulate rainfall on movement of potassium through undisturbed top soil cores. *Australian Journal of Soil Research* 28: 857-867.

Young, R.D., Westfall, D.G. and Colliver, G.W. 1985. Production, marketing and use of phosphorus fertilizer. In *Fertilizer Technology and Use.* (Ed. O.P. Engelstad), Soil Science Society of America Inc., Madison, USA.

Zucker, A. and Zech, W. 1985. Sulfur status of four uncultivated soil profiles in Northern Bavaria. *Geoderma* 36: 229-240.

Appendix 3.1 Results from NaOBr digestions of plant material compared with median values published by the International Plant-Analytical Exchange, Wageningen Agricultural University, Netherlands.

Batch	Number	Materials analysed	Total-S concentration (Y)[2]	Median (X)	MAD2[1]
			.....mmole kg^{-1}.....		
March-April, 1988 (33)[3]					
	821	Bouvardia plant	99(1.98)[4]	98	7.5
	868	Vine leaf	42(2.28)	42	5.5
	757	Spinach	136(4.75)	127	6.5
	757	Spinach	128(2.25)	126	6.0
	869	Lettuce	74(2.53)	71	3.0
	565	Barley (grain)	43(0.81)	44	4.0
May-June, 1988 (40)					
	757	Spinach	118(1.34)	131	6.0
	854	Potato tuber	47(2.87)	44	6.0
	841	Endive	111(3.45)	110	5.0
	870	Leek	150(4.56)	140	10.0
	556	Letuce	49(0.78)	47	3.0
September-October, 1988 (31)					
	551	Pea (grain)	81(3.40)	75	3.0
	872	Gladiolus	36(1.46)	32	3.0
	662	Oil plam (leaf)	60(4.66)	56	3.0
	677	Maiz (plant)	38(1.84)	37	3.5
	722	Amaryllis (shoot)	110(2.54)	95	5.0
	757	Spinach	141(4.87)	128	7.0
January-February, 1989 (33)					
	890	Cauliflower	214(1.59)	207	25.0
	891	Courgettes	80(2.18)	75	6.0
	875	Cabbage	222(4.12)	217	16.0
	875	Cabbage	229(4.90)	220	10.0
	873	Gladiolus leaf	38(1.04)	34	5.0
	911	Tabac leaf mixture	142(3.65)	138	11.0

[1] *MAD2 = Median of Absolute Deviations (median of the absolutes of the observations minus their median) calculated after omitting unusual values*

[2] *Regression of Y on X; $Y = 1.7 + 1.02X$; $R^2 = 0.99$*

[3] *= Number of laboratories from other countries which had reported the analysis*

[4] *= Numbers in brackets are standard deviations*

Appendix 3.2 Calculation for ^{35}S enrichment of fertilizer materials

(a) Divide the desired liquid scintillation counting rate (**B**, Bequerels), [suppose the desired counting is 16.67 Bq or 1000 cpm] by counting efficiency (**CE**),[90%], of the cocktail mixture, this yields the amount of radioactivity (A_t) required per sample vial.

A_t = **B/CE** Bq
= 16.67*100/90 Bq

Where

A_t = the desired count rate in **V** ml of extractable S (CaP-S) from the top 0-2.5 cm soil at the end of the experiment cpm or Bq per ml

(b) Calculate the initial activity of (A_o) of ^{35}S (half life, $t_{1/2}$ = 87.4 days) when the duration of the experiment is **T** days [150].

A_o = A_t*EXP(**T***0.693/$t_{1/2}$) Bq
= 18.52*EXP(150*0.693/87.4) Bq

(c) From the aliquot of extractant counted (**V** ml), [1.00 ml], and the soil to extractant ratio [**S** g soil : **E** ml extr. soln. = 5:40], calculate radioactivity per g of soil (AS_o) at time 0 (t_o).

AS_o = A_o*E/(**V***S) Bq g^{-1} soil
= 60.83*40/(1*5) Bq g^{-1} soil

(d) Calculate the total amount of radioactivity to be added per gram of soil (AF_o) if, at steady, state extractable S makes up **X** percent [2%] of total S.

AF_o = AS_o*100/**X** Bq g^{-1} soil
= 486.66*100/2 Bq g^{-1} soil

(e) Calculate the total amount of radioactivity (AP_o) required from the desired agronomic specification.

AP_o = AF_o*W*1000 Bq
= 24333.15*0.442*1000 Bq

where

W = soil dry weight to 2.5 cm depth (of a soil core 15 cm in diameter) Kg

This is a total amount needed for applying into a soil core of 15 cm in diameter.

Therefore specific activity of fertilizer (SA_f) is

SA_f	=	$AP_o/(R*D/10)$	Bq g^{-1} S
	=	$10.75*10^6/(0.0177*30/10)$	Bq g^{-1} S

where

D	=	soil area to be fertilized [15 cm diameter] i.e. 0.0177 m^2	m^2
R	=	rate of S fertilization (30 kg S ha^{-1})	kg ha^{-1}

Appendix 3.3 The manufacturing of ^{35}S labelled superphosphate

Phosphate rock used in these experiments was a mixture of two unreactive phosphate rocks, Nauru Island and Christmas Island grade A (15% P) at the ratio of 1:1. The stoichiometric equation for the reaction between apatite rock and sulphuric acid is as follows:

$$Ca_{10}(PO_4)_6F_2 + 7H_2SO_4 + 3H_2O \rightarrow 3Ca(H_2PO_4)_2.H_2O + 7CaSO_4 + 2HF$$

According to the equation, the ratio of phosphate rock (molecular weight=1008 g) to superphosphate (1608 g) is about 1:1.6, then:

Amount of superphosphate required		=	**S**	g
Weight of 1:1; Christmas:Nauru RP required		=	**S**/1.6	g
		=	**A**	g
Weight of sulphuric acid (100%) required for 100% acidulation[a]				
		=	**S**/1.6*0.62	g
		=	**D**	g
Weight of 98% sulphuric acid (w/w) required				
		=	**D**/0.98	g
	or	=	**W**	g
		=	**W**/1.84	ml

Where 1.84 is specific gravity of sulphuric acid. The acid is normally diluted to 65% (w/w) by addition of water. Therefore, the amount of water (**B**) needed to be added to make 65% sulphuric acid:

	B	=	98***W**/65	g
	or	=	98***W**/65	ml

Therefore the superphosphate is prepared by mixing '**B**' g of water and '**W**' g of sulphuric acid (98% w/w) to '**A**' g of phosphate rock. Before mixing, the dilute acid is heated to about 65-70 ^{o}C. After mixing, the mixture is dried at room temperature (20 ^{o}C).

[a] Based on 0.62 acid rock ratio; Bolan *et al.*, (1987)

Appendix 4.1 Activities of ^{35}S and ^{32}P in pasture per unit area (square centimetre) as influenced by different application rates of ^{35}S and ^{32}P labelled dung and distances of pasture from dung.

Distances	Dung D1	D2	D3	Means
PART A (FIRST HARVEST)	..... ^{35}S KBq cm^{-2}			
R1	0.11	0.32	0.45	0.29
R2	0.01	0.02	0.06	0.03
R3	0.00	0.00	0.01	0.00
R4	0.00	0.00	0.00	0.00
Means	0.03	0.09	0.13	
Lsd 5% Dung = 0.03; distance = 0.04; within dung = 0.06				
C.V. % = 59.74				
PART B (SECOND HARVEST)	..... ^{35}S KBq cm^{-2}			
R1	0.09	0.25	0.36	0.23
R2	0.02	0.03	0.08	0.04
R3	0.01	0.01	0.02	0.01
R4	0.01	0.01	0.01	0.01
Means	0.03	0.08	0.12	
Lsd 5% Dung = 0.03; distance = 0.04; within dung = 0.24				
C.V. % = 45.1				
PART C (FIRST HARVEST)	..... ^{32}P KBq cm^{-2}			
R1	0.02	0.06	0.13	0.07
R2	0.01	0.03	0.05	0.03
R3	0.00	0.01	0.01	0.01
R4	0.00	0.00	0.01	0.00
Means	0.01	0.02	0.05	
Lsd 5% Dung = 0.01; distance = 0.01; within dung = 0.06				
C.V. % = 45.1				

Appendix 4.2 Influence of dung application rates and distances of pasture from ^{35}S and ^{32}P labelled dung on the percent recovery of ^{35}S and ^{32}P by pasture.

Distances	Dung D1	D2	D3	Means
PART A (FIRST HARVEST)		..... % ^{35}S		
R1	1.95	2.81	1.99	2.25
R2	0.16	0.24	0.34	0.24
R3	0.17	0.10	0.13	0.13
R4	0.07	0.02	0.03	0.04
Means	0.59	0.79	0.62	
Lsd 5% Dung = ns; distance = 0.25; within dung = 0.87				
C.V. % = 51.38				
PART B (SECOND HARVEST)		..... % ^{35}S		
R1	1.54	2.23	1.59	1.79
R2	0.37	0.36	0.44	0.39
R3	0.55	0.46	0.33	0.49
R4	0.30	0.17	0.10	0.19
Means	0.69	0.81	0.62	
Lsd 5% Dung = ns; distance = 0.25; within dung = 0.44				
C.V. % = 49.1				
PART C (FIRST HARVEST)		..... % ^{32}P		
R1	0.47	0.73	0.78	0.66
R2	0.24	0.44	0.37	0.35
R3	0.24	0.30	0.35	0.29
R4	0.22	0.13	0.11	0.15
Means	0.29	0.40	0.40	
Lsd 5% Dung = ns; distance = 0.13; within dung = 0.23				
C.V. % = 50.4				

Appendix 4.3 Specific activity of ^{35}S or ^{32}P in pastures (S.A.) and the percentage of plant S and P derived from dung (%SDFD and %PDFD) calculated using the S.A. of ^{35}S and ^{32}P in the total pool (TP) and extractable pool (EP), CaP-S and Olsen-P, of dung samples.

		Harvest 1			Harvest 2			Harvest 1		
		^{35}S			^{35}S			^{32}P		
		S.A.	%SDFD		S.A.	%SDFD		S.A.	%PDFD	
			TP	EP		TP	EP		TP	EP
Treatments		KBq mg^{-1} S	%	%	KBq mg^{-1} S	%	%	KBq mg^{-1} P	%	%
D1	R1	21.8	1.83	0.18	8.4	0.70	0.07	2.8	1.41	1.13
	R2	1.8	0.15	0.02	1.7	0.14	0.01	1.3	0.63	0.50
	R3	0.4	0.03	0.00	0.5	0.05	0.01	0.3	0.14	0.11
	R4	0.3	0.03	0.00	0.4	0.03	0.00	0.4	0.18	0.14
D2	R1	59.7	5.02	0.51	19.7	1.65	0.17	7.7	3.77	3.00
	R2	4.8	0.40	0.04	2.4	0.20	0.02	3.6	1.76	1.41
	R3	0.9	0.07	0.01	0.9	0.07	0.01	1.1	0.52	0.41
	R4	0.2	0.01	0.00	0.4	0.03	0.00	0.4	0.21	0.17
D3	R1	63.1	5.30	0.54	27.6	2.23	0.24	14.4	7.07	5.64
	R2	10.3	0.87	0.09	5.5	0.45	0.05	5.7	2.81	2.24
	R3	1.5	0.13	0.01	1.4	0.12	0.01	2.3	1.14	0.91
	R4	0.4	0.03	0.00	0.4	0.03	0.00	0.8	0.41	0.32

Appendix 4.4 Percentage of plant S (PART A and B) and P (PART C) derived from dung applied at different rates (%SDFD and %PDFD)[a] in pasture at different radial distances away from dung.

Distances	Dung weights (g) 0.5	1.0	2.0	Means
PART A (FIRST HARVEST)		..... %SDFD .....[a]		
R1	0.188	0.514	0.543	0.415
R2	0.016	0.041	0.089	0.049
R3	0.003	0.008	0.013	0.008
R4	0.003	0.001	0.004	0.003
Means	0.052	0.141	0.162	
Lsd 5% Dung = 0.045; distance = 0.015; within dung = 0.08				
C.V. % = 59.74				
PART B (SECOND HARVEST)		..... %SDFD .....[a]		
R1	0.071	0.167	0.238	0.159
R2	0.014	0.021	0.047	0.027
R3	0.005	0.007	0.011	0.008
R4	0.003	0.003	0.004	0.003
Means	0.023	0.050	0.075	
Lsd 5% Dung = 0.014; distance = 0.016; within dung = 0.028				
C.V. % = 45.1				
PART C (FIRST HARVEST)		..... %PDFD .....[a]		
R1	1.13	3.00	5.65	3.26
R2	0.49	1.41	2.25	1.38
R3	0.11	0.41	0.90	0.47
R4	0.14	0.16	0.32	0.21
Means	0.47	1.24	2.28	
Lsd 5% Dung = 0.014; distance =0.016; within dung = 0.96				
C.V. % = 57.2				

[a] *calculated using specific activities of ^{35}S and ^{32}P in the exchangeable pool of dung S and P*

Appendix 4.5 Diffusion of sulphate and phosphate in soil.

The initial flux of either phosphate or sulphate from under a moist dung pellet out into the soil can be described by the following equation (Nye and Tinker, 1977; Barber, 1984):

$$Fp = [-Dp\theta f(\delta C_p/\delta S_p)]*\delta C_p/\delta X \quad mm^2\ sec^{-1}$$

where

F_p	=	flux of $H_2PO_4^=$ or $SO_4^=$	$mm^2\ sec^{-1}$
D_p	=	diffusion coefficient of $H_2PO_4^=$ (or $SO_4^=$) in water	$mm^2\ sec^{-1}$
θ	=	volumetric soil water content	1
f	=	impedance factor	
$\delta C_p/\delta S_p$	=	reciprocal of buffer power; unit change, C, ($\mu g\ mm^{-3}$ solution) in solution $H_2PO_4^=$ or $SO_4^=$ concentration with a unit change in the amount of soil surface, S, ($\mu g\ mm^{-3}$ soil) $H_2PO_4^=$ (or $SO_4^=$)	
$\delta C_p/X$	=	concentration gradient between soil solution affected by the dung to the bulk soil solution. For the purpose of calculation, **X**, is considered to be 1 mm	

Assumption:
It is assumed that, for a 2 g dung pellet which contains 275 and 5454 mg S and P/kg, respectively, inititally covers an area of 10 cm^2 and a volume of 10 cm^3 and when wet contains 40% moisture. Then;

soluble sulphate was measured at 0.55 mg/10 cm^3, i.e. 0.055 mg/cm^3 given a dung solution containing 138 $\mu g\ S\ mm^{-3}$ and similarly;

suluble phosphate was measured at 11.0 mg/10 cm^3 i.e. 1.1 mg/cm^3 given a dung solution containing 2750 $\mu g\ P\ mm^{-3}$.

The following table presents parameters used for theoretical comparison of sulphate and phosphate flux away from dung.

Ion	D	θ	f	$\delta C/\delta S$	$\delta C/\delta X$	F
	$mm^2\ s^{-1}$				$\mu g\ mm^{-3}\ mm^{-1}$	$\mu g\ mm^{-1}\ s^{-1}$
$H_2PO_4^=$	$1*10^{-3}$	0.3	0.3	0.015[a]	2750[c]	$3.7*10^{-3}$
$SO_4^=$	$1*10^{-3}$	0.3	0.3	0.830[b]	138[d]	$10.3*10^{-3}$

[a] *Sorn-srivichai, 1985;* [b] *Appendix 7.14;* [c] *and* [d] *Table 4.1 and see assumption above;*

This simple calculation suggests that 2-3 times more sulphate should initially diffuse away form the dung pellet than phosphate. The different rates of diffusion through soil do not explain why relatively more ^{32}P than ^{35}S was recovered at greater radial distances from dung.

Appendix 4.6 Calculation for area of a paddock influenced by sheep dung.

A. Stock Unit (SU)

SU	=	**DM*PU/RQ**	su ha^{-1}
where			
DM	=	dry matter production[a]	kg ha^{-1} $year^{-1}$
PU	=	pasture utilization	percent
RQ	=	dry matter requirement/SU/year	kg
Therefore			
SU	=	12000*70/100/550	su ha^{-1}
	=	15.27 ≅ 15	su ha^{-1}

B. Quantity of dung (PY)

1. number of patches

PY	=	**ND*SU*365**	patch $year^{-1}$
where			
ND	=	number of excretions per day[b]	
PY	=	6*15*365	
	=	32850	patch $year^{-1}$

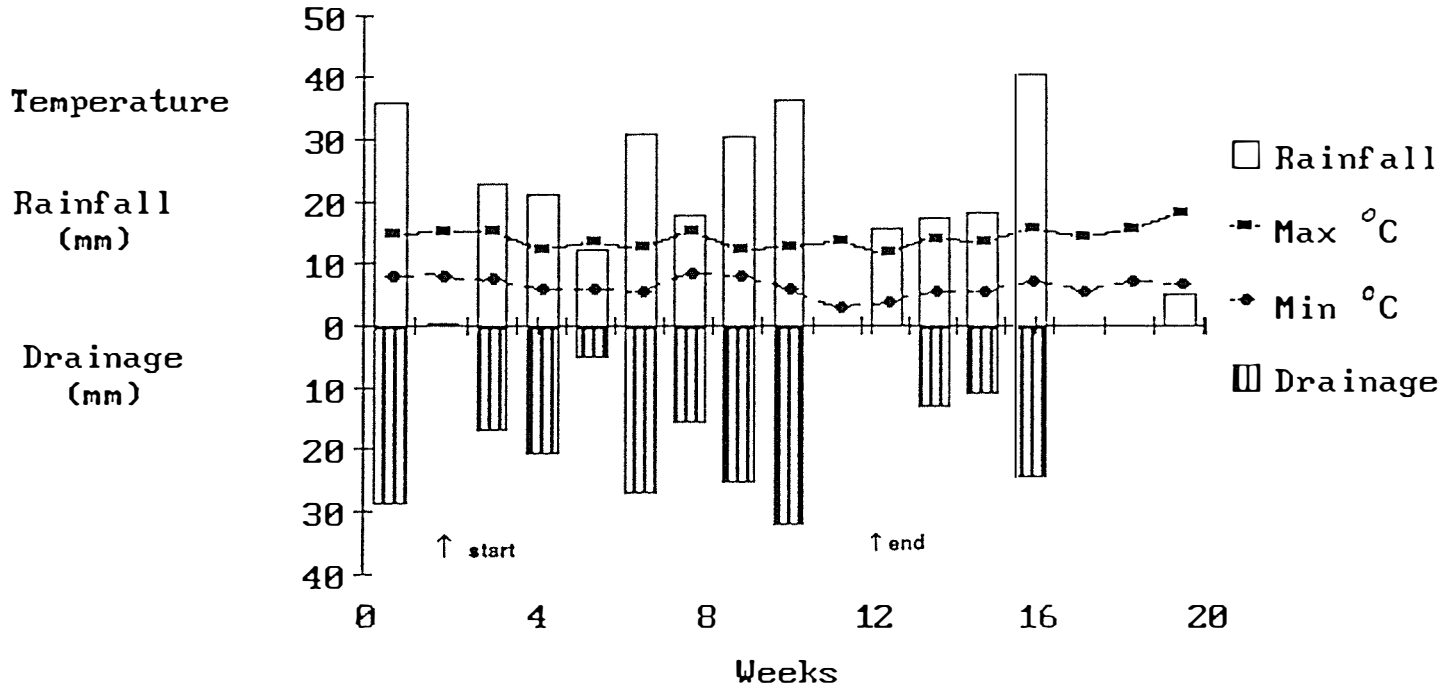
2. dung weight (**M**)

M	=	**PY*E**	kg ha^{-1} $year^{-1}$
where			
E	=	mass excreted per patch[b]	kg day^{-1}
M	=	32850*0.1	
	=	3285	kg ha^{-1} $year^{-1}$

C. Fraction of paddock influenced by dung (PI)

PI	=	π*(**Rp**+**Rd**)^2*PY/10000	
where			
Rp	=	radius of dung patch[c]	metre
Rd	=	extended radius of area of influence	metre
PI	=	22/7*(0.064+0.10)^2*82125/10000	
	=	0.2775	
or	=	27.75 percent of paddock	

[a] *Horne, D. 1985*; [b] *and* [c] *Cleland, A. Personal communication*



Appendix 4.7 Weekly rainfall (Rain), drainage water (Drainage), average maximum (Max) and minimum (Min) temperature during the field trial period (June 27, 1985 - August 29, 1985).

Appendix 5.1 Recovery of ^{35}S from ^{35}S labelled gypsum S^{o} fertilizers after application onto the surface of soil cores.

S Applied mg S core^{-1}	Recovery percentage of total ^{35}S %
Gypsum	
12.5	69.1
25.0	65.2
50.0	72.1
Mean	68.8
F-test	ns
C.V. %	14.3
Elemental S[1]	
12.5	69.4
25.0	77.5
50.0	72.4
Mean	73.1
F-test	ns
C.V. %	16.2

ns = not significant

[1] *particle size = <10μm*

Appendix 5.2 Cumulative S uptake by pasture and the percentage and amount of plant S derived from fertilizers (SDFF) with time.

		Days after fertilizer application				
	Fertilizers	30	60	90	120	150
Cumulative plant S uptake				.....mg S core^{-1}.....		
	Superphosphate	14.2	28.3	41.6	51.3	69.5
	Elemental S[1]	12.6	28.1	41.4	55.8	71.8
	F-test	ns	ns	ns	ns	ns
	C.V. %	21.7	13.1	17.6	11.7	19.2
%SDFF				.....%.....		
	Superphosphate	28.9	23.4	18.5	17.4	13.5
	Elemental S[1]	19.3	15.7	16.9	13.4	10.6
	F-test	***	***	ns	ns	ns
	C.V. %	11.1	8.2	8.1	15.2	14.2
Cumulative S derived from fertilizers				.....mg core^{-1}.....		
	Superphosphate	4.1	6.6	7.7	8.9	9.3
	Elemental S[1]	2.4	4.4	6.9	7.5	7.6
	F-test	***	***	ns	ns	ns
	C.V. %	29.5	13.0	16.9	16.6	10.6
Number of replications		20	16	12	8	4

*** = significant at 0.1% level; ns = not significant

[1] particle size = <10μm

Appendix 5.3 Total CaP-S in three soil layers; CaP-S as percentage of S derived from, PART A, fertilizers (number in parentheses) and PART B, native extractable S and PART C, total extractable S in 0-10 cm depth of soil cores.

Fertilizers & Layers	Days after fertilizer application					
	0	30	60	90	120	150
PART A, Total CaP-S (% of CaP-S derived from fertilizers)[a]						
Superphosphate			.....mg S layer^{-1}.....			
Top	-	22.4(27)	7.4(15)	6.0(8)	5.9(7)	5.8(9)
Middle	-	16.9(12)	2.9(21)	3.8(5)	4.4(5)	3.3(3)
Bottom	-	18.4(13)	3.5(17)	4.0(5)	4.5(7)	3.9(3)
Elemental S[1]						
Top	-	18.9(20)	10.7(26)	7.5(13)	7.7(10)	6.9(11)
Middle	-	16.2(6)	4.7(19)	5.1(10)	5.1(6)	4.0(3)
Bottom	-	16.2(13)	4.2(17)	5.0(6)	4.9(5)	4.5(4)
Lsd 5%						
Within type[c]	-	2.9	1.7	0.9	1.0	1.6
C.V. %	-	10.7	20.6	11.8	12.3	23.1
PART B, Native CaP-S[b]						
Superphosphate			.....mg S layer^{-1}.....			
Top	7.4	16.3	6.3	5.5	5.5	5.3
Middle	3.5	14.8	2.3	3.6	4.2	3.2
Bottom	3.5	16.1	2.9	3.8	4.2	3.8
Elemental S[1]						
Top	7.4	15.2	7.9	6.5	6.9	6.1
Middle	3.5	15.2	3.8	4.6	4.8	3.9
Bottom	3.5	15.6	3.6	4.7	4.7	4.3
Lsd 5%						
Within type[c]	-	ns	1.4	0.8	0.9	1.3
C.V. %	-	11.0	21.8	11.8	12.0	12.4
PART C, Total CaP-S (0-10 cm)						
			.....mg S core^{-1}.....			
Superphosphate	14.4	57.8	13.9	13.9	14.8	13.1
Elemental S[1]	14.4	51.4	19.7	17.6	17.8	15.4
F-test	-	ns	*	*	*	*
C.V. %	-	7.8	10.5	9.0	14.1	13.1

** = significant at 5% level; ns = not significant;* [1] *particle size = <10 µm*

[a] calculated as $100 * (\text{CaP-}^{35}\text{S} / \text{SAF}) / \text{CaP-S}$

[b], calculated as $\text{CaP-S} - (\text{CaP-}^{35}\text{S} / \text{SAF})$ mg core^{-1}

where

SAF = Specific activity of ^{35}S in labelled fertilizers (see Table 5.1)

CaP-^{35}S = Amounts of ^{35}S activity in CaP-S extraction

CaP-S = Amounts of extractable S,

[c], *for comparison of means within each fertilizer type*

Appendix 5.4 Cumulative ^{35}S uptake in herbage and total amount of ^{35}S in 0-10 cm soil depth at five harvesting times.

	Fertilizers	Recovery percentage %				
Days		30	60	90	120	150
HERBAGE						
	Superphosphate	6.4	12.9	13.3	15.7	16.3
	Elemental S[1]	3.6	8.1	11.1	13.2	13.6
	F-test	*	*	*	ns	ns
	C.V. %	28.2	13.0	17.5	16.6	10.6
SOIL						
	Superphosphate	43.7	26.7	24.1	23.3	22.9
	Elemental S[1]	59.7	53.3	45.6	42.5	42.3
	F-test	*	*	*	*	*
	C.V. %	7.5	13.2	12.6	4.4	16.9
TOTAL (soil+herbage)						
	Superphosphate	50.9	39.6	37.4	39.0	39.2
	Microfine-S^0	64.4	61.3	56.6	55.8	55.8
	F-test	*	*	*	*	*
	C.V. %	7.5	11.1	12.6	5.3	9.7
UNACCOUNTED FOR (losses beyond 10 cm)						
	Superphosphate	49.1	60.4	62.6	61.0	60.8
	Elemental S[1]	35.6	39.7	44.4	44.2	44.2

** = significant at 5% level; ns = not significant*

[1] *particle size = <10μm*

Appendix 5.5 Recovery of total ^{35}S in three layers of soils at five harvest times.

Fertilizers	Layers	Percent recovery %				
Days after application		30	60	90	120	150
Superphosphate						
	Top	24.6	15.5	14.2	13.5	13.9
	Middle	9.5	5.9	5.1	5.4	5.4
	Bottom	9.6	5.3	4.7	4.4	4.6
Elemental S[1]						
	Top	48.8	39.6	31.3	29.1	28.5
	Middle	6.9	8.8	8.5	8.8	8.9
	Bottom	4.6	4.8	5.8	4.6	4.8
Lsd 5% within type[a]		3.5	3.2	3.9	1.6	3.3
C.V. %		13.7	16.3	22.3	9.7	20.7

** = significant at 5% level; ns = not significant*

[a] *for comparison among means within each fertilizer type*

[1] *particle size = <10μm*

Appendix 5.6 Organic ^{35}S expressed as a percentage of total ^{35}S activity **applied** (PART A) and as a percentage of total ^{35}S **remaining** (PART B) in three soil layers at five harvests.

Fertilizer	Layers	Days after fertilizer application 30	60	90	120	150
PART A				.....% of applied.....		
Superphosphate						
	Top	13.9	13.4	13.2	12.8	12.1
	Middle	5.8	4.8	4.8	5.1	5.1
	Bottom	5.6	4.2	4.5	3.9	4.3
Elemental S[1]						
	Top	39.8	33.9	29.9	27.7	27.1
	Middle	5.1	7.1	7.7	8.2	8.6
	Bottom	3.2	3.7	5.3	4.2	4.6
Lsd 5% Within type[a]		14.5	7.6	13.4	2.1	11.2
C.V. %		21.1	16.9	17.9	9.8	22.3
PART B				.....% of remaining.....		
Superphosphate						
	Top	56.9	86.6	93.5	95.3	93.3
	Middle	60.6	81.2	94.6	93.8	94.3
	Bottom	57.9	78.7	92.6	90.4	93.9
Elemental S[1]						
	Top	82.4	85.9	92.9	95.3	95.0
	Middle	73.2	80.3	90.0	93.3	96.9
	Bottom	69.7	75.2	91.6	90.4	94.6
Lsd 5% Within type[a]		12.6	4.3	2.7	1.9	1.9
C.V. %		12.5	15.9	2.0	1.3	1.3

* = *significant at 5% level; ns = not significant*

[a] *for comparison of means within each fertilizer type*

[1] *particle size = <10μm*

Appendix 5.7 Carbon-bonded ^{35}S expressed as a percentage of total ^{35}S activity applied (PART A) and as a percentage of total ^{35}S remaining (PART B) in three soil layers at five harvests.

Fertilizers	Layers	Days after fertilizer application 30	60	90	120	150
PART A				.....% of applied.....		
Superphosphate						
	Top	11.2	8.8	8.6	9.8	9.2
	Middle	4.7	2.5	1.4	2.4	2.3
	Bottom	3.7	1.4	1.1	0.9	1.2
Elemental S[1]						
	Top	34.8	21.3	23.1	25.6	25.0
	Middle	3.3	4.3	5.8	5.6	5.7
	Bottom	0.9	0.8	2.0	1.6	1.8
Lsd 5% Within type[a]		18.4	12.2	7.9	3.7	6.0
C.V. %		30.0	36.6	27.5	17.3	22.2
PART B				.....% of remaining.....		
Superphosphate						
	Top	46.0	57.1	60.7	72.6	70.8
	Middle	48.8	43.1	27.4	43.6	43.5
	Bottom	36.5	25.6	18.2	19.8	24.6
Elemental S[1]						
	Top	72.0	53.9	74.2	88.3	88.4
	Middle	47.8	48.4	67.6	62.3	63.3
	Bottom	20.6	16.1	31.9	31.8	33.7
Lsd 5% Within type[a]		23.9	16.1	23.1	19.4	15.8
C.V. %		35.4	26.1	32.9	24.3	19.4

* = significant at 5% level; ns = not significant

a for comparison of means within each fertilizer type

1 particle size = <10µm

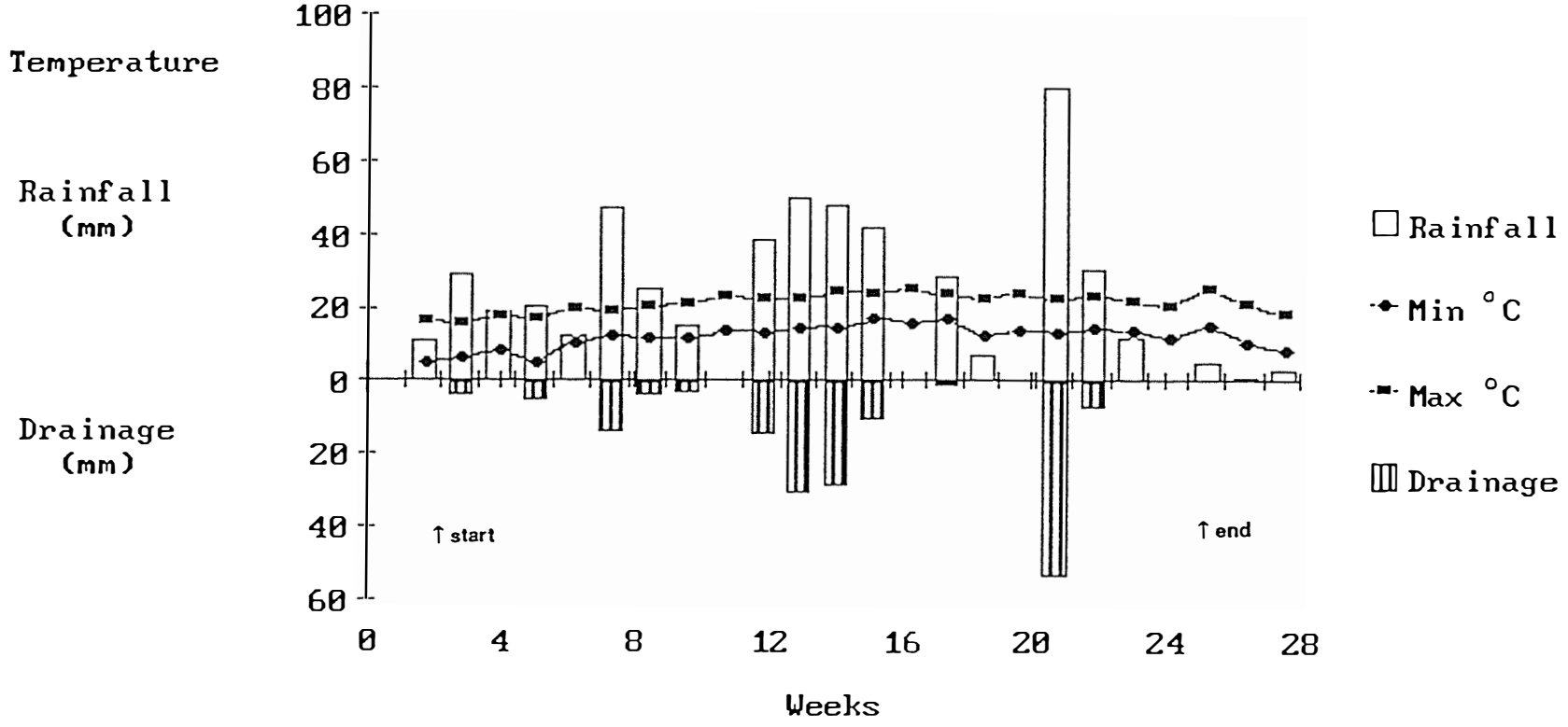
Appendix 5.8 ^{35}S Ester sulphate expressed as a percentage of total ^{35}S activity **applied** (PART A) and as a percentage of total ^{35}S **remaining** (PART B) in three soil layers at 5 harvests.

Fertilizers	Layers	Day after fertilizer application 30	60	90	120	150
PART A				.....% of applied.....		
Superphosphate						
	Top	2.7	4.6	4.6	3.1	2.8
	Middle	1.1	2.3	3.4	2.7	2.8
	Bottom	1.9	2.8	3.4	3.0	3.4
Elemental S[1]						
	Top	4.9	12.7	5.8	2.1	2.1
	Middle	1.8	2.8	1.9	2.6	2.9
	Bottom	2.3	2.9	3.3	2.6	2.8
Lsd 5% Within type[a]		2.4	13.5	ns	ns	ns
C.V. %		44.4	53.4	33.3	45.5	43.3
PART B				.....% of remaining.....		
Superphosphate						
	Top	10.8	29.4	32.4	22.6	22.4
	Middle	11.8	38.8	67.3	50.3	50.7
	Bottom	21.5	53.1	74.3	70.6	69.4
Elemental S[1]						
	Top	10.4	31.9	17.9	7.1	6.6
	Middle	25.4	31.9	22.4	30.9	33.2
	Bottom	49.1	59.1	59.7	58.5	60.9
Lsd 5% Within type[a]		16.7	15.0	22.6	19.5	15.8
C.V. %		48.6	24.4	32.9	32.3	25.8

** = significant at 5% level; ns = not significant*

[a] *for comparison of means within each fertilizer type*

[1] *particle size = <10µm*



Appendix 5.9 Weekly amount of rainfall (Rain) and drainage water (Drainage), average maximum (Max) and minimum (Min) temperature during the field trial period from October 1985-March 1986.

Appendix 6.1 Total S and ^{35}S activity taken up by herbage from ^{35}S labelled superphosphate and microfine S^0 treated plots in five harvests.

Fertilizers	Days after fertilizer application				
	30	60	90	120	150
TOTAL S	..... mg S core^{-1}				
Superphosphate	14.2	14.4	13.3	13.8	13.2
microfine S^0	12.6	15.5	13.3	14.4	15.9
F-test	ns	ns	ns	ns	ns
C.V. %	22.9	18.8	19.5	35.3	36.4
^{35}S *ACTIVITY*	.....KBq core^{-1}				
Superphosphate	1174	967	511	255	155
Microfine S^0	757	1028	577	315	245
F-test	*	*	*	ns	ns
C.V. %	29.4	13.0	17.5	16.6	10.6
Number of replicates	20	16	12	8	4

** = significant at 5% level; ns = not significant*

Appendix 7.1 Cumulative sulphur taken up by pastures (PART A) and cumulative dry matter yield, DM, (PART B) in glasshouse trials (average of seven replications).

Treatments		Days after fertilizer application					
		30	60	90	120	150	180
PART A, CUMULATIVE S UPTAKE				..... g m^{-2}			
Ramiha	SS	0.40	0.66	0.93	1.15	1.30	1.74^{a}
	LL	0.33	0.48	0.69	0.89	1.02	1.34^{ab}
	SS/PR	0.40	0.57	0.78	0.99	1.12	1.47^{ab}
	SS/gr	0.38	0.53	0.73	0.93	1.09	1.50^{b}
	PR	0.40	0.57	0.77	0.98	1.13	1.59^{b}
	Ctrl	0.40	0.56	0.72	0.89	1.02	1.35^{b}
F-test		ns	ns	ns	ns	ns	**
C.V. %		22.4	22.2	18.1	16.9	15.9	15.7
Tokomaru	SS	0.39^{a}	0.58^{a}	0.80^{a}	1.01^{a}	1.16^{a}	1.53^{a}
	MM	0.27^{b}	0.42^{b}	0.63^{b}	0.77^{b}	0.88^{b}	1.15^{b}
	LL	0.25^{b}	0.41^{b}	0.61^{b}	0.75^{b}	0.87^{b}	1.19^{b}
	SS/PR	0.27^{b}	0.44^{b}	0.66^{b}	0.84^{b}	0.97^{b}	1.29^{b}
	SS/gr	0.27^{b}	0.39^{b}	0.62^{b}	0.80^{b}	0.93^{b}	1.25^{b}
	PR	0.29^{b}	0.43^{b}	0.63^{b}	0.79^{b}	0.89^{b}	1.21^{b}
	Ctrl	0.24^{b}	0.32^{c}	0.51^{c}	0.59^{c}	0.68^{c}	0.97^{c}
F-test		*	**	**	**	**	**
C.V. %		31.9	27.4	20.1	17.0	16.5	15.4
PART B, CUMULATIVE DM				..... g m^{-2}			
Ramiha	SS	88	193	333	421	480	593
	LL	83	180	313	393	453	544
	SS/PR	103	192	315	403	462	562
	SS/gr	101	188	298	377	439	545
	PR	100	194	315	409	471	608
	Ctrl	113	214	325	414	472	593
F-test		ns	ns	ns	ns	ns	ns
C.V. %		18.2	13.1	15.4	16.2	14.1	13.8
Tokomaru	SS	106	210	319	410	494	610
	MM	79	173	283	362	435	532
	LL	81	185	298	384	467	590
	SS/PR	77	170	292	379	455	564
	SS/gr	89	174	294	376	450	555
	PR	94	197	319	409	478	604
	Ctrl	85	167	271	338	402	506
F-test		ns	ns	ns	ns	ns	ns
C.V. %		14.5	22.1	16.8	14.8	14.6	14.3

, ** and * = significant at 5, 1 and 0.1% level, respectively; ns = not significant; mean separation by DMRT at 5% level denoted by letters*

Appendix 7.2 Cumulative sulphur taken up (PART A) and cumulative dry matter yield, DM, (PART B) of pastures in Field trials (average of three replications).

Treatments		Days after fertilizer application					
		30	60	90	120	150	180
PART A, CUMULATIVE S UPTAKE				..... g m^{-2}			
Ramiha	SS	0.15	0.20	0.35	0.50	0.58	0.68
	LL	0.15	0.20	0.37	0.48	0.55	0.63
	SS/PR	0.17	0.22	0.39	0.49	0.55	0.62
	SS/gr	0.23	0.29	0.47	0.56	0.61	0.70
	PR	0.11	0.16	0.27	0.35	0.38	0.42
	Ctrl	0.12	0.16	0.29	0.38	0.43	0.55
F-test		ns	ns	ns	ns	ns	ns
C.V. %		33.0	28.1	22.6	21.7	23.1	21.9
Tokomaru	SS	0.05	0.06	0.20	0.30	0.34	0.38
	MM	0.08	0.11	0.27	0.36	0.39	0.44
	LL	0.07	0.11	0.27	0.37	0.40	0.45
	SS/PR	0.10	0.10	0.31	0.42	0.47	0.53
	SS/gr	0.08	0.10	0.29	0.39	0.44	0.50
	PR	0.12	0.13	0.31	0.38	0.42	0.46
	Ctrl	0.08	0.10	0.23	0.31	0.34	0.37
F-test		ns	ns	ns	ns	ns	ns
C.V. %		43.7	46.1	28.9	26.1	27.3	26.5
PART B, CUMULATIVE DM				..... g m^{-2}			
Ramiha	SS	102	132	236	301	350	408
	LL	106	146	244	303	353	416
	SS/PR	106	140	244	300	334	378
	SS/gr	160	202	311	361	390	447
	PR	76	112	195	240	261	296
	Ctrl	98	127	196	266	304	404
F-test		ns	ns	ns	ns	ns	ns
C.V. %		33.3	28.1	22.6	21.7	23.1	21.9
Tokomaru	SS	35	45	130	194	202	238
	MM	67	82	198	267	295	322
	LL	68	89	201	275	305	341
	S/PR	72	87	210	279	311	353
	SS/gr	67	84	208	280	305	350
	PR	60	108	193	249	327	359
	Ctrl	64	83	182	238	264	293
F-test		ns	ns	ns	ns	ns	ns
C.V. %		47.1	48.5	34.1	29.9	29.7	28.8

ns = not significant

Appendix 7.3 Sulphur concentration of pastures in glasshouse (PART A) and field (PART B) trials as influenced by different particle size of elemental S.

Treatments		Days after fertilizer application					
		30	60	90	120	150	180
PART A, GLASSHOUSE[1]				..... %			
Ramiha	SS	0.47	0.23[a]	0.20[a]	0.25[a]	0.26[a]	0.39[a]
	LL	0.38	0.14[c]	0.17[b]	0.25[b]	0.21[c]	0.34[b]
	SS/PR	0.41	0.19[b]	0.17[b]	0.23[b]	0.23[b]	0.36[a]
	SS/gr	0.38	0.17[b]	0.18[b]	0.25[a]	0.25[a]	0.40[a]
	PR	0.40	0.18[b]	0.17[b]	0.23[b]	0.24[b]	0.33[b]
	Ctrl	0.34	0.17[c]	0.15[c]	0.19[c]	0.21[c]	0.27[c]
F-test		ns	**	*	*	**	**
C.V. %		13.6	28.5	11.4	13.2	13.6	13.4
Tokomaru	SS	0.36	0.18[a]	0.20[a]	0.24[a]	0.17[a]	0.32[a]
	MM	0.33	0.17[a]	0.19[b]	0.18[b]	0.15[b]	0.26[b]
	LL	0.31	0.15[b]	0.19[b]	0.16[c]	0.14[b]	0.26[b]
	SS/PR	0.35	0.17[a]	0.19[b]	0.21[a]	0.18[a]	0.31[a]
	SS/gr	0.31	0.14[c]	0.19[b]	0.22[a]	0.18[a]	0.31[a]
	PR	0.32	0.14[c]	0.17[c]	0.17[c]	0.15[b]	0.26[b]
	Ctrl	0.29	0.14[c]	0.14[c]	0.16[c]	0.14[b]	0.29[b]
F-test		ns	*	*	**	**	***
C.V. %		12.5	16.5	13.6	16.3	13.8	13.6
PART B, FIELD TRIAL[2]				..... %			
Ramiha	SS	0.15	0.15	0.15	0.22	0.17	0.17[a]
	LL	0.14	0.13	0.16	0.18	0.14	0.13[b]
	SS/PR	0.16	0.15	0.17	0.18	0.14	0.16[a]
	SS/gr	0.16	0.14	0.16	0.19	0.14	0.16[a]
	PR	0.14	0.14	0.13	0.17	0.14	0.13[b]
	Ctrl	0.13	0.13	0.15	0.17	0.13	0.12[c]
F-test		ns	ns	ns	ns	ns	**
C.V. %		9.7	11.4	9.7	12.7	16.0	11.8
Tokomaru	SS	0.14	0.11	0.17	0.17	0.17	0.16[a]
	MM	0.12	0.13	0.14	0.14	0.14	0.13[b]
	LL	0.12	0.13	0.15	0.14	0.12	0.13[b]
	SS/PR	0.15	0.11	0.16	0.15	0.16	0.15[b]
	SS/gr	0.11	0.12	0.16	0.16	0.15	0.14[b]
	PR	0.13	0.13	0.13	0.13	0.13	0.13[b]
	Ctrl	0.12	0.11	0.13	0.14	0.12	0.12[c]
F-test		ns	ns	ns	ns	ns	**
C.V. %		15.3	12.1	15.3	17.7	15.6	9.5

, ** and * = significant at 5, 1 and 0.1% level respectively; ns = not significant; mean separation by DMRT at 5% level denote by letters; [1] and [2] average of seven and three replications, respectively*

Appendix 7.4 Cumulative percentage of ^{35}S taken up by pastures in glasshouse (PART A) and field (PART B) trials at six sampling times.

Treatments		Days after fertililzer application					
		30	60	90	120	150	180
PART A, GLASSHOUSE TRIALS[1]				..... %recovery			
Ramiha	SS	3.1[a]	8.6[a]	12.5[a]	14.9[a]	16.6[a]	20.5[a]
	LL	0.5[c]	1.1[c]	1.9[c]	2.5[c]	2.9[c]	4.1[c]
	SS/PR	1.9[b]	4.8[b]	7.7[b]	10.3[b]	11.7[b]	14.7[b]
	SS/gr	1.6[b]	3.9[b]	6.2[b]	8.4[b]	10.3[b]	13.7[b]
F-test		*	***	***	***	***	***
C.V. %		42.5	44.4	36.5	32.9	29.4	24.7
Tokomaru	SS	1.8[a]	4.0[a]	6.3[a]	8.6[a]	10.6[a]	13.9[a]
	MM	0.4[c]	0.9[c]	1.4[c]	1.9[c]	2.5[c]	3.5[c]
	LL	0.2[c]	0.5[c]	0.8[c]	1.0[c]	1.3[c]	1.9[c]
	SS/PR	0.8[b]	2.6[b]	4.7[b]	6.9[ab]	8.9[ab]	12.6[ab]
	SS/gr	0.8[b]	2.1[b]	4.0[b]	5.8[b]	7.7[b]	10.4[b]
F-test		**	**	***	***	***	***
C.V. %		28.5	41.2	34.6	34.9	32.2	28.7
PART B, FIELD TRIAL[2]				..... % recovery			
Ramiha	SS	1.4[a]	1.8[a]	4.6[a]	6.6[a]	8.0[a]	9.8[a]
	LL	0.26b	0.3[b]	0.8[b]	1.1[b]	1.4[b]	1.7[b]
	SS/PR	1.4[a]	1.8[a]	4.2[a]	5.7[a]	6.6[a]	8.1[a]
	SS/gr	1.5[a]	1.9[a]	4.4[a]	5.8[a]	6.6[a]	8.3[a]
F-test		**	**	***	***	***	***
C.V. %		27.5	28.2	31.1	31.8	35.3	32.5
Tokomaru	SS	0.5[a]	0.6[a]	2.2[a]	3.3[a]	3.7[a]	4.2[a]
	MM	0.2[b]	0.2[b]	0.6[b]	0.9[b]	1.0[b]	1.1[b]
	LL	0.1[b]	0.1[b]	0.3[b]	0.5[b]	0.5[b]	0.6[b]
	SS/PR	0.6[a]	0.7[a]	2.7[a]	3.9[a]	4.6[a]	5.7[a]
	SS/gr	0.4[ab]	0.4[ab]	1.9[a]	2.7[a]	3.2[a]	3.9[a]
F-test		*	***	**	***	***	***
C.V. %		54.0	45.5	32.1	29.2	35.1	30.4

, ** and * = significant at 5, 1 and 0.1% level, respectively; mean separation by DMRT at 5% level denoted by letters; [1] and [2] average of seven and three replications, respectively*

Appendix 7.5 Percentage of plant S cumulatively derived from fertilizers (%SDFF) in glasshouse (PART A) and field (PART B) trials.

Treatments		Days after fertilizer application					
		30	60	90	120	150	180
PART A, GLASSHOUSE TRIAL[1]				..... % SDFF			
Ramiha	SS	23.8[a]	39.0[a]	40.2[a]	38.8[a]	38.1[a]	35.2[a]
	LL	4.2[c]	7.3[c]	8.3[c]	8.4[c]	8.6[c]	9.3[c]
	SS/PR	14.9[b]	26.2[b]	29.3[b]	31.5[b]	31.7[b]	30.5[b]
	SS/gr	12.1[b]	21.5[b]	24.8[b]	26.4[b]	27.9[b]	26.8[b]
F-test		**	***	***	***	***	***
C.V. %		35.2	33.5	26.7	23.0	21.2	18.3
Tokomaru	SS	14.1[a]	20.6[a]	23.7[a]	23.4[a]	27.3[a]	27.3[a]
	MM	5.3[c]	6.5[c]	6.9[c]	7.6[b]	8.4[b]	9.3[b]
	LL	3.0[c]	4.0[c]	4.0[c]	4.2[b]	4.4[b]	4.6[c]
	SS/PR	9.4[b]	17.5[ab]	21.5[ab]	24.5[a]	27.1[a]	28.7[a]
	SS/gr	8.5[b]	15.2[b]	19.1[b]	21.6[a]	24.6[a]	24.6[a]
F-test		*	**	***	***	***	***
C.V. %		28.7	34.5	32.4	31.1	28.1	22.2
PART B, FIELD TRIAL[2]				..... %SDFF			
Ramiha	SS	27.7[a]	26.8[a]	39.0[a]	39.5[a]	41.0[a]	43.1[a]
	LL	4.5[c]	4.3[c]	6.2[b]	7.0[b]	7.3[b]	8.0[b]
	SS/PR	25.7[a]	24.8[a]	32.1[a]	34.7[a]	35.7[a]	38.9[a]
	SS/gr	19.6[b]	19.3[b]	27.6[a]	29.9[a]	31.4[a]	34.7[a]
F-test		**	***	***	***	***	***
C.V. %		13.1	14.7	23.1	19.1	18.7	15.2
Tokomaru	SS	29.5[a]	28.7[a]	33.2[a]	32.0[a]	32.4[a]	33.5[a]
	MM	7.0[c]	6.4[c]	7.1[c]	7.3[c]	7.3[c]	7.5[c]
	LL	3.4[c]	4.0[c]	4.0[c]	4.1[c]	4.2[c]	4.4[c]
	SS/PR	15.4[b]	14.7[b]	24.7[b]	27.3[a]	28.8[a]	32.1[a]
	SS/gr	15.4[b]	12.9[b]	19.4[b]	20.3[b]	21.4[b]	23.4[b]
F-test		***	**	***	***	***	***
C.V. %		28.1	26.2	17.8	14.7	12.6	10.1

, ** and * = Significant at 5, 1, and 0.1% level, respectively; mean separation by DMRT at 5% level denoted by letters; [1] and [2] average of seven and three replications, respectively*

Appendix 7.6 Percentage of oxidized elemental $^{35}S^{o}$ cumulatively taken up by pastures[1] in glasshouse and field trials (average of three replications).

Treatments		Days after fertilizer application				
		30	60	90	180[2]	Field 180
				... % ...		
Ramiha	SS	6.2[a]	13.8	19.8[a]	26.2[a]	10.0[a]
	LL	1.9[b]	8.6	10.6[b]	25.7[a]	1.7[b]
	SS/PR	4.0[a]	8.5	14.5[a]	21.0[b]	8.3[a]
	SS/gr	3.7[a]	12.9	10.4[b]	19.4[b]	8.5[a]
F-test		*	ns	*	**	***
C.V. %		35.8	25.6	22.8	16.7	32.8
Tokomaru	SS	3.4	6.4	8.8[a]	20.8[a]	4.3[a]
	MM	2.8	5.5	5.9[b]	16.8[b]	1.1[b]
	LL	1.6	3.8	5.3[b]	9.4[c]	0.6[b]
	SS/PR	1.4	6.6	13.0[a]	21.1[a]	5.9[a]
	SS/gr	1.1	5.2	8.1[b]	17.1[b]	4.0[a]
F-test		ns	ns	*	***	**
C.V. %		50.1	49.6	28.4	20.1	33.5

, ** and * = significant at 5, 1 and 0.1%, level respectively; ns = not significant; mean separation by DMRT at 5% level denoted by letters*

[1] *calculated as described in Section 7.3.9* and briefly:

% $^{35}S^{o}$ oxidized taken up = [$\sum$ % of ^{35}S taken up] * 100 / [100 - (% residual $^{35}S^{o}$)]

[2] *average of seven replications*

Appendix 7.7 Percentage recovery of extractable ^{35}S (CaP-S) in three soil layers of Ramiha and Tokomaru soils at six sampling times in glasshouse trials and after 180 days in the field trials (average of three replications).

Treatments		Days after fertilizer application 30	60	90	180[1]	Field 180
RAMIHA				.....% recovered		
Top	SS	21.1	11.5	13.1	17.3	5.1
	LL	1.0	1.4	1.3	3.2	2.0
	SS/PR	12.7	12.4	13.2	23.8	6.0
	SS/gr	7.6	10.9	5.3	14.8	6.1
Middle	SS	0.2	0.3	0.3	1.0	1.9
	LL	0.3	0.1	0.1	0.1	0.2
	SS/PR	0.1	0.3	1.3	1.4	2.9
	SS/gr	0.1	0.1	0.3	0.5	2.3
Bottom	SS	0.1	0.1	0.5	0.2	3.9
	LL	0.1	0.2	0.1	0.1	0.1
	SS/PR	0.1	0.1	0.7	0.3	4.0
	SS/gr	0.8	0.1	0.3	0.2	4.4
F-test	Layer	**	**	**	**	**
Lsd 5% within layer		5.4	3.5	3.7	5.2	1.1
C.V. %		78.1	41.2	41.0	44.4	55.1
TOKOMARU				.....% recovered		
Top	SS	10.1	8.7	12.1	11.3	4.2
	MM	1.0	3.0	2.0	2.3	1.0
	LL	1.1	1.0	1.0	0.7	0.8
	SS/PR	14.3	10.7	6.1	11.6	5.9
	SS/gr	10.6	2.5	8.7	7.8	4.2
Middle	SS	0.1	0.1	0.5	0.6	1.3
	MM	0.0	0.1	0.2	0.1	0.2
	LL	0.0	0.0	0.1	0.1	0.1
	SS/PR	0.0	0.2	0.4	0.8	1.9
	SS/gr	0.1	0.1	0.8	0.5	1.2
Bottom	SS	0.1	0.2	0.9	0.9	0.9
	MM	0.2	0.1	0.1	0.1	0.1
	LL	0.1	0.1	0.1	0.1	0.1
	SS/PR	0.1	0.2	0.3	0.3	1.6
	SS/gr	0.1	0.2	0.7	0.4	0.9
F-test	Layer	**	**	**	**	**
Lsd 5% within layer[2]		4.1	3.2	1.8	3.1	1.2
C.V. %		87.5	76.7	40.4	45.3	40.8

[1] *average of seven replications;* [2] *Lsd at 5% level*

Appendix 7.8 Concentration of soil extractable S (CaP-S) in three layers of Ramiha soil at six sampling times in glasshouse trials and after 180 days in the field trials (average of three replications).

Treatments		Days after fertilizer application 30	60	90	180[1]	Field 180
RAMIHA		..... mg kg^{-1}				
Top	SS	54.4	54.4	46.9	36.1	27.7
	LL	20.8	26.6	19.2	21.0	19.7
	SS/PR	57.1	56.0	44.3	44.8	22.9
	SS/gr	41.6	64.5	29.3	54.1	25.6
	PR	32.5	32.3	19.6	21.7	24.0
	Ctrl	26.1	29.3	17.6	22.6	19.2
Middle	SS	14.4	18.1	14.4	21.9	20.1
	LL	14.4	18.7	9.6	18.5	19.7
	SS/PR	16.0	20.2	13.7	24.0	24.0
	SS/gr	16.0	17.1	15.5	17.6	19.7
	PR	13.3	18.6	13.8	19.9	20.1
	Ctrl	15.5	19.2	16.5	19.9	19.8
Bottom	SS	14.9	18.1	19.7	25.8	26.3
	LL	15.4	22.4	18.1	22.1	21.8
	SS/PR	16.5	22.9	17.1	29.0	29.3
	SS/gr	16.0	19.7	18.1	23.3	24.0
	PR	14.4	24.0	17.6	24.9	21.9
	Ctrl	20.2	21.8	20.8	24.6	20.3
F-test	Layer	***	***	***	***	***
Lsd 5% within layer[2]		15.1	9.2	13.5	14.2	5.6
C.V. %		35.5	16.2	37.3	25.1	14.5

[1] *average of seven replications; *** = significant at 0.1% level;* [2] *Lsd at 5% level*

Appendix 7.9 Concentration of soil extractable S (CaP-S) in three layers of Tokomaru soil at six sampling times in glasshouse trials and after 180 days in the field trials (average of three replications).

Treatments		Days after fertilizer application 30	60	90	180[1]	Field 180
TOKOMARU		..... mg kg^{-1}				
Top	SS	33.1	39.5	45.9	33.8	15.5
	MM	17.6	29.3	15.5	15.4	12.3
	LL	17.1	20.3	14.9	11.2	10.7
	SS/PR	41.1	38.4	20.8	35.4	19.2
	SS/gr	33.3	25.6	30.4	28.1	17.7
	PR	25.6	19.2	19.2	12.6	17.7
	Ctrl	14.9	18.1	11.7	13.7	11.7
Middle	SS	6.4	7.5	8.5	12.0	13.3
	MM	9.6	8.5	6.4	10.1	11.2
	LL	9.6	6.9	5.3	9.1	13.3
	SS/PR	12.8	10.3	4.8	9.8	14.9
	SS/gr	11.2	9.6	6.4	9.4	16.0
	PR	10.1	8.0	5.3	7.7	13.8
	Ctrl	8.0	8.5	5.3	9.8	14.9
Bottom	SS	7.4	6.9	9.6	9.8	11.2
	MM	9.7	10.7	7.5	10.7	8.5
	LL	8.5	8.0	7.5	11.6	11.8
	SS/PR	8.5	8.0	6.9	11.4	11.2
	SS/gr	8.0	8.5	6.9	12.8	11.7
	PR	7.4	9.1	6.9	13.7	13.3
	Ctrl	6.4	6.9	6.9	14.8	11.3
F-test	Layer	***	***	***	***	***
Lsd 5% within layer[2]		13.5	9.1	6.2	6.5	5.6
C.V. %		47.1	31.8	26.5	24.5	23.4

[1] *average of seven replications;* *** *= significant at 0.1% level;* [2] *Lsd at 5% level*

Appendix 7.10 Percentage recovery of total ^{35}S in three soil layers in glasshouse trials and after 180 days in the field trials (average of three replications).

Treatments		Days after fertilizer application 30	60	90	180[1]	Field 180
RAMIHA				.....% recovered		
Top	SS	111.2	89.2	86.9	69.1	51.1
	LL	129.3	118.1	109.6	96.3	81.3
	SS/PR	99.4	100.2	92.3	93.1	52.8
	SS/gr	96.6	85.5	85.3	77.3	51.9
Middle	SS	1.2	1.7	3.1	5.4	13.4
	LL	0.8	0.5	0.2	1.1	5.5
	SS/PR	1.3	0.4	4.2	5.9	18.4
	SS/gr	0.4	0.9	0.9	2.1	15.5
Bottom	SS	2.5	0.7	1.4	0.9	8.9
	LL	1.9	1.2	0.2	0.4	0.9
	SS/PR	3.8	1.2	1.9	1.1	10.0
	SS/gr	1.7	1.9	0.8	0.4	11.3
F-test	Layer	***	***	***	***	***
Lsd 5% Within layer		16.5	14.9	5.8	10.7	13.9
C.V. %		25.1	26.7	10.1	21.9	30.7
TOKOMARU				.....% recovered		
Top	SS	93.8	98.6	86.4	73.2	67.4
	MM	116.8	121.2	100.4	89.6	73.5
	LL	118.3	120.1	139.6	88.5	83.1
	SS/PR	105.4	106.5	103.2	81.4	68.8
	SS/gr	108.1	105.5	76.6	70.6	85.3
Middle	SS	10.3	0.9	1.4	14.7	20.7
	MM	6.4	1.1	1.8	5.7	18.1
	LL	3.8	1.4	1.5	12.3	16.9
	SS/PR	4.9	1.9	2.4	13.3	19.8
	SS/gr	1.9	0.5	1.3	11.3	25.2
Bottom	SS	10.9	1.5	0.6	6.9	6.8
	MM	13.8	9.8	0.8	6.5	8.8
	LL	4.5	1.1	0.3	3.9	5.5
	SS/PR	6.2	2.7	0.8	5.2	12.8
	SS/gr	10.6	1.4	1.3	5.5	8.2
F-test	Layer	***	***	***	***	***
Lsd 5% Within Layer[2]		18.5	15.6	12.6	15.6	13.9
C.V. %		27.9	24.8	21.1	29.9	30.7

[1] *average of seven replications; *** = significant at 0.1% level;* [2] *Lsd at 5% level*

Appendix 7.11 Sulphur concentration, cumulative sulphur uptake and dry matter yield of pastures on two soils at three samplings in glasshouse trials.

Treatments		Sulphur concentration			Cumulative S uptake			Cumulative dry matter yield		
Days		30	60	90	30	60	90	30	60	90
		... % ...			.. g m^{-2} ..			.. g m^{-2} ..		
Ramiha	GP	0.44[a]	0.49[a]	0.45[a]	0.57[a]	1.23[a]	1.67[a]	130	267	391
	SSP	0.60[a]	0.46[a]	0.59[a]	0.64[a]	1.21[a]	1.67[a]	106	231	348
	Ctrl	0.34[b]	0.17[b]	0.15[b]	0.40[b]	0.56[b]	0.72[b]	113	214	325
F-test		***	***	***	**	***	***	ns	ns	ns
C.V. %		10.1	11.7	9.2	18.9	18.2	17.1	18.2	16.9	16.4
Elemental S (SS)		0.47	0.23	0.20	0.40	0.66	0.93	88	193	333
Tokomaru	GP	0.44[a]	0.40[a]	0.41[a]	0.54[a]	1.08[a]	1.45[a]	125	269[a]	347[a]
	SSP	0.44[a]	0.48[a]	0.40[a]	0.50[a]	1.45[a]	1.69[a]	112	248[a]	382[a]
	Ctrl	0.29[b]	0.14[b]	0.14[b]	0.24[b]	0.32[b]	0.51[b]	86	168[b]	272[b]
F-test		***	***	***	***	***	***	ns	*	*
C.V. %		9.6	6.8	9.3	28.2	26.2	27.6	27.5	22.6	23.7
Elemental S (SS)		0.36	0.18	0.20	0.39	0.58	0.80	106	210	319

*, ** and *** = significant at 5, 1 and 0.1% level, respectively; ns = not significant; mean separation by DMRT at 5% level denoted by letters

Appendix 7.12 Cumulative ^{35}S taken up by pasture and percentage of plant sulphur derived from labelled fertilizers (%SDFF) after application of ^{35}S labelled gypsum and superphosphate in glasshouse trials.

Treatments		Cumulative ^{35}S uptake			%SDFF		
Days		30	60	90	30	60	90
			... % ...			... % ...	
Ramiha	GP	9.4	17.5	22.6	47.7	42.4	37.9
	SSP	7.1	18.5	19.6	34.3	37.4	35.9
F-test		ns	ns	ns	ns	ns	ns
C.V. %		11.6	11.8	10.8	9.9	6.9	7.4
Elemental S (SS)		3.1	8.6	12.5	23.8	39.0	40.2
Tokomaru	GP	8.3	14.3	19.4	49.8	48.6	42.3
	SSP	7.9	16.9	22.4	48.6	44.5	40.1
F-test		ns	ns	ns	ns	ns	ns
C.V. %		14.2	20.3	16.7	27.9	24.1	17.5
Elemental S (SS)		1.8	4.0	6.3	14.1	20.6	23.7

ns = not significant at 5% level

Appendix 7.13 Phosphate extractable S concentration and amounts present in three soil depths of two soils 90 days after application of gypsum and superphosphate.

Treatments		Concentration		Amount	
		Ramiha	Tokomaru	Ramiha	Tokomaru
		mg kg^{-1}		mg $layer^{-1}$	
GP	Top	95.2	66.8	29.2	28.6
	Middle	14.4	11.2	8.3	7.6
	Bottom	12.4	6.0	16.2	10.7
SSP	Top	91.2	71.2	26.5	28.7
	Middle	18.8	6.6	10.8	4.2
	Bottom	14.8	5.2	20.1	9.1
Ctrl	Top	17.7	11.7	5.8	5.6
	Middle	16.5	5.3	10.6	3.3
	Bottom	20.8	6.9	29.9	10.0
F-test					
Treatments		**	**	**	**
Within treatments[1]		20.5	30.1	7.2	7.5
C.V. %		27.5	35.3	21.7	25.4

*** = significant at 0.1% level; [1] Lsd at 5% level*

Appendix 7.14 Sulphate retention (%) and simple relationship between amounts of adsorbed sulphate (mg S kg^{-1}) and sulphate concentration in solution (mg S l^{-1}) for three layers of Ramiha and Tokomaru soils.

Soils	Layers	S retention %	Relationships $(Y = a + bX)_a$	R^2 %
Ramiha	Top	12.1	3.8 + 0.6X	95.8
	Middle	28.0	2.2 + 1.4X	89.0
	Bottom	29.1	2.6 + 1.9X	98.3
Tokomaru	Top	24.1	3.4 + 1.5X	98.1
	Middle	28.2	4.0 + 1.8X	99.4
	Bottom	31.1	2.1 + 2.3X	94.6

[a] *Y = adsorbed S (mg S kg^{-1}); X = solution concentration (mg S l^{-1})*

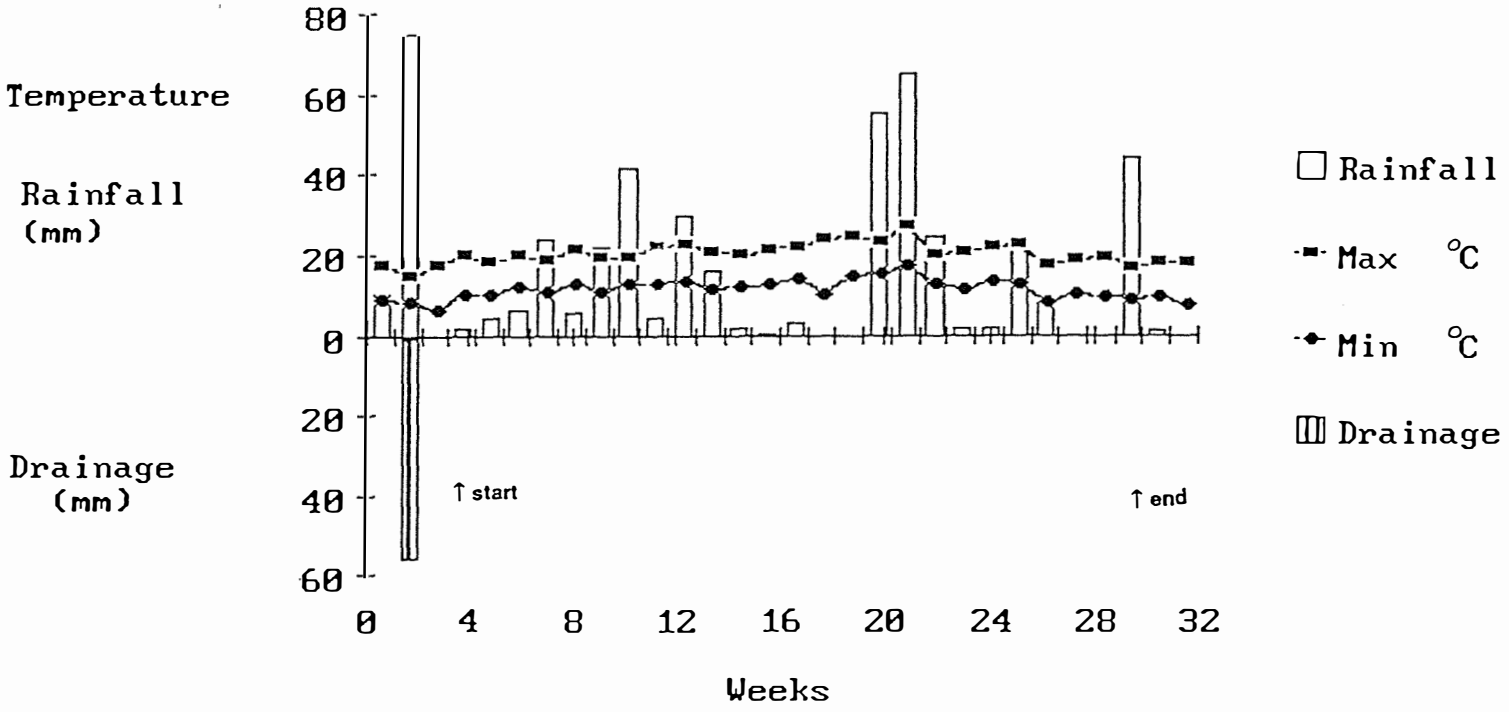
Appendix 7.15 Recovery of $^{35}S^{o}$ from two sizes elemental S at day 0 through $NaHCO_3$ dry digestion (total ^{35}S) and acetone extraction (total $^{35}S^{o}$ activity and S^{o}) after application onto the surface of soil cores.

Particle sizes	Rate applied mg S core^{-1}	Recovery percentage: Total ^{35}S %	Total $^{35}S^{o}$ %	Total S^{o} %
<0.150 mm	12.5	71.6	82.3	96.9
	25.0	74.2	77.7	91.3
	50.0[a]	76.7	80.7	87.2
	Mean	74.2	80.2	91.9
	F-test	ns	ns	ns
	C.V.: %	17.1	16.7	14.3
0.250-0.500 mm	12.5	91.1	93.5	83.7
	25.0	101.8	90.8	91.4
	50.0[a]	88.6	78.2	90.6
	Mean	95.8	87.2	88.6
	F-test	ns	ns	ns
	C.V. %	21.3	19.1	13.2

[a], *equivalent to approximately 30 kg S ha^{-1}*

remark: $^{35}S^{o}$ activity applied at 3.3 MBq g So and negligible amount of $^{35}S^{o}$ were detected in the phosphate extractable S

ns = not significant at 5% level



Appendix 7.16 Weekly rainfall (Rain) and drainage water (Drainage), average maximum (Max) and minimum (Min) temperature during November 1987 - June 1988.