ROLE OF ORGANIC SULPHUR IN SUPPLYING SULPHATE FOR PLANT GROWTH

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ABSTRACT

An open system of incubation was developed to measure sulphur mineralisation in a wide range of New Zealand soils. During 10 weeks of incubation at 30 °C, the amount of sulphur mineralised varied from 2.9 to 26.8 μg sulphur g^{-1} soil (3.4 to 32.2 kg ha^{-1}).

In an attempt to explain the variation in sulphur mineralisation between soils correlations were examined between the amounts of mineralised-SO_4^{2-} and some individual soil chemical properties. C-bonded forms of sulphur (C-S) in soils showed the best single factor correlation with the amounts of mineralised-SO_4^{2-} and this relationship was able to account for up to 63% of the variation in mineralised-SO_4^{2-} between the soils. These findings were further supported by a series of experiments in which C-bonded sulphur was identified as a major contributor to mineralised-SO_4^{2-}. The best multiple correlation was obtained from the combination of C-bonded sulphur and the C:N ratio of the soil and this relationship accounted for 71% of the variation in sulphur mineralisation between the soils.

The dichotomous model of soil sulphur cycling as proposed by McGill and Cole (1981) was tested by altering the amounts of readily available C,N and S in the soil. Addition of C to soils resulted a significant decrease in the mineralisation of C-bonded forms of sulphur. This would suggest that mineralisation of C-bonded forms of sulphur is controlled by the availability of metabolisable C in soils. In addition, this study showed that soil micro-organisms could mineralise C-bonded sulphur to satisfy their sulphur or possibly nitrogen requirements. It was also found that in the presence of low levels of SO_4^{2-} increased microbial activity (where C was added) did not necessarily result in the mineralisation of HI-reducible forms of sulphur. In most cases, microbes apparently selectively mineralised more C-bonded than HI-reducible forms of sulphur.

Sulphur-35 was used as a tracer both in a carrier-free form and with sulphate as a carrier to examine the cycling of sulphur in a closed incubation system. Soil treatment prior to the addition of sulphur-35 i.e. preconditioning, air-drying or adding glucose showed marked differences in the rate of sulphur-35 incorporation into the soil and also affected the nature of the sulphur-35 incorporation into organic fractions. Recovery of sulphur-35 in the microbial biomass showed that a considerable amount of sulphur is incorporated through the biomass. In some cases the amount of sulphur cycled through the biomass reached 90% of the total incorporation. Addition of sulphate as carrier decreased the amount of sulphur-35 incorporation.

Reincubation of the labelled soil, where sulphur-35 was incorporated into organic fractions during the original incubation showed that the longer the sulphur remained in organic fractions the less it was mineralised.

A technique was developed to remove the HI-reducible sulphur from the soil organic sulphur fraction. The method effectively removed more than 98% of the HI-reducible sulphur from the soil. Such a separation enabled the study of sulphur mineralisation characteristics from C-bonded sulphur.

In a field experiment, the effects of seasonal variation on sulphate and microbial biomass-S levels in soils were assessed. Attempts were made to explain these variations through changes in rainfall and temperature. The amounts of sulphur held in microbial biomass tissues was higher in the autumn and summer seasons compared to winter season.

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DECLARATION OF ORIGINALITY

This thesis reports the original work of the author except where otherwise stated.

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CONTENTS

CHAPTER THREE

MATERIALS AND METHODS

CHAPTER EIGHT

MINERALISATION OF SULPHUR RECENTLY INCORPORATED INTO SOIL ORGANIC MATTER

CHAPTER NINE

REMOVAL OF HI-REDUCIBLE SULPHUR FROM SOIL ORGANIC MATTER AND SUBSEQUENT MINERALISATION OF THE REMAINING C-BONDED SULPHUR

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND

It is well established that the bulk of the sulphur present in surface soils occurs mainly in organic forms and that it becomes available for plant uptake only if it is mineralised to inorganic sulphate by soil microorganisms. It has also been shown that when inorganic sulphate is added to the soil it can become incorporated into soil organic matter fractions, a process referred to as immobilisation. Many workers (e.g. Freney *et al.*, 1971; Goh and Tsuji, 1979; Maynard *et al.*, 1983; and McLaren *et al.*, 1985) have shown that the processes of mineralisation and immobilisation occur simultaneously in the soil. The net result of these biological processes has a significant effect on the availability of soil sulphur to plants.

Until recently, the use of superphosphate in New Zealand as the major phosphate fertilizer has ensured substantial inputs of plant available sulphur into soils on a regular basis. Against this background, soil organic matter has probably been viewed as having limited importance as a source of sulphur for plant uptake. However, the biological turnover of sulphur (involving mineralisation and immobilisation) could still have had a significant influence in controlling the plant availability of sulphur added to soil. The present economic condition of the agricultural sector in New Zealand is encouraging farmers to use high analysis phosphatic fertilizers which do not contain sulphur (e.g. triple superphosphate, reactive phosphate rock, diammonium phosphate). This substantially increases the importance of soil organic sulphur and its ability to release sulphate-S for plant uptake. Therefore, understanding of sulphur mineralisation and immobilisation becomes extremely important.

In the past, sulphur fertilizer recommendations for pasture maintenance were made on the basis of field trials. Optimum fertilizer rates were determined on the basis of either

sulphur uptake by plants or increase, in plant yield. An alternative to this approach was provided by Sinclair and Saunders (1984), who by using available information from the field experiments and by making some gross assumptions, proposed a sulphur cycling model which has been widely used by the New Zealand Ministry of Agriculture and Fisheries (M.A.F) in a computerised fertilizer advisory service (CFAS). One of the assumptions made in this model is that sulphur mineralisation and immobilisation in soils reaches an equilibrium after a certain period of pasture development. Such an assumption is very simplistic and the validity of this assumption is also questionable because studies by Sorn-Srivichai (1980) have shown that the extent of sulphur mineralisation in soils having similar pasture development indices varies greatly. There is very limited information available concerning the mineralisation and immobilisation of sulphur in New Zealand soils. Indeed, the CFAS model also ignores the fact that during the course of the year, variations in sulphur availability to plants might well result from changes in the rate and extent of biological sulphur transformations.

At present, in spite of a significant number of studies on sulphur mineralisation and immobilisation in soils (e.g. Williams, 1967; Freney *et al.*, 1971; Maynard *et al.*, 1983), it is difficult to predict likely variations in soil sulphur availability. There is still considerable uncertainty regarding the extent of biological turnover of soil sulphur (Freney *et al.*, 1975; Tillman, 1983). Improvement in the prediction of the availability of soil organic sulphur will require a firm understanding of the factors controlling processes such as mineralisation and immobilisation and identification of measurable parameters which could be used to assess availability.

Soil sulphur status is currently assessed by the M.A.F by determining the level of extractable sulphate-S in the soil. This test determines the amount of sulphate present at the time of sampling, but does not distinguish between that derived from previous fertilizer additions and that produced by mineralisation of organic matter. Whether there is any relationship between the extractable sulphate level and the potential of soil to mineralise sulphate is unknown. An improvement of knowledge in this area could help to improve the interpretation of the M.A.F soil sulphate test.

Overall, it is clear that improvement of sulphur fertilizer requirements and efficiency of sulphur fertilizer use requires a much better understanding of the transformations of sulphur in the soil.

1.2 OBJECTIVES

The studies reported in this thesis were designed to examine the magnitude of sulphur mineralisation in New Zealand soils and also to improve the general understanding of transformations of organic and inorganic sulphur in soils. The main aims of the study were as follows;

1. To develop a suitable method which could give a consistent measurement of mineralisable sulphur in a wide range of soils.

2. To determine those soil properties most likely to give a good indication of the mineralisation of soil sulphur i.e. to examine correlations between individual soil properties and mineralisable sulphur in soils.

3. To identify the sources of mineralisable organic sulphur in soils.

4. To examine the influence of carbon and nitrogen on the mineralisation of sulphur.

5. To examine the rate of sulphur cycling in soils by means of labelling with sulphur-35.

6. To compare the mineralisation characteristics of recently incorporated sulphur and native sulphur in soils.

7. To investigate the role of the soil biomass in sulphur cycling.

8. To measure natural variation with time of soil sulphate-S and biomass-S levels in field soils.

CHAPTER TWO

LITERATURE REVIEW

2.1 INTRODUCTION

Sulphur is an essential element for biological systems. The importance of sulphur as an essential element for plant growth has been recognised since the middle of the 19th century (Salm-Horstman, 1851). Plants obtain most of their sulphur from the soil. The availability of sulphur for plant uptake varies from soil to soil. Such variations are caused by the differences in soil parent material, external sulphur inputs to soils and the mineralisation of sulphur from organic sulphur forms. In most agricultural soils, surface soils contain more than 95 percent of the sulphur in organic forms. Soil micro-organisms play an important role in the release of this sulphur for plant growth. Since the 1950's many facets of this important nutrient have been investigated, covering a wide range of topics such as soil sulphur chemistry, biochemistry, microbiology and the agronomic benefits of this essential element.

The literature cited in this review covers relevant areas such as the functions of sulphur in plant growth and the nature of soil sulphur. Attention has also been given to a common problem in sulphur research, that is, analytical techniques to determine the different forms of sulphur in plants and soils. Since the main thrust of the study described in this thesis has been to examine the processes and factors involved in soil sulphur transformations, particularly the role of soil microorganisms, the literature on these aspects of the study has been discussed in more detail. Models of sulphur cycling in soils are also discussed.

2.2 SULPHUR IN PLANTS

Sulphur compounds have been identified as important constituents of plant tissues. The most abundant sulphur containing compounds found in plants are cysteine, methionine

and sulphoquinovosyl-diglyceride (Thompson *et al.*, 1986). Other sulphur-containing compounds are also found in plants, but in lesser amounts, for example, iron-sulphur proteins, thiamine, biotin, coenzyme A, lipoic acid and glutathione etc. The most essential sulphur components however are the sulphydryl-containing amino acids, cysteine and methionine. Cysteine plays an important role in the structuring of proteins by virtue of the disulphide (S-S) cross linkages, which form through oxidation of the sulphydryl groups of two adjoining cysteinyl residues. The sulphydryl groups are also involved in the identification and attachment of substrates to enzymes. These sulphur-containing amino acids have been identified as intermediates in the synthesis of other important compounds within the cell e.g. S-adenosyl methionine donates a methyl group in the synthesis of many cellular compounds including chlorophyll, flavanoids and sterols (Huxtable, 1986). Synthesis of the vitamin thiamine also involves methionine, while cysteine plays an important role in synthesis of the coenzyme A. Sulphur is also a major component of iron-sulphur (Fe-S) proteins (Lovenberg, 1977), in which an Fe-S centre acts as an electron carrier. Many Fe-S proteins are found in nature, some of which are soluble and some non-soluble and membrane bound. The soluble Fe-S proteins are called ferrodoxins (Bhome and Boger, 1982). There are two combinations of Fe-S (2Fe-2S, 4Fe-4S) centres involved in electron transport. The 2Fe-2S ferrodoxin is part of the electron transport chain of photosynthesis (Avron, 1981). Fe-S proteins with 4Fe-4S centres are known as bacterial types (Bhome and Boger, 1982) and are prominent in N_2 fixation and other processes in bacteria. These 4Fe-4S centres are present in plant sulphite reductase and nitrate reductase. They are also present in the cell membrane where they function in photosynthesis and respiration.

Sulpholipids are another type of organic compound required in the synthesis of cellular membrane, especially the foundation of chlorophyll in the chloroplast lamellae (Harwood, 1980). A wide range of sulphur containing compounds (Table 2.1) have significant influence on the flavour, odour and toxicity of a number of plants which may protect the plants from diseases, insects and other herbivores. For example, mercaptopropane is responsible for the aroma of onion, dimethyl sulphide for the odour of cabbages and thiocyanate for the toxic characteristics of certain cruciferous plants (Anderson, 1975).

Table 2.1 Sulphur compounds found in various plant species (From Thompson *et al.*, 1986).

Compound	Source
1. Methane thiol	Coffee (Coffea arabica)
	Tea (Camellia sinensis)
2. Propane-1-thiol	Onion (Allium cepa)
3. Methane dithiol	Plume albizzia (Albizzia lophantha)
4. Isobutyric acid 3,3'-dithiol	Asparagus (Asparagus officinalis)
5. Dimethyl sulphide	Cabbage (Brassica oleracea)
6. Dibutyl sulphide	Cabbage
7. β-Methylthiopropionate	Pineapple (Ananas comosus)
8. Dimethyl disulphide	Onion and Cabbage
9. Diethyl disulphide	Cabbage
10. Methyl, ethyl disulphide	Cabbage
11. Ethyl, n-propyl disulphide	Cabbage
12. n-Propyl, n-butyl disulphide	Cabbage
13. di-n-Propyl disulphide	Cabbage
14. Methyl, n-propyl disulphide	Garlic (Allium sativum)
15. Methyl, allyl disulphide	Garlic
16. n-Propyl, allyl disulphide	Garlic and Cabbage
17. Diallyl disulphide	Garlic

2.2.1 Sulphur uptake by plants

Sulphur is taken up by plants either through the leaves or roots. There is evidence of atmospheric SO_2 absorption by a number of plants. Fried (1949) showed that alfalfa (Medicago sativa L.) absorbed $^{35}SO_2$ through the leaves and converted it into organic sulphur compounds in the plant. Olsen (1957) reported that cotton plants (Gossipium hersutum L.) grown in SO_2 rich atmosphere absorbed as much as 30% of the total sulphur from the atmospheric sulphur.

Sulphur in soil solution as the sulphate ion (SO_4^{2-}) is taken up by plant roots. Sulphate ions reach the apparent free space in the roots by diffusion and mass flow. Sulphate enters the plant in the root hair region and diffuses through the cortex. Proof of this was provided by Cacco *et al.*, (1977) who found that SO_4^{2-} transport into roots was directly correlated with the fraction of the root which was covered with hairs, indicating that root hairs are a major site of SO_4^{2-} entry into plants. From the roots, sulphate is actively accumulated in the primary endodermis. It is then transported to the vessels of the xylem and moves in the transpiration stream to the leaves, where it is inserted into mesophyllic cells. Sulphate reduction and the incorporation of sulphur into organic forms occurs primarily in the leaves, with the chloroplast as the major site of reduction (Thompson *et al.*, 1986).

The kinetics of sulphate uptake by plant roots is poorly understood and the studies conducted to date show considerable disagreement (Vange *et al.*, 1974; Thoiron *et al.*, 1980). Sulphate transport into cells is against both chemical and biochemical potential gradients and is therefore an energy dependent process. Epstein (1976) suggested that specific transport proteins, known as "**permeases**", are required as carriers to transport SO_4^{2-} into cells.

In roots, maximum sulphate transportation was observed in the presence of a high concentration of Ca^{2+} (Smith, 1978). This may have increased the affinity for sulphate, which could mean direct interaction with a sulphate permease. Also Ca^{2+} may exert an indirect neutralization of surface potential resulting in a greater accessibility of sulphate to the permease (Roomans *et al.*, 1979). There are several reports on the inhibitory

effects of sulphate analogous compounds. A comprehensive report by Vange *et al.*, (1974) showed the inhibitory effect of structurally similar compounds on sulphate uptake in the order of CrO_4^{2-} >SeO_4^{2-} >MoO_4^{2-}, and >WO_4^{2-}.

The relationship between the supply of sulphate and its uptake is questionable, especially when sulphate is present in higher amounts than necessary for maximum plant growth. For example, reports from Petersson (1966) and Radet (1966) show sulphate uptake proportional to its supply until maximum growth is reached, with very little or no further uptake at higher concentrations. Contrary to this report, Jones (1962) showed a continuous uptake of sulphate. There are also suggestions that this relationship may vary from species to species and may depend on the growth of the plants (Jones, 1964).

2.2.2 Distribution and Redistribution of sulphur in plants

The distribution of absorbed sulphate ions and the redistribution of sulphur within the plant seems to depend on the sulphur status of the plant and the age of the plant. The climatic factors which affect the physiology of plants also play a major part in the above process. Generally, during the early growth of plants, sulphur is translocated to the younger leaves. When plants mature the foliar sulphur concentration decreases. Rominger et at., (1975) found that the sulphur concentration in alfalfa (Medicago sativa L.) dropped from 0.34 to 0.22% after eight weeks growth. In a comparative study, Bouma et at., (1972) demonstrated that subterranian clover plants grown initially under different sulphur levels showed remarkable differences in the distribution of the sulphur when they were subsequently provided with adequate sulphur. Plants grown initially under sufficient sulphur levels showed an increase of 26-33% of the total uptake of sulphur mainly in newly formed shoots, compared with plants grown initially under sulphur deficient conditions where most of the increase in sulphur uptake was recorded in old leaves. However, in each case, roots exercised a powerful demand for sulphur, accumulating 37-42% of the increased sulphur uptake.

The dynamic nature of plant sulphur uptake, accumulation of sulphur in the plant tissues and the aging factor which have been illustrated above highlight the limitations on the use of the foliar analysis for diagnostic purposes.

The redistribution or remobilisation of the stored sulphate and reduced sulphur (methionine and cysteine) in plant tissues are of particular interest at the time of fruit formation, and also when plants suffer from sulphur stress. It appears that developing reproductive tissues receive much of their sulphur from remobilisation of sulphur from vegetative tissues. Friedrich and Schrader (1978) supplied maize plants with $^{35}SO_4^{2-}$ until silking. At silking, plants were removed from S-labelled nutrient solutions and placed in nutrient solutions with and without nitrate (NO_3-N). Considerable amounts of $^{35}SO_4^{2-}$ that accumulated in vegetative tissues before silking, rapidly mobilised during silking. At maturity, ears from plants deprived of N at silking, contained 73% of their total sulphur as ^{35}S as compared to 56% in plants continuously supplied with N. This study also revealed that most of the remobilisation of labelled sulphur was in the SO_4^{2-} form. In a tissue culture study, tobacco cells degraded free methionine and cysteine dramatically within 7 days of sulphur deprivation (Smith, 1980). This suggests that reduced sulphur compounds can also be degraded perhaps by specific enzymes, and if the need arises sulphate can be mobilised to the area of demand.

2.2.3 Sulphur deficiency in plants

When plants are unable to obtain sufficient sulphur to meet their nutritional requirements they show visual symptoms of sulphur deficiency in their appearance, colour and growth. Some plants show sulphur deficiency earlier than others, depending on their sulphur requirements, which differ greatly among species, cultivars, stage of growth and season of growth (Thompson *et al.*, 1970). The halophyte plants are known to have the highest sulphur requirement, followed by plants from the cruciferae and lilliacae families. The legumes, cotton and tobacco are grouped as having intermediate requirements, whereas small grains and maize require the least sulphur. Sulphur deficiencies are difficult to distinguish because the symptoms are rather similar to N deficiency. As with nitrogen, sulphur also decreases plant growth and causes chlorosis of leaves. In cruciferous plants , chlorosis starts with interveinal yellowing of new leaves and spreads gradually over the entire leaf area. A prolonged deficiency causes reddening and purpling in the petioles, stems and leaves of many crops (McLachlan, 1978) . Leaf cupping and upright leaf structure is often associated with sulphur deficiency.

Table 2.2 Critical values for sulphur concentration in different plants (From Andrew, 1977).

Species	Critical S concentration (%)
White clover (Trifolium repens)	0.18
Kenyan white clover (T. semipilosum)	0.17
Lucerne (Medicago sativa)	0.20
Barrel medic (M. trunculata)	0.20
Annual medic (M. denticulata)	0.20
Phasy bean (Macroptilium lathyriodes)	0.17
Siratro (M. atropurpureum)	0.15
Kuru vine (Desmodium intottum)	0.17
Silver leaf desmodium (D. uncinatum)	0.17
Townsville stylo (Stylosanthes humilis)	0.14
Lotononis (Lotononis bainesii)	0.15
Neonotonia (Neonotonia wightii)	0.17

There are two common methods used to confirm the visual observation of sulphur deficiency. The first method is to measure the total sulphur and sulphate contents in plant tissues (Ulrich, 1952; Dow and Roberts, 1982). If sulphur levels fall beyond predetermined critical values then addition of sulphur fertilizer is necessary. The interpretation of such results needs to take into account the plant part sampled , tissue age and growth rate (Jones *et al.*, 1980). The second method is to assess the sulphate level in top-soils as an index of available sulphur. The usefulness of these tests is often debatable (Tabatabai, 1982), hence using soil sulphate level alone for fertilizer recommendations is often not advisable (Cornforth *et al.*, 1983). A comprehensive pot and field study conducted by Andrew (1977) on temperate and tropical legumes determined critical values (Table 2.2) of sulphur. When plant sulphur reached the critical level, an addition of remedial sulphur was required. The sampling included the whole plant tops rather than specified plant parts such as leaf or stem. Optimum sampling time was suggested to be just before the flowering stage of growth. The critical values determined in the pot trials gave consistent responses when related to field experiments. However these critical values can be of limited use because the trials were lacking a grazing component which would have a significant impact on the responses in pasture systems. The sampling time suggested by Andrew (1977) is also likely to be too late for any supplementary fertilizer to have a marked affect on plant growth.

2.2.4 Sulphur responses in New Zealand agriculture

Sulphur deficiency and responses to sulphur fertilizers in the New Zealand agriculture has been reviewed by Saunders and Cooper (1983). The earliest recorded study in New Zealand was conducted by McConnell (1914) who found that application of elemental sulphur to various crops and pastures increased yield. Since then a number of studies (Table 2.3) have been carried out to assess the application of sulphur fertilizers. Most of the early work was conducted in the southern parts of New Zealand where sulphur responses were more prominent. A number of studies conducted in the northern part of the country (Blackmore at al., 1969; Douglas and Risk, 1981; Pigot *et al.*, 1984) also showed sulphur responses but of lesser magnitude. The reasons for such wide ranging deficiencies and responses of sulphur reported throughout the New Zealand are due to

Table 2.3 Sulphur responses in New Zealand soils.

Crop	Area	Sulphur rate (kg ha^{-1})	% increase* in yield	Reference
Lucerne	Central Otago	94.0	A	Tennet and Duff (1929)
Turnip	North Otago	50.0	C	McLeod (1961)
	Westland			
Clover	Wanganui	50.0	A & B	Blackmore *et al.*,(1969)
	Canterbury		B	Clifford & White(1986)
	Eastland,Matapiro	50.0	A	Nguyen (1982)
	Taupo	50.0	A	Nguyen (1982)
Rape seed	North Otago		A	McLeod (1961)
Mixed	Bay of Plenty	25.0 & 50.0	S	Cottier & Hewitt (1975)
-pasture	Canterbury		B	Clifford & White (1986)
	Wairarapa			
Grass	Taupo	50.0	A	Nguyen (1982)
Barley	South Canterbury	12.0 & 24.0	C	McLeod (1961)
French beans	North Otago		S	Lobb (1954)

* A = above 40 , B = 10-40 , C = below 10 % yield increase (dry matter), S = significant response in vigour, improved colour,

a combination of factors such as; low atmospheric input of S, low sulphur in rainwater, high leaching of sulphate and soils with low sulphate adsorption properties. The present trend of fertilization in New Zealand agriculture is biased towards high analysis fertilizer which are rich in phosphorus and contain very little, or no sulphur. If the present trend of fertilization continues, then it is likely that the list of sulphur responsive areas in New Zealand will grow appreciably.

2.3. GEOCHEMISTRY OF SULPHUR

Sulphur is introduced into the soil by the weathering of plutonic rocks and also from volcanic eruptions which tend to bring massive amounts of sulphur rich molten material to the earth surface (Migdisov *et al.*, 1983). Most of the sulphur in these molten rocks occurs as sulphide ions (S^{2-}, HS^-) and $H_2S_{(g)}$. In the presence of metallic ions and hydrogen and oxygen atoms, sulphide ions form a wide range of sulphur bearing minerals for example, pyrite (FeS_2), gypsum ($CaSO_4.2H_2O$), anhydrite ($CaSO_4$), epsomite ($MgSO_4.7H_2O$), chalcopyrite ($CuFeS_2$) etc. Ronov (1980) found that more than 90% of sulphur in sedimentary rocks was in the form of pyrite. Besides the above mentioned sulphur minerals, there are significant amounts of sulphur found as elemental sulphur (S^0) in volcanic deposits and over salt domes. Gypsum and calcite ores also contain substantial amount of elemental sulphur. A number of soil microorganisms including those from genus Thiobacillus, are known to oxidise the elemental sulphur to sulphate-S.

2.4 FORMS OF SULPHUR IN SOILS

Sulphur in soils can be divided into two major forms . The first form is chemically inorganic in nature (e.g. sulphate, and sulphides). The second form is organic sulphur (such as cysteine, methionine, sulpholipids and various other compounds either found in living organisms or formed by decomposition of organic matter). Before organisms lived on the earth's surface, most of the sulphur would have been in the inorganic sulphide form. With the introduction of oxygen into the atmosphere sulphides were oxidised to sulphate which was later transformed into organic fractions by living organisms. When these organisms die the transformed organic sulphur is returned to the soil surface. The contribution of organic sulphur from dead tissues is such that in most arable land in

humid and temperate regions, organic sulphur constitutes between 90 to 95% of the total sulphur in the top-soil.

2.4.1 Inorganic sulphur

Inorganic forms of sulphur are an important proportion of total sulphur in the soil. Plant growth depends on the availability of inorganic sulphate. An adequate supply of sulphate-S assures good plant growth. However, the presence of excess amounts of inorganic sulphur in reduced forms, especially sulphides can cause toxicity to many plants. Sulphate is the dominant form of inorganic sulphur present in most freely drained soils while sulphides are mainly found in anaerobic/waterlogged/poorly drained soils. On the basis of its solubility and state of oxidation, inorganic sulphur can be separated into four distinct forms, which are discussed in the next four sections.

2.4.1.1 *Water-soluble sulphate ($W\text{-}SO_4^{2-}$)*

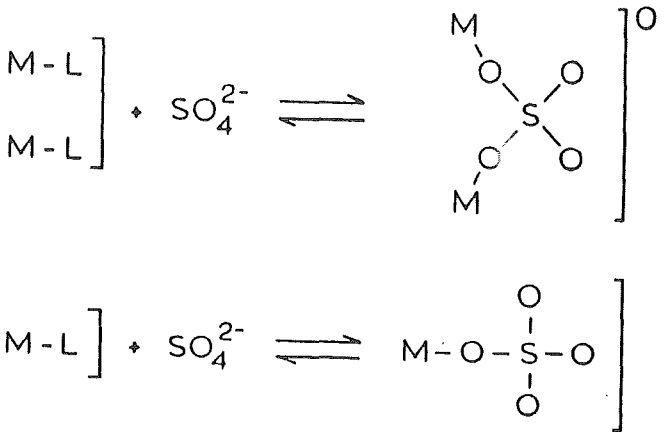
In strict terms, water-soluble sulphate is the sulphate present in the soil solution and which can be extracted from the soil with pure distilled water (Spencer and Freney, 1960; Fox *et al.*, 1964; Walker and Doornenbal, 1972). It represents the most readily plant available form of sulphur. However, many workers (Roberts and Koehler, 1968; Probert 1976) have also used extractants such as 0.01 M $CaCl_2$ or 0.1 M LiCl to remove readily available sulphate from soils. The concentrations of the reagents in these extractants are very low, thus the SO_4^{2-} extracted will be mainly water-soluble sulphate. Walker (1972) found no significant difference in extracting capacity between water and 0.15% $CaCl_2$. Since Ca^{2+} is a flocculating ion, use of this solution will minimise the extraction of colloidal clay particles and soluble organic matter.

The level of water-soluble sulphate can change very quickly in soils. Sulphate ions may be adsorbed by soil colloids (see section 2.4.1.2) or if soils are lacking anion-retentive sites, it may leach downwards or move laterally out of the soil profile. When it stays in the soil solution, it is readily utilized by plants and soil micro-organisms. Generally sulphate levels within the soil profile increase with depth (Williams, 1974; Probert, 1977). Soils from semi-arid regions have high concentrations of sulphate in sub-soils. In some cases, it may

reach as high as 10% of the total sulphur (Stace *et al.*, 1968). However, Tabatabai and Bremner (1972b) reported a reverse trend down the profile where sulphate levels decreased down the profile.

2.4.1.2 *Adsorbed sulphate*

The amount of sulphate adsorbed in soils depends on the number of adsorption sites available. Aluminium and iron oxides are the two major components involved in sulphate adsorption by soils. The adsorption reaction of sulphate can be pictured in equations (I) and (II) ; where M is the Fe or Al ion and L is an OH^- or OH_2 ligand. The initial charge of the site could be either positive or neutral.



(Adapted from Bohn *et al.*, 1986)

Sulphate adsorption is dependent on the soil pH, the amount of adsorbed sulphate decreases with increasing pH from acidic to neutral soils (Fox *et al.*, 1971; Gebhardt and Coleman, 1979; Marsh *et al.*, 1983). The main reason for low adsorption of SO_4^{2-} between pH 6-7 (neutral) is due to a low electrostatic potential at the adsorption plane. In highly weathered soils, sulphate adsorption usually increases with depth due to the presence of high amounts of exchangeable Al^{3+} and low base saturation (Singh *et al.*, 1980; Fox, 1982).

The weaker adsorption of sulphate ions compared to phosphate ions in soils is the basis of methods for extracting adsorbed sulphate from soil (Ensminger, 1954; Barrow, 1969). Most workers have used phosphatic solutions ($Ca(H_2PO_4)_2$, KH_2PO_4, NaH_2PO_4), containing 500 μg P ml^{-1}. When these solutions are introduced to the soil, phosphate ions displace SO_4^{2-} ions from retention sites into the solution. The same principle applies in the release of adsorbed-SO_4^{2-} when phosphatic fertilizers are applied to the soil (Gilman and Fox, 1980). The quantity of adsorbed sulphur depends on the nature of soil, climate, cultivation practices and fertilization. Adsorbed sulphur has been regarded as plant available sulphur (Hassan *et al.*, 1970) and the process of adsorption in some cases is regarded as a beneficial process because it enhances the availability of both current and residual fertilizers.

2.4.1.3 *Mineral sulphates*

This form of sulphate is prominent in calcareous soils, where it is deposited as insoluble crystals of calcium sulphate ($CaSO_4$). An early study by Williams and Steinbergs (1962) reported up to 2000 μg sulphur ml^{-1} in some calcareous soils from Australia. Some times the proportion of this type of sulphur may reach as high as 90-95% of the total sulphur (Williams, 1974). Theoretically, it is possible that sulphate could occur in soils as Al and Fe sulphates (Adam and Hajak, 1978). To demonstrate this, Singh (1967) and Kodama and Singh (1972) formed complex sulphates basaluminite ($Al_4(OH)_{10}SO_4.5H_2O$) and alunite ($KAl_3(OH)_6(SO_4)_2$) when they reacted aluminium hydroxide solution with SO_4^{2-} in the presence of clay minerals. Their occurrence in well-aerated soils however has not been demonstrated to be of any significance. Soils from semi-arid regions are likely to contain some mineral sulphate eg. $BaSO_4$, $CaSO_4$, Na_2SO_4.

2.4.1.4 *Sulphide sulphur*

Considerable amounts of sulphur can be reduced to sulphides under anaerobic conditions. Sulphur reduction is commonly observed in poorly-drained and waterlogged soils and also in tidal swamplands. Although traces of sulphite, thiosulphate and elemental sulphur are found in anaerobic soils (Smittenberg *et al.*, 1951), sulphides make up the bulk of the reduced compounds (Brummer *et al.*, 1971). In peaty soils where

anaerobic conditions are prevalent down the profile, Lowe and Bustin (1985) recorded heavy accumulation of reduced sulphur (FeS_2) in the anaerobic zone. The sulphide concentration is critical for high water requiring crops e.g. (rice) and also for crops grown under waterlogged conditions e.g. (Jute) because it causes toxicity to these plants. In well-aerated soils this form of sulphur is almost non-existent.

2.4.2 Organic sulphur

Organic sulphur in soils is the product of its inhabitants (plants, animals and microorganisms). A wide range of sulphur organic compounds have been identified in these living organisms. Thus there is potential for these compounds to be found in soils at some stage during the life or death of the organisms (Freney, 1986). The sulphur containing amino acids cystine, cysteine and methionine contribute greatly to the organic sulphur pool in soils. Another major group of organic sulphur compounds in soils are the ester sulphates. These two groups of organic sulphur compounds dominate the nature of organic sulphur in soils. Sulpholipids (SQD) are also found in soils but to a lesser extent. Analytical methods to date have been unable to characterize the precise contributions from various bio-organic compounds. The reasons for this can be attributed to: (i) minor sulphur contributing compounds are found in low concentrations which may be difficult to detect, (ii) unlike in a pure medium, soil is a complex material full of heterogeneous organisms, plant tissues and dead animal tissues where the use of any hydrolytic or depolymerising extractant is likely to degrade sulphur compounds.

At present, soil organic sulphur has been fractionated into two main groups of compounds on the basis of their reducibility by hydriodic acid (HI). The fraction which can be reduced is characterized as the HI-reducible fraction. The fraction which can not be reduced by HI is referred to as carbon-bonded organic sulphur.

2.4.3 HI-reducible Sulphur

The HI-reducible fraction was first determined by Freney (1958), who defined this fraction as those organic sulphur compounds that are reduced to H_2S by a reducing mixture of hydriodic, formic and hypophophorous acids (HI-reducing mixture) . He also concluded

that the HI-reducing mixture reduces only those compounds which contain the C-O-S linkage (ester sulphate), C-N-S linkage (sulphamate), the second sulphur in S-sulphocysteine (-C-S-S) and also some organic sulphites, such as dimethyl sulphite or diethylsulphite. In another study, assessing the recovery of added sulphur compounds, Freney (1961) showed that compounds possessing ester linkages (heprin and agar) were recovered completely by the HI-reducing mixture. Now there is general consensus (Cooper 1972; Freney *et al.*, 1970; Lowe and DeLong 1963) that this fraction is largely composed of ester sulphates. Studies by Freney *et al.*, (1970) confirm that amino sulphur, where sulphur is bonded directly to carbon, is not reduced by HI. Hence a clear separation can be made between amino sulphur and other forms of sulphur.

2.4.3.1 *Origin of soil HI-reducible sulphur (ester sulphates)*

On average 50% of the organic sulphur in surface soils is in HI-reducible forms (Tabatabai, 1982). It is believed to consist mainly of ester sulphate compounds. However, there is no direct evidence for the presence of such compounds in soils. There has been no isolation of any significant amounts of ester sulphate compounds from soils. In contrast, a number of living organisms are known to contain appreciable amounts of ester sulphate (Fitzgerald, 1976). Soil micro-organisms are believed to be one of the major contributors to this form of sulphur. Micro-organisms can synthesise ester sulphates, both extracellularly and also within the cellular body. Taylor and Novelli (1961) isolated a soil bacterial strain which was able to synthesise a sulphated heteropolysaccharide-protein complex. This compound contained ester sulphate groups. Burns and Wynn (1975) reported that a fungal species (Aspergillus oryzae) was able to synthesis a number of arylsulphate esters. These workers also found that the fungus A. oryzae contained phenyl arylsulphotransferase enzyme which was thought previously to be exclusively found only in mammals. The presence of this enzyme indicates that ester sulphate of phenols could be present in these organisms. A very significant ester sulphate is choline O-sulphate, which has been found in fungi (Bellenger *et al.*, 1968; Catalfomo *et al.*, 1973), algae (Ikawa *et al.*, 1973), lichens (Feige and Simonis, 1968) and plants (Nissen and Benson 1964; Thompson *et al.*, 1970). Despite the lack of identification of ester sulphates in soil their presence in these microorganisms clearly indicates the likely input of ester sulphates by micro-organisms. Fitzgerald (1976) suggested that due to the presence of a wide

range of microbes in soils which are equipped with different enzyme systems, there could be a variety of ester sulphates formed in soils through microbial action. Besides these, the occurrence of sulphated thioglycosides in cruciferous plants (Virtanen 1965) with N-O-SO_3^- linkages and sulphur bearing lipids linked as ester sulphates (Goren, 1971; Kate, 1970) are also significant sources of ester sulphate in soils. Mammals also contribute a large proportion to this organic sulphur pool. Dodgson and Rose (1975) estimated roughly that human excreta alone would contribute approximately 50 tonnes of sulphur daily in the form of ester sulphates. Besides this major contribution there are other ester sulphates of animal origin which are likely to make their way into soils. These include heparin from polyhexose sulphate esters, lactose 6-O-sulphate and sulphate esters of glycoproteins (Fitzgerald, 1976).

2.4.3.2 *Distribution of HI-reducible sulphur in soils*

The distribution of HI-reducible sulphur varies from soil to soil. Reported values range from as low as 30% to a high of 80% of the total organic sulphur in soils (Williams and Steinbergs, 1959; Freney, 1961; Cooper, 1972; Bettany *et al.*, 1973; Neptune *et al.*, 1975; Biederbeck, 1978). This variation can be attributed to the nature of the organic input into soils, climatic factors and the degree of microbial activity. Bettany *et al.*, (1973) reported that the proportion of HI-reducible sulphur in soil varies due to the nature of the decomposable organic material combined with moisture and temperature. There are conflicting reports about the distribution of HI-reducible sulphur in the soil profile. In some soils from Iowa, U.S.A. (Tabatabai and Bremner, 1972b), the proportion of HI-reducible sulphur increases down the profile while in another study in Australia, Williams (1975) found no proportional change in the organic sulphur fractions with depth. In any case, movement of organic material from surface soil to sub-surface could be a deciding factor in proportional differentiation of organic sulphur in soil profiles. Thus, the increase in the amount of HI-reducible sulphur down the profile noted by Tabatabai and Bremner (1972b) could have been due to movement of soluble organic matter containing HI-reducible forms of sulphur. The distribution of HI-reducible sulphur is also associated with soil particle size. The amount of HI-reducible sulphur increases from sand to fine clays (Anderson *et al.*, 1983). Bettany *et al.*, (1979) reported that the fulvic acid fraction of organic matter is rich in HI-S, and contained up to 84% of the total sulphur as HI-S. In a

gel permeation chromatographic study, Swift *et al.* (1988) found that HI-reducible sulphur was associated with high molecular weight (humic materials) and in an extraction fractionation study Freney *et al.*, (1969) reported that a relatively high proportion of HI-reducible sulphur was present in the humic fraction of organic matter. Thus, it is debatable as to how much and what proportion of HI-reducible sulphur is bound to different organic matter fractions. These differences may also be due to the sample preparation and the origin of samples. Environmental conditions and cultivation pratices also influence the distribution of HI-S in soil. Studies by Bettany *et al.*, (1979) have showed that an environmental gradient which affected microbial activity also influenced the distribution of HI-S in soils. Soil from a drier climatic gradient had more HI-S in the organic matter than soil from the moist climate. Agricultural practices and land use can also effect the HI-S distribution in soils. McLaren and Swift (1977) examined the effects of long term cultivation on the native organic sulphur in some Scottish soils. These workers compared pairs of soils taken from long-term pasture and adjacent continuously cultivated sites. Twenty years of cultivation had decreased the HI-reducible forms of sulphur by 25% in cropped soils compared to pasture soils.

2.4.4 Carbon-bonded sulphur

An attempt to determine this fraction of sulphur directly was developed by Lowe and DeLong (1963) in which Raney Nickel (alloy) and sodium hydroxide are used for the desulphurisation of carbon-bonded sulphur which, in the presence of HCl, it is converted to sulphide. The hydrogen sulphide is then entrapped in an absorbing solution of and determined colorimeterically by following the Johnson and Nishita (1952) procedure. Raney Ni can reduce all the sulphur bonded to carbon in mercaptans, and it can also reduce carbon-bonded sulphur from amino acids, sulphoxides and sulphonic acids attached to an aromatic nucleus.

Freney *et al.*, (1970) suggested an indirect approach of calculating carbon-bonded sulphur (C-S), that is to subtract the amount of HI-reducible sulphur from the total organic sulphur (Figure 2.1). Since then many workers (Maynard *et al.*, 1983; David *et al.*, 1982; McLaren *et al.*, 1985) have used this calculation based on the assumption that the non-

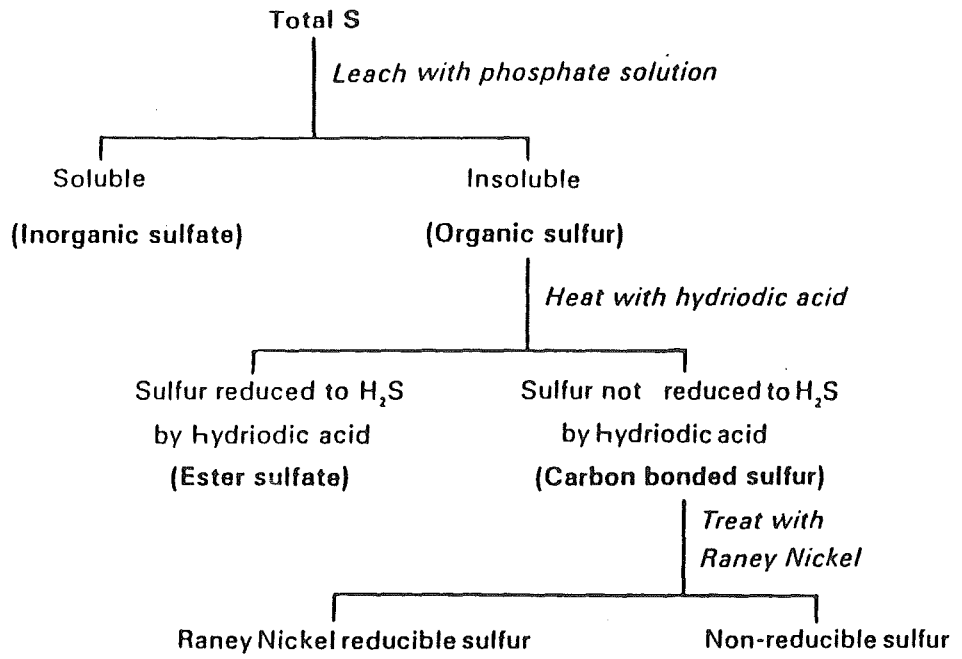


Figure 2.1 Flow diagram of the procedure to differentiate and determine the forms of sulphur in soils.
(Freney, 1986)

HI-reducible sulphur fraction consists entirely of carbon-bonded forms of sulphur. With our present knowledge, this assumption is possibly an oversimplification. Carbon-bonded sulphur values calculated as described above, are always higher than the Raney Nickel values. This suggests that either the Raney Nickel method underestimates the carbon-bonded sulphur or the subtraction method is inaccurate. Theoretically, the sums of Raney Ni-S and HI-reducible sulphur should be slightly higher than the total organic sulphur values because both of these methods can also determine the reduced inorganic forms $S_2O_3^{2-}$, $S_2O_4^{2-}$, $S_4O_6^{2-}$, SO_3^{2-} and S^0. However these forms of sulphur may not be contributing greatly to the total sulphur in well-drained and well-aerated soils (Nor and Tabatabai, 1976). Extensive work by Freney *et al.*, (1970) explains some reasons why Raney Nickel underestimates C-S in soils. They investigated the effectiveness of the Raney Nickel method in reducing various compounds containing C-S bonds (e.g., thioacetamide, methionine, methionine sulphoxide, methionine sulphones, cystine, cysteic acid and taurine). They concluded that the Raney Nickel method failed to reduce taurine, cysteic acid and methionine sulphones and the presence of metallic cations (Fe and Mg) interfered with the Raney Nickel sulphur determinations. Recoveries of the added C-bonded forms of sulphur were also dependent on the amount of alloy used, time allowed for reduction, the concentration of alkali and the amount of sulphur dissolved in the alkali. In most reported cases, recovery of organic sulphur as the sum of HI-S and Raney Ni-S have seldom reached above 90% . Freney *et al.*, (1970) found that only 4 soils out of 15 tested exceeded >90% recovery. Lowe (1965) had even lower rates of recovery, only four out of 30 soils showed >90% recovery of total organic sulphur. It would appear that the above mentioned low recoveries were associated with the presence of aliphatic sulphur compounds (aliphatic sulphones or sulphonic acids) which could not be reduced by the Raney Ni .

A series of consistent low recoveries of total organic sulphur in soils led Lowe (1964) to believe that there could be another group of sulphur compounds, referred to as "**inert-organic sulphur**" which cannot be reduced by either HI-reducing mixture or Raney Nickel. Because of its resistance to either of the reducing reagents, these compounds have been regarded as a stable fraction of organic sulphur, which is resistant to degradation and hence least important for plant growth (Lowe, 1964, Lowe and DeLong, 1965; Biederbeck 1978). If this is true then aliphatic sulphones and sulphonic

acids which are non-reducible by Raney Ni (Freney *et al.*, 1970) would be regarded as inert-organic sulphur compounds. If these compounds are end products of any soil-biological process or added by the particular vegetation, then one would expect the level of this fraction will rise with time. However, there is no evidence to support the "inert-carbon-S compounds" hypothesis. It is possible that this fraction of C-S is more resistant than the other two fractions (HI-S and C-S) and may take a longer time to degrade into simple molecules. It may be appropriate to call this fraction a **non-reducible** organic carbon fraction rather than naming it as inert organic sulphur.

2.4.4.1 *Origin of carbon-bonded sulphur*

The abundance of the amino acids cysteine and methionine in living cells is well known and hence these compounds are likely to be major sources of carbon-bonded sulphur in soils. However sulpholipids are also an important source of C-S in soils, particularly (6-Sulphoquinovosyldiglyceride) (SQD) which is an important constituent of chloroplast membranes. Harwood and Nicholls (1979) reported that sulphur involved in SQD structuring may constitute between 33 to 50% of the total sulphur in green leaves. There are several other aliphatic compounds found in plant tissues where sulphur is directly bonded to carbon. For example, thiomethylether in the roots of Flaneria repanda (Bohlmann and Kleine, 1963), thiophane compound in the roots of Baeria aristata, Cav. (Bohlmann *et al.*, 1964), a-terthienyl in the petals of Tagetes erecta L. (Zechmeister and Sease, 1947). The amount of these compounds in most soils will be marginal as only a continuous deposition of plant tissues containing aliphatic compounds would result in substantial aliphatic C-S in soils. Even if they do exist in determinable quantities, the Raney Ni method perhaps will not reduce such compounds (Freney *et al.*, 1970).

2.4.4.2 *Distribution of carbon-bonded sulphur in soils*

The distribution of Raney Nickel reducible sulphur (NiC-S) in mineral soils varies from 5 to 55% of the total sulphur. Since this fraction is associated with organic carbon, it's not surprising that organic soils have higher proportion of NiC-S and total C-S in surface soils. In Canadian soils, Lowe and DeLong (1962) found that organic soils contained 47-58% NiC-S compared with 12-35% in mineral soils. Freney *et al.*, (1970) reported 22-54% NiC-S

in Australian soils and Neptune *et al.*, (1975) found a considerably lower amount of this fraction in Brazilian soils, ranging from 5-12% . Generally forest soils contain higher proportion of C-S compared with crop or pasture soils. Long-term cropping can also decrease the C-S from soil organic matter. Studies by McLaren and Swift (1977) and McLachalan and DeMarco (1975) have shown between 75 and 80% decreases in C-bonded forms of sulphur resulting from the long-term cultivation. In addition, in a pot experiment Freney *et al.*, (1975) showed a decrease in the C-bonded forms of sulphur due to mineralisation and plant uptake sulphur.

The C-bonded sulphur also decreases from surface to subsurface soils (Lowe 1965; Tabatabai and Bremner, 1972b). Lowe (1965) also separated fractions of polysaccharides as C-S from Canadian soils but it constituted only 2% of the total sulphur. A finding of small amounts of sulpholipids (0.29-0.45% of the total sulphur) has been reported by Chae and Tabatabai (1981), and slightly higher proportions (0.5-3.5%) were found in British Columbian soils (Chae and Lowe, 1980). In both cases surface soils contained more lipid sulphur than subsurface soils. The amount of non-reducible sulphur varies considerably depending on the origin of organic matter.

2.5 THE SULPHUR CYCLE

Figure 2.3 shows the overall cycling of sulphur in soils. This cycle can be divided into two subcycles. One which involves the external factors such as sulphur added as fertilizer, animal waste, and sulphur brought by acid rain, therefore it is called **external cycling**. In other words this part of the sulphur cycle take place above the soil surface. The other part of the sulphur cycle deals with the internal transformations below the soil surface and hence it is called **internal cycling**. Althogh many types of transformations are shown in the internal cycling of sulphur in Figure 2.2, the most important are the transformations of organic sulphur to sulphate-S and vice-versa. The release of SO_4^{2-} from organic sulphur is known as sulphur **mineralisation** and the incorporation of soil SO_4^{2-} into organic sulphur is known as **immobilisation** of sulphur. Both of these processes are carried out by soil micro-organisms. The balance between the two processes determines the availability of sulphate-S for plant growth. These two processes play an important role in the internal cycling of sulphur within the soil system. The dynamics of the internal cycling

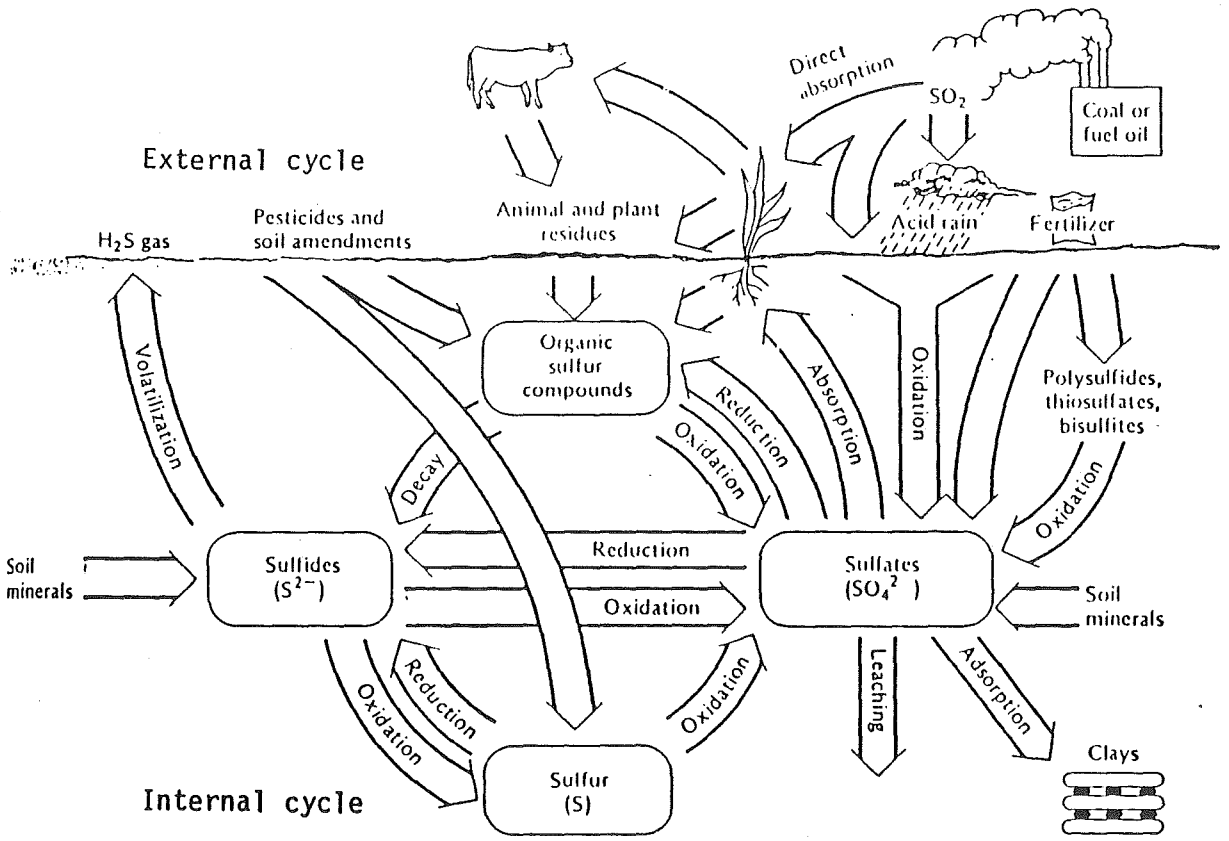


Figure 2.2 An overall view of sulphur cycling in soils.

(Brady, 1984).

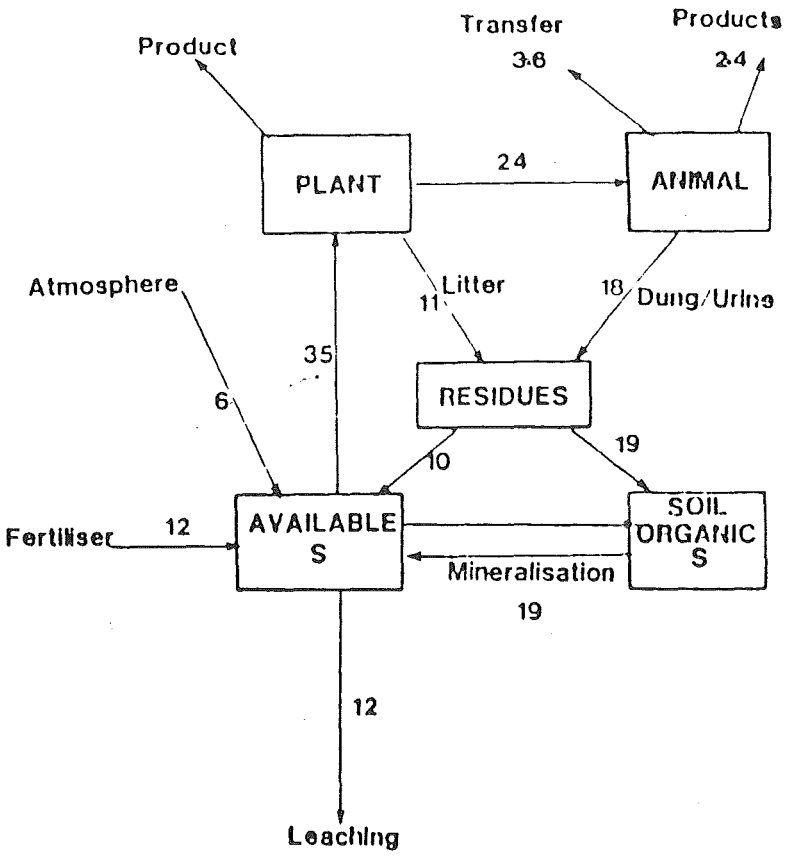


Figure 2.3 Sulphur cycling model for established pasture grazed by dairy cattle. Numbers shown in the figure indicate the transfer rate of sulphur between the pool as kg S ha^{-1} $year^{-1}$. (Tillman, 1983)

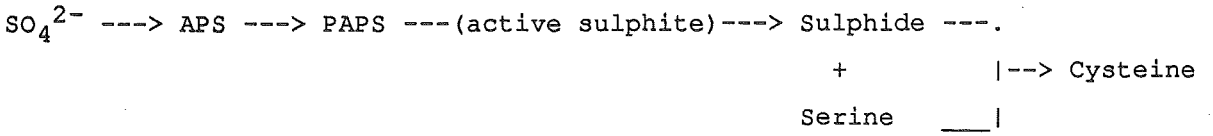
to some extent is effected by the external gain and loss of sulphur in soils. In field conditions, the over all cycling of sulphur is determined by the interactions of the external and internal cycles of sulphur. Sulphur cycling shown in Figure 2.2, broadly describes the sulphur inputs and outputs in soil systems. The determination of sulphur inputs and losses from the external sources are relatively easy to measure compared to internal transformations. However, for practical purposes a quantitative understanding of these two types of cycles is essential for modelling sulphur movement through the soil-plant system.

Tillman (1983) presented an integrated model of sulphur cycling in a grazed pasture (Fig. 2.3). The input and outputs of sulphur have been pooled into boxes which make it easier to quantify the overall gains and losses. This model suggests that the losses of sulphur occurring due to leaching would have to be supplemented to avoid deficiency. The model assumes that the formation of organic sulphur compounds in soil and the release of sulphate from soil organic sulphur is at equilibrium. Information available for the Tokomaru silt loam soil casts doubt on the validity of equilibrium state assumptions since a large variation in organic sulphur contents have been reported (Sorn-Srivichai, 1980). Tillman (1983) suggested that an urgent priority should be given to develop a method to measure sulphur mineralisation in soils. In the context of most temperate mineral soils, where more than 90% of the total sulphur is found in organic forms, even a small percentage release of sulphur will have a considerable effect on the plant available sulphur status. Therefore, sulphur mineralisation/immobilisation processes in temperate soils hold the key to the overall availability of sulphate-S for plant growth.

2.6 SULPHUR MINERALISATION AND IMMOBILISATION IN SOILS

The microbiological transformation of sulphur in soil is a complex process. It comprises mineralisation and immobilisation. Both of these processes are concurrent in soils (Freney *et al.*, 1971; Maynard *et al.*, 1983). In the last two to three decades, a number of studies have been conducted to improve the understanding of sulphur mineralisation and immobilisation, yet still the processes involved in the mineralisation of sulphur are poorly understood (Scott, 1985). A descriptive explanation of sulphur transformation has been presented by McGill and Cole (1981) (see section 2.7). It is believed that mineralisation

of sulphur results either (a) release from organic materials during oxidation of C by soil organisms to provide energy or (b) release controlled by the supply and need of the microbial population. Only the excess SO_4^{2-}-S which is not required by micobes becomes available for plant uptake. There are large numbers of sulphur-containing amino acids, esters and other organic sulphur compounds such as thiols, thioether, steroid sulphates, lipid sulphates and glycoprotiens which can provide carbon as well as sulphur. The exact process of sulphur mineralisation is not well understood, however it is known that microbes can use their enzyme systems to use a number of these compounds as substrates (Huxtable, 1986; Dodgson *et al.*, 1982). Sulphur liberated by microbial action is an important source for plant available sulphur. In agricultural systems with low or zero sulphur fertilizer input, this source of sulphur is extremely important for plant growth. Mineralisation of sulphur is likely to occur when microbes are in short supply of easily metabolisable carbon or sulphate-S. On the other hand, if carbon and nitrogen are available in high amounts relative to sulphur then immobilisation of inorganic sulphate sulphur is likely to take place. The pathway of sulphate immobilisation is obscure. One of the immobilisation pathways for sulphate is the formation of cysteine which is generally believed to involve a two-step process. Sulphate-S is transformed into energy-rich nucleotides, APS (adenosine 5'-phosphosulphate) and PAPS (3'-phosphoadenosine-5'phosphate). The PAPS is reduced to sulphide and then combined with the amino acid serine to form cysteine.



(From Scott, 1985)

Two methods are used to study sulphur mineralisation and both have some advantages and some limitations. The most common method is to measure the net change in the level of sulphate during incubation of soil in a container such as a conical flask. This method is referred to as a **closed incubation system**, the mineralised sulphate remaining

within the system during the incubation period. The amount of extractable sulphate is measured before and after the incubation to determine the net mineralisation. Results obtained using this incubation system have often given very low levels of mineralisation (Barrow, 1961; Williams, 1967; Kowalenko and Lowe, 1975b; Maynard *et al.*, 1985). In some case, an initial flush of mineralisation has been recorded (Williams, 1967) but thereafter very slow mineralisation or even some immobilisation has been observed. In other studies, the pattern of sulphate release has been reversed, where initially it was marked with immobilisation of sulphate followed by slow mineralisation of sulphate (Barrow, 1961; Kowalenko and Lowe, 1975b). Some studies have shown a rapid mineralisation in the first two weeks followed by a plateau (Swift, 1983). Such diverse results from the above studies show the complex nature of sulphur mineralisation in soil systems. Generally, closed incubation studies have measured relatively small amounts of net mineralisation. This is mainly due to the cyclic nature of sulphur transformations where mineralised sulphur is continuously incorporated back to organic sulphur. Therefore, it is difficult to measure total potentially mineralisable sulphur. Use of radio-isotopic sulphur (^{35}S) in closed incubation systems has been helpful in making some headway, especially in the understanding of sulphate immobilisation into organic forms and tracing the movement of added sulphur in that system (Freney *et al.*, 1971; McLaren *et al.*, 1985). The concurrence of sulphur mineralisation and immobilisation which had been assumed previously, became evident from isotopic sulphur-35 studies, e.g. McLaren *et al.*, (1985) reported immobilisation of added $^{35}SO_4^{2-}$ into organic forms and at the same time observed mineralisation of native organic sulphur to $^{32}SO_4^{2-}$.

The second method used in sulphur mineralisation studies is called an **open incubation system**. This method was initially developed for N mineralisation studies (Legg *et al.*, 1971). Tabatabai and Al-Khafaji (1980) reported a successful adaptation of an open system incubation for sulphur where mineralised sulphate is periodically removed during the incubation. An open system of incubation gives much higher rates of mineralisation compared with the closed system. In a comparative study Maynard *et al.*, (1983) reported an approximately 10 fold higher sulphur mineralisation in an open incubation system than a closed system. The open system simulates the field situation where mineralised sulphur is readily removed by plant uptake, leached downwards or may be adsorbed on to soil colloidal components. Use of this method of incubation system has

been very limited, however it does hold much promise to explore various aspects of sulphur mineralisation.

2.6.1 Factors affecting sulphur mineralisation and immobilisation

Being a microbially induced process, mineralisation/immobilisation of sulphur is regulated by those physical and chemical factors which influence the growth of micro-organisms. The factors which have been investigated include soil moisture, temperature, pH, availability of substrate, and alternate drying and wetting. The presence of growing plants has also been shown to affect mineralisation.

2.6.1.1 *Effect of moisture and temperature*

The effect of moisture and temperature on the mineralisation of other nutrients such as C and N has frequently been demonstrated (eg., Campbell, 1978). It is often difficult to single out one factor as being more important than the other. To assess the influence of moisture and temperature, most of the studies have either kept the temperature constant and varied the moisture level or vice-versa. Williams (1967) conducted a detailed study on a group of eastern Australian soils. When soils were incubated at 30 °C and varying moisture levels, sulphur mineralisation decreased sharply above and below the 100 cm tension (field capacity (FC)) moisture level. The low levels of sulphur mineralisation at low moisture contents could have been due to a low level of enzymatic activity (Cooper 1972) and also low solubility of the substrate which may decrease the level of mineralisation. On the other hand, at high moisture contents, decreased sulphur mineralisation was due to poor aeration. In another study, conducted in England to examine sulphate immobilisation and sulphide production, Choudhury and Cornfield (1967a) found a high level of immobilisation of inorganic sulphate in the presence of added organic carbon-rich sources. The rate of immobilisation was highest when moisture was maintained between 20 to 60% water holding capacity (WHC) moisture content. When soils were over-saturated at 133% WHC moisture level it caused a substantial reduction of sulphate to sulphide. In both studies it is noticeable that moisture levels above field capacity were not favorable for sulphur mineralisation.

When soils maintained at a constant moisture level (FC) were incubated in a closed system for 64 days at 10, 20 and 30 °C , Williams (1967) found that sulphur mineralisation was suppressed at 10 °C, and resulted in no mineralisation, but sulphur mineralisation almost doubled at 20 °C and tripled at 30 °C. Choudhury and Cornfield (1967b) found similar results in an incubation study. They reported that the level of mineralisation increased from 20 to 40 °C but fell sharply when the temperature was raised from 40 to 50 °C. Such a high temperature would normally deter soil microbial activity, hence resulting in a decline in sulphur mineralisation. However, studies by Keer (1981) showed some mineralisation at 5 and 10 °C. In an open column mineralisation study Tabatabai and Al-Khafaji (1980) reported that cumulative sulphur mineralisation was linearly related with time of incubation over a 28 week period. The average rate of sulphur mineralisation was three times higher at 35 °C than 20 °C. In contrast to these reports, Nicolson (1970) reported that an increase in temperature from 10 to 20 °C showed no effect on sulphur mineralisation, suggesting that soil temperature in that range has no effect on sulphur mineralisation. The reason for this result is probably due to two main factors. Firstly, the moisture level of 12% WHC chosen by Nicolson (1970) for incubation was very low and possibly restricted the enzymatic activity. Secondly, the soil used in the study was sandy and had a poor organic carbon and sulphur status which would have deterred microbial growth. In conclusion, soil moisture and temperature have significant effect on sulphur mineralisation and immobilisation. For favourable mineralisation, an optimum soil moisture and temperature is necessary. From the reported studies it seems, moisture ranging from 50 to 100% FC and temperature between 30-40 would be suitable for maximum sulphur mineralisation.

2.6.1.2 *Effect of drying and wetting*

Drying and wetting of the soil has appreciable effects on sulphate-S production from soil organic sulphur. There are a number of reports showing that air-drying releases considerable amounts of sulphate (Barrow, 1961; Williams, 1967; Kowalenko and Lowe, 1975a; David *et al.*, 1982). The mechanism of this phenomenon is not exactly clear. It is believed that this release could be the function of chemical hydrolysis of organic sulphur compounds which is sometimes associated with the Birch phenomenon (Williams, 1967). Kowalenko and Lowe (1975a) noted that sulphur transformation during air drying was not

related to soil microbiological activity, as measured by CO_2 evolution. This strengthens the belief that this phenomenon is of nonbiological nature. The higher the temperature of drying, the higher the amount of sulphur released as extractable sulphate-S (Barrow, 1961; Williams, 1967; David *et al.*, 1982). When moist soils were oven dried at 100 °C the amount of KH_2PO_4 extractable SO_4^{2-} rose from 4.6 to 32.7 $\mu g\ g^{-1}$ soil (Williams, 1967). Not only sulphate-S content increases on drying but increases in HI-S forms of sulphur have been noted as well (David *et al.*, 1982). This increase was related to a decrease in carbon-bonded forms of sulphur. When air-dried soils are moistened, a flux of sulphate is a common feature in closed system incubation studies (Barrow, 1961; Williams, 1967), possibly caused by the "Birch effect". Barrow (1961) suggested that such a flux of sulphate-S after wetting a dry soil would be a factor encouraging quick plant growth in a number of Australian soils. Repeated events of drying and moistening have also given an increase in sulphate-S contents (Williams, 1967). Such an increase is unlikely to occur in temperate countries. However, it may have some value for tropical and monsoon countries, where high temperature and frequent rains are common but there is a lack of evidence to support the substantial release of SO_4^{2-} in field conditions.

2.6.1.3 *Effect of carbon, nitrogen and sulphur relationships*

Organic forms of N and sulphurin soils are often related to the amount of organic carbon, and transformations of any one of these elements usually also involves simultaneous transformations of the others. Walker (1957) believed that where soil organic sulphur is highly correlated to total N, the rates of mineralisation of N and sulphur should occur in a similar ratio to the ratio of N to sulphurin the soil organic matter. This hypothesis has been widely rejected because it failed to fit the results from several other studies. The ratio of mineralised nitrogen to sulphur has been found to be wider than that of N to sulphurin soil organic matter (Williams, 1967; Haque and Walmsey, 1972; Kowalenko and Lowe, 1975b). In contrast, there are cases where the ratio of mineralised N to sulphur was smaller than that found in the soil organic matter (Nelson, 1964; Tabatabai and Al-Khafaji 1980; Maynard *et al.*, 1983). Such contradictory results raise the point of the likely usefulness of N:S ratios in predicting the mineralisation behavior of one or the other element. Freney (1962) suggested that N and sulphur could occur in different fractions of organic matter and be mineralised at different rates. Thus, until a

better fractionation of organic sulphur is achieved, the distribution of C:N:S will reveal very little about the proportional mineralisation of these elements. That is why workers have failed to get any significant relationship between C:N, C:S, N:S ratios with the amount of sulphur mineralised (Swift, 1977; Tabatabai and Al-Khafaji 1980). However, in general, soils with wide C:N:S ratios have resulted in low sulphur mineralisation (Haque and Walmsey, 1972; Kowalenko and Lowe, 1975b). There could be some use of C:S ratios as suggested by Barrow (1960). If the C:S ratio is <200 then it is likely to encourage sulphur mineralisation and if this ratio exceeds 400 then inorganic S is likely to be immobilised. This is a crude assumption and needs more clarification. Not only the amount of organic carbon but the nature of the organic carbon will also influence sulphur mineralisation and immobilisation. Saggar *et al.* (1981b) showed the addition of cellulose caused considerable immobilisation of sulphate-S. McLaren *et al.* (1985) reported that addition of glucose as a source of organic carbon doubled the incorporation of $^{35}SO_4$ into organic forms. These two studies show that addition of metabolisable C can affect the transformation of sulphur considerably.

2.6.1.4 *Effect of soil pH*

Soil pH effects the growth of microbial populations. Low pH favours fungal growth , so that under acidic conditions sulphur mineralisation will mainly be performed by fungi and under less acidic to neutral pH range it would be induced by both bacteria and fungi. Studies by Tabatabai and Al-Khafaji (1980) have shown an inverse relationship between soil pH and the amount of sulphur mineralised, mineralisation of sulphur decreasing with an increase in soil pH. In contrast, Williams (1967) showed that the addition of lime, which increases soil pH, increased the net mineralisation of sulphur. In the same study, when soils were treated with HCl to lower the pH, smaller amounts of sulphate were released from the soils. The effects of liming, which increases soil pH also increases sulphate concentration in soil solution. Such increases include mineralised and the desorbed sulphate which is released from the sorbed site due to increase in soil pH. Such increases have often been confused with mineralisation. Freney and Stevenson (1966) have attempted to explain the possible reasons for the increase in soluble sulphate content when soils are treated with lime;

(a) higher mineralisation of sulphur from soil organic matter by bacteria growing better in a more favorable environment,

(b) higher release of sulphate from soil organic matter by chemical hydrolysis at alkaline pH,

(c) increase in soil pH causes release of adsorbed sulphate from soil exchangeable sites and often lime ($CaCO_3$) contains sulphate-S as an impurity which may increase sulphate-S in soils,

2.6.1.5 Effect of plants

The presence of plants generally increases sulphur mineralisation in soils. Glasshouse experiments have shown increased levels of sulphur mineralisation in cropped soils compared with uncropped soils (Spencer and Freney 1960; Nicolson, 1970; Freney *et al.*, 1975; Tsuji and Goh, 1979; Maynard *et al.*, 1985). The amount of sulphur mineralisation caused by the presence of plants may vary from soil to soil depending on the soil organic sulphur status and the plants internal demand for sulphur. Maynard et al. (1985) reported a several fold increase in net mineralised sulphur in cropped compared with uncropped soils. This increase has been attributed mainly to a "rhizosphere effect". This effect is regarded as of great significance in the cycling of sulphur in the soil-plant system. It is believed that there is greater microbial activity associated in the rhizosphere of plants and/or the excretion of enzymes by plant roots which catalyze the degradation of soil organic sulphur compounds.

2.6.2 Source of mineralisable organic sulphur

In order to develop a satisfactory soil test for predicting sulphur mineralisation, it is necessary to know which fractions of organic sulphur are mineralisable. There is very limited research being conducted to assess such a pool and consequently the process of sulphur mineralisation is not well understood. In addition, the present methods used for fractionating organic sulphur appear unsatisfactory. Some early work was carried out by Frederick *et al.*, (1957), who showed that soil microbes grown in culture could

Table 2.4 Sulphate recovered from organic sulphur compound in soil perfusates. (Frederick *et al,.* 1957)

Compounds	Sulphate-S (% of the S of the added Compound)		
	2 wks	4 wks	6 wks
Cystine	19	80	92
Taurine	67	86	100
Na-taurocholate	70	73	97
Thiamine	5	7	9
Methionine	1	2	1
Thiourea	1	2	1
Phenylthiourea	0	2	1
K-ethyl xanthate	1	0	1
Sulphathiozole	0	0	0
Sulphonmethane	0	0	0

mineralise a number of organic compounds, including cystine into sulphate (Table 2.4). However, apart from cystine and methionine, other compounds listed in Table 2.4 have not been found in soil organic fractions. Also these soil amino acids may not occur in such a free state as used by Frederick and co-workers. To investigate the process of sulphur mineralisation and the extent of mineralisation a reverse approach was adopted, whereby radio-active $^{35}SO_4$ was added to soils to follow the cycling of the applied sulphate and to study the dynamics of this system (Freney *et al.*, 1971; McLaren *et al.*, 1985). Both of these group of workers have found that in a closed system, following the incorporation of applied $^{35}SO_4$ an apparent equilibrium is reached within 40-60 days, where immobilisation and mineralisation of $^{35}SO_4$ occur at the same rate and the distribution of radioactive sulphur-35 stays constant in both organic sulphur forms (HI-S and C-S). McLaren *et al.* (1985) extended this type of study further when they removed excess inorganic sulphate from ^{35}S incubated soils before re-incubation. They found that about 40-60% of the recently incorporated organic sulphur was mineralisd within 7 weeks. However, they did not record the input of the individual organic fractions to the re-mineralised sulphur.

The recent works of Fitzgerald and co-workers (Fitzgerald and Andrew 1984; Fitzgerald *et al.*, 1983, 1984; Strickland and Fitzgerald, 1983) have shown that carbon-bonded S compounds which are likely to be found in the soils can be mineralised into sulphate and also sometimes converted into ester sulphates. Fitzgerald *et al.* (1984) showed that 45% of added L-methionine was mineralised to sulphate within 48 hours of incubation. Strickland and Fitzgerald (1983) reported the mineralisation of sulphoquinovose (6-sulpho-6-deoxyglucose) to sulphate. Approximately 20% of the added 6-sulphoquinovose was mineralised to sulphate after 48 hours incubation. Not only C-S compounds but HI-S can also be mineralised with similar ease. Houghton and Rose (1976) found that a number of sulphate esters were hydrolysed to sulphate by soil enzymes. From the above reports it is evident that both forms of sulphur can be mineralised to sulphate. A noticeable aspect in all experiments is the speed of mineralisation. Most of the transformation occurs between 1 and 48 hours. Thus a quick and periodic assessment is necessary to estimate the nature of the transformation. Despite the knowledge of mineralisation of a variety of soil organic sulphur fractions it is not clear whether some are mineralised more easily than the others. Freney *et al.* (1975) made an attempt to investigate this problem by

incorporating ^{35}S into soil organic sulphur and then using that recently ^{35}S labelled organic sulphur for plant growth. They measured the ^{35}S and ^{32}S distribution in the HI-reducible and carbon-bonded forms of sulphur before and after the plant growth and determined that the recently incorporated HI-S decreased considerably and found little increase in the carbon-bonded sulphur. Similar results have been reported by Tsuji and Goh (1979). This tends to suggest that HI-S forms of sulphur represents a mineralisable pool of organic sulphur. However, this could not be confirmed by Freney *et al.* (1975) because native sulphur (^{32}S) showed a reverse trend where plants utilised more carbon-bonded S than the HI-reducible sulphur. Field studies by McLaren and Swift (1977) and McLachlan and DeMarco (1975) show approximately 75 to 80% decrease in the amounts of native C-bonded forms of sulphur due to long-term mineralisation caused by continuous cultivaton. This would tend to suggest that C-bonded form of sulphur is representing mineralisable forms of sulphur in soil.

In conclusion, the studies show that both the HI-reducible and carbon-bonded sulphur represent mineralisable sulphur as Freney *et al.* (1975) found that plant could utilise sulphur from both organic forms of sulphur. With the present division of organic sulphur based on analytical procedures, it seems difficult to single out any one fraction which is more mineralisable than the other.

2.7 SULPHUR MINERALISATION MODELS

Very few attempts have been made to model the S mineralisation and immobilisation processes. This is due to lack of data and also inconsistency in data caused by the differences in incubation techniques (Maynard *et al.*, 1983), soil physical, chemical and biological characteristics. As was discussed in 2.6.1.3, despite the strong association between soil organic sulphur and organic nitrogen there is rarely any resemblance in their mineralisation pattern (Kowalenko and Lowe 1975a; Biederbeck, 1978). The mineralisation and immobilisation of sulphur in surface soils is a continuous process. The controlling factors of each of these processes are not well known. It has been observed that addition of metabolisable carbon encourages immobilisation of inorganic sulphur (Saggar *et al.*, 1981b) but at the same time, microbes may also be involved in sulphur mineralisation. David et al. (1983) suggested a schematic model (Fig.2.4) which shows

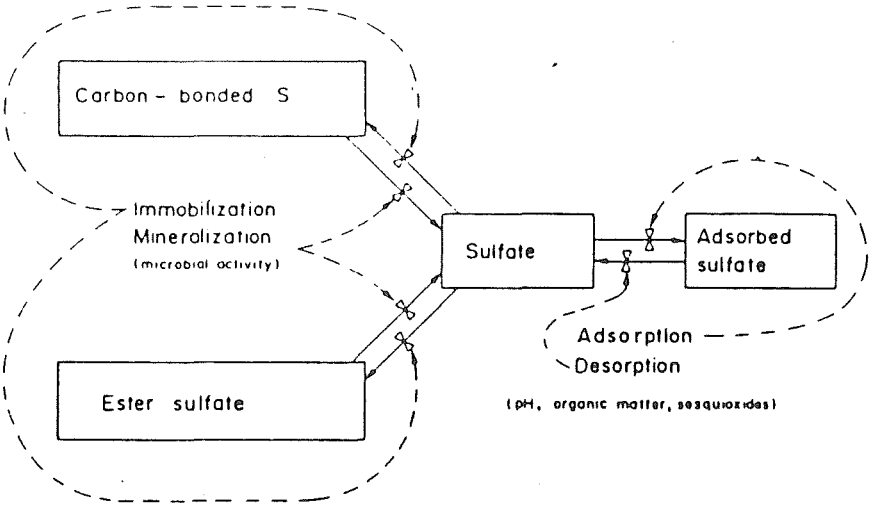


Figure 2.4 Schematic model of sulphur transformations in surface soils. (David *et al.*, 1983)

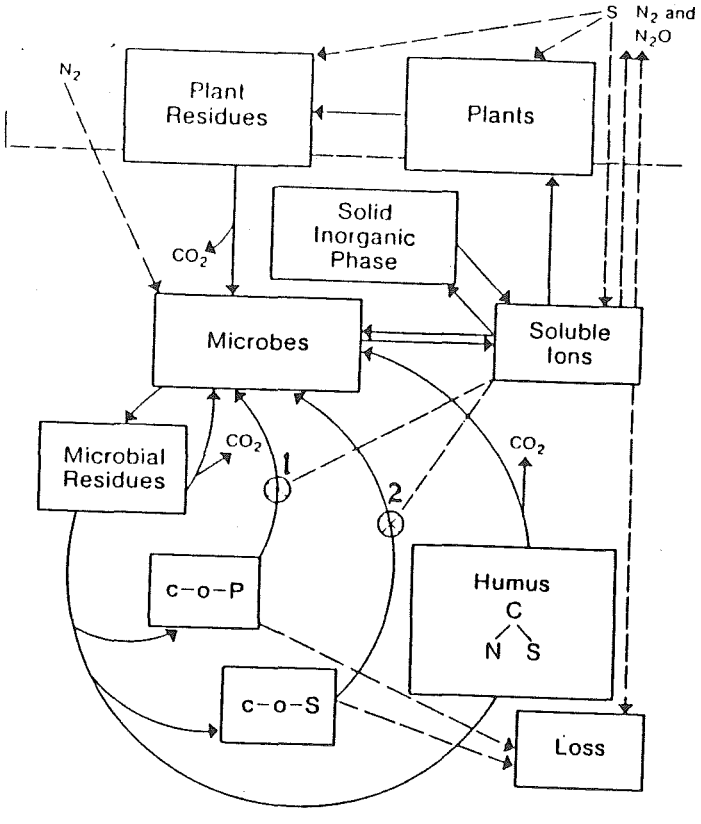


Figure 2.5 Schematic illustration of interrelations of C, N, S and P cycling within the soil-plant system. Mineralisation of ester sulphate and phosphate is controlled by the concentration of sulphate and phosphate in soil solution shown as 1 and 2.

(McGill and Cole, 1981)

the possible sulphate flux from one sulphur pool to another. Most of the closed system mineralisation studies will tend to agree with this flow model. The model lacks definition and does not explain the pattern of sulphur mineralisation in relation to other nutrients. A conceptual model to explain the relative mineralisation of sulphur and N was presented by McGill and Cole, 1981 (Fig. 2.5). The model is based on the stoichiometric relationship between C, N, S and organic P (P_O) in soil organic components. It assumes that the processes involved for the mineralisation of these elements are common. On the basis of these assumptions the model explains the dichotomous system operating in the mineralisation of C, N, S and P_O. Elements which are directly bonded to carbon in humus material (N and C-bonded S) would be mineralised as a result of C oxidation to CO_2. This process is purely microbial and thus it has been called **biological** mineralisation. In this case, N and S will be mineralised regardless of the sulphur status of the soil and the production of nitrate and sulphate will solely depend on microbial demand for carbon. However, those elements which exist as esters may be mineralised by extracellular or periplasmic hydrolytic enzymes. The mechanism of this process is controlled by the end products. When sulphate has been produced in sufficient quantities, the enzyme hydrolysis of non carbon-bonded sulphur will stop. Because of enzymatic involvement, this type of mineralisation has been referred as **biochemical** mineralisation. It follows that soil organisms, including plant roots, may preferentially hydrolyse the HI-S if they have a need for sulphur. Although, this model has helped in explaining the results from the past studies. However, the two processes of mineralisation suggested in the model, biological and biochemical have not been examined in detail. Thus their occurence in soil system needs further investigation.

2.8 SULPHATASE ENZYMES (IN SOIL MICROORGANISMS)

Sulphatase enzymes are a group of enzymes which are largely responsible for the hydrolysis of ester sulphates. These enzymes are found in a number of soil fungi and bacteria and plant roots. Sulphatase enzymes are found within the cellular bodies and are also located extracellularly. Sulphatase enzymes have become an important aspect of enzyme studies, not only for their contribution in soil sulphur cycling, but also for commercial and industrial purposes . These enzymes are active over a wide range of pH and a variety of organic compounds. Research on sulphatase enzymes in soils has been

concentrated mainlt on only one group of enzymes; namely arylsulphatases (Tabatabai and Bremner, 1970a; Cooper, 1972; Speir, 1977b). However it is well known that soil microbes can exude several kind of sulphatase enzymes. Payne and Feisal (1963) isolated Pseudomonas bacteria from soils which were rich in alkylsulphatase enzymes. These enzymes play an important role in the biodegradation of detergents which usually contain long chains of primary or secondary alkyl sulphate esters. The detergents are added in such a great quantity to our soil and water that if enzymatic degradation had not occurred then the contamination would have created a major hazard to our environment. The discovery of choline sulphatase enzymes in Aspergillus species and Pseudomonas aeruginosa was confirmed by Lee (1977) and Lucas *et al.*, (1972) respectively. This enzyme is responsible for the hydrolysis of choline sulphate, in which sulphate is liberated from the choline ester. Payzae and Korn (1956) discovered that the Flavobacterium heparinum bacteria isolated from soil could degrade heparine and heparan polymers. The enzymes involved were named heprinase and heparitin sulphatase lyase. The degradation of these polymers is marked by the liberation of sulphate ions from the polymeric chain. Another enzyme arylsulphatase, which has been found in Aspergillus species, E. Coli, Aerobactor aerogenes, and Pseudomonas species (Dodgson *et al.*, 1982), catalyses the hydrolysis of ester sulphates. Despite the evidence of a wide range of sulphatase enzymes found in soil microbes, only arylsulphatase activity has been determined with any confidence and consistency (Tabatabai and Bremner, 1970a; Speir and Ross, 1978). The details of this particular enzyme will be discussed in section 2.8.1 of this review.

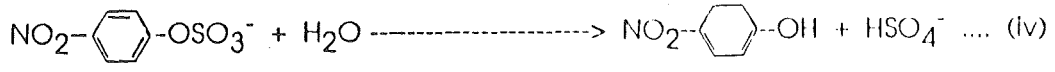
Besides the above mentioned sulphatase enzymes there are a number of other enzymes found in living organisms performing specific functions. Activity of these above mentioned enzymes (apart from arylsulphatase) have been determined mainly in controlled environments. The discovery of these enzymes in soils has been restricted either by the lack of sensitive methods for determining small fractions of enzymes or the problems associated with denaturing these enzymes when they are purified. The latter problem may be a major cause of concern in soil enzyme research.

2.8.1 Soil arylsulphatase activity and sulphur mineralisation

It is obvious from the above evidence (2.6.1) that soil sulphatase enzymes may contribute substantially to the release of sulphur by the degradation of soil organic sulphur compounds. Since there are considerable amounts of soil organic sulphur found in ester sulphate forms (see section 2.4.1) , the possibility of sulphatase enzymes utilizing such compounds was first realized by Tabatabai and Bremner (1970a), who reported finding arylsulphatase activity in some soils from Iowa, U.S.A. Since then several workers have reported the occurrence of this enzyme in soils throughout the world (Cooper, 1972; Kowalenko and Lowe, 1975a; Thornton and McLaren 1975; Lee and Speir, 1979; Stott and Hagedorn, 1980; Haynes, 1987). Arylsulphatase is believed to activate the hydrolysis of arylsulphate by cleaving the O-S bond (Spencer, 1958). The reaction can be represented as;

$$R.OSO_3^- + H_2O \xrightarrow{\text{Arylsulphatase}} R.OH + H^+ + SO_4^{2-} \quad \text{.....(iii)}$$

where R is an aryl compound. The determination of arylsulphatase activity is based on the above reaction using potassium p-nitrophenol sulphate as a substrate (Tabatabai and Bremner, 1970a). Soil is incubated at 37 °C with this ester sulphate from which arylsulphatase activates the release of p-nitrophenol which is yellow. The sample is then extracted with NaOH solution and the p-nitrophenol measured colorimetrically. The action of the enzyme is expressed in equation (iv) where the OSO_3^- ions are hydrolysed to HSO_4^-. The choice of substrate used by these workers is the same as Robinson *et al.*, (1952) and Whitehead et al. (1952), who determined the activity of arylsulphatase in microbes. The enzyme is most active in the pH range of 4.5 to 6.2 . Arylsulphate hydrolysis can occur either intracellularly or in the periplasmic space, depending on the organisms involved and the nature of the substrate (Dodgson and Rose, 1975).



p-Nitrophenol sulphate (Colourless)

p-Nitrophenol (Yellow colour)

Discovery of arylsulphatase in soils was thought to provide information about the level of sulphur mineralisation from ester sulphates. Cooper (1972) and Lee and Speir (1979) found that arylsulphatase activity was significantly correlated with the HI-S forms of soil sulphur. Cooper (1972) indicated a possible implication of this enzyme in an initial rapid release of sulphate upon wetting the soils. An experiment by Speir (1977b) seemed to validate this assumption where he reported a significant relationship between sulphur uptake by ryegrass plants and arylsulphatase activity. In another experiment, Lee and Speir (1979) again found a strong relationship between sulphur uptake and total organic-S, HI-S and sulphatase activity. This relationship between sulphur uptake and the tested sulphur components was due to the highly inter-related nature of sulphur components. Thus if one sulphur component gives a significant correlation then the others will naturally also correlate well. In contrast to these reports, other workers (Tabatabai and Bremner, 1972b; Kowalenko and Lowe, 1975a) conducting incubation experiments in the absence of plants failed to find any relationship between arylsulphatase activity and levels of S mineralisation. In a plant-free incubation study, Kowalenko and Lowe (1975a) found a significant drop in the levels of arylsulphatase as the incubation proceeded. The absence of plants could have contributed to this phenomenon. Plants would have continuously removed the mineralised sulphate and also phosphate, the presence of which has detrimental effects on the sulphatase enzyme activity (Al-Khafaji and Tabatabai, 1979). The activity of arylsulphatase has been highly correlated with soil organic matter by several workers (Cooper, 1972; Speir, 1977b; Sarathchandra and Perrott, 1981). Also the activity of arylsulphatase varies depending on the source of organic matter (Perucci and Scarponi, 1984). Tabatabai and Bremner (1970b) found a marked difference in arylsulphatase activity with soil depth. The decrease was

associated with the organic matter which also decreased with the soil depth. Speir (1977b) conducted a survey of arylsulphatase activity in different New Zealand soils and reported a significantly higher activity in wetter podzolic soils than in soils from the drier end of the climosequence. These two studies show that arylsulphatase activity depends on the availability of substrate. Drier soils, where substrate is less likely to move down the profile showed low enzymatic activity in lower horizons compared to the soils from a wetter climate where movement of organic material into lower horizons is likely to introduce higher amounts of substrate, hence higher level of enzymatic activity were found.

Studies conducted on the storage of soil samples before the determination of enzymatic activity, revealed that moist soil samples, when stored at above 5 °C temperature lead to a decrease in arylsulphatase activity (Tabatabai and Bremner, 1970b). These workers suggested that a suitable temperature to store such samples would be -10 °C which would maintain the arylsulphatase activity. However in another study, Speir and Ross (1975) found that soil samples stored at -20 °C temperature lost a considerable amount of arylsulphatase activity and they recommended that air-dry storage at 4 °C is a better way to preserve the arylsulphatase activity. Tabatabai and Bremner (1970b) showed that air-drying of moist samples increased the arylsulphatse activity. This increase could have been caused by the breakdown of soil aggregates which provided greater accessibility of substrates for arylsulphatase activity. Al-Khafaji and Tabatabai (1979) showed that the application of trace elements (25 μmole/g soil) to soils, inhibited the activity of arylsulphatase enzyme. The elements Ag, Hg, B, V and Mo were most effective, causing more than 50% loss in the activity. Inhibition in the activity of this enzyme was also caused by MoO_4^{2-}, WO_4^{2-}, AsO_4^{3-}, and PO_4^{3-}. However, anions such as NO_2^-, NO_3^-, Cl^- and SO_4^{2-} were non-inhibitory and showed no effect on the activity of arylsulphatase. In studies of pure cultures of many bacteria and fungi formation of arylsulphatase is suppressed by addition of sulphate (Rammler *et al.*, 1964; Fitzgerald, 1976).

In conclusion, knowledge about the function of arylsulphatase in soil is rather limited. Its ability to hydrolyse p-nitrophenol sulphate is no proof of its effectiveness on natural soil substrates. It is most active in the pH range between 4.5 and 6.2. Thus soils outside this

pH range may have high sulphur mineralisation but show low levels of arylsulphatase activity. The values determined for this enzyme are dependent on many factors such as soil treatment, storage temperature, moisture level, presence of trace elements etc. In the light of present knowledge about soil enzymes, it seems unwise to simply correlate only the activity of arylsulpatase enzyme to sulphur mineralisation in soils while there are possibly many other enzymes involved in the process of organic sulphur mineralisation. This could also be one of the reasons for contrasting results being reported from different studies.

2.9 SOIL MICROBIAL BIOMASS

The existence of abundant microbial life in soils is well known. Their adaptability and diversity to degrade organic matter has been well documented by Schnitzer and Khan (1978). The microbial degradation of those organic compounds where sulphur is a constituent is determined basically by two factors; firstly the need to use structurally bonded carbon for energy purposes and secondly for the requirement of sulphur either for body tissues or to fulfill metabolic and physiological needs. The degradation of organic sulphur compounds is performed with the help of selective and non-selective enzymes secreted by the microorganisms. It would be very difficult if not impossible, to account for the activity of each enzyme and establish a relationship with the level of sulphur mineralisation. That is why a simplistic approach like measuring only arylsulphatase activity which might be considered an indirect measurement of microbial activity has not given consistent results (2.6.2). Thus a direct biochemical approach, initiated by Jenkinson (1976), to determine the microbial status of soil as a measure of microbiological activity at any given time has gained much wider acceptance (Spier, 1979; Sarathchandra *et al.*, 1984). There has been general agreement among workers that the amount of microbial biomass is an indicator of the dynamics of the biochemical changes in soils, and a repository of plant nutrients which are more **labile** than the bulk of the soil organic matter (Jenkinson and Ladd 1981; Ladd *et al.*, 1981; Van Veen *et al.*, 1984; Powlson *et al.*, 1987). The latter part of this assumption has invoked interest in estimating the **labile** pool of soil nutrients including sulphur, held in the microbial biomass.

2.9.1 Determinations of soil microbial biomass-S by biochemical methods

Initially biochemical determinations were carried out for carbon and nitrogen (Anderson and Domsch, 1978; Eiland, 1983; Sparling *et al.*, 1986) but later on were adapted for phosphorus and sulphur (Hedley and Stewart, 1982; Saggar *et al.*, 1981a).

A biochemical method for determining biomass sulphur (B_S) was first reported by Saggar *et al.*, (1981a), who used a biocidal treatment for determining the B_S in soils. They applied the same biocidal agent as Jenkinson and Powlson (1976) used in their fumigation incubation method (FI) to kill soil microbes. Unlike the Jenkinson and Powlson method however, $CHCl_3$ was added in liquid form to soil samples @ 1 ml g^{-1} soil rather than $CHCl_3$ applied into vapour form. Also the length of the treatment was restricted to only two hours compared with 18-24 hours fumigation. Microbially released sulphur was extracted either by 10 mM $CaCl_2$ or 0.1 M $NaHCO_3$ solution (1:5 soil:solution ratio). The combination of biocidal treatment and the two extractants managed to recover between 40 to 44% bacterial sulphur and 33 to 40% fungal sulphur. The $NaHCO_3$ solution was the more efficient extractant, and always extracted more sulphur than $CaCl_2$ from both bacteria and fungi. Taking the percentage recovery of the added microbial S and assuming the microbial population distribution as suggested by Anderson and Domesch (1978), a biomass S recovery constant (K_S) of 0.35 for $CaCl_2$ and 0.41 for $NaHCO_3$ were calculated. These K_S values were then used to calculate the actual biomass sulphur in soils (equation v).

$$\text{Biomass S} = \frac{\text{total extracted S after fumigation} - \text{total extracted S without fumigation}}{K_S} \quad \text{.....(v)}$$

Saggar *et al.*, (1981a) reported a value of 2.3% of the total organic sulphur held in microbial cells. This is in agreement with the reported values by Maynard *et al.*, (1983) and Strick and Nakas (1984). Both of the extractants used by Saggar *et al.*, (1981a) were

Table 2.5 Microbial biomass sulphur (B_S) and active sulphur pool in soils.

References	Estimation* method	Biomass-S[1]	Nature of experiment	Country
Kowalenko (1978)	CB	1.3	Field experiment	Canada
McLaren et al. (1985)	CA	3-6	Incubation	Scotland
Saggar et al., (1981a)	DB	2.3	Incubation	Canada
Maynard et al., (1983)	DB	1-3	Incubation	Canada
Strick and Nakas (1984)	DB	2.2	Incubation	USA
Haynes (1987)	DB	<1.0	Field experiment	New Zealand

* CA calculated active pool, CB calculated biomass, DB determined biomass.

1. calculated as percentage of organic S in surface soils.

equally effective in recovering sulphur from pure microbial cultures and also when cultures were mixed with soils. However, Strick and Nakas (1984) are critical of using either of the two extractants in soils which have high sulphate adsorption and suggested that use of a phosphatic extractant (NaH_2PO_4) would be more appropriate in extracting microbial sulphur. Another criticism of the Saggar *et al.*, (1981a) method is the way $CHCl_3$ has been applied to the soils, enabling it to dissolve some of the native organic sulphur and also unoxidised elemental sulphur, thus, this method may overestimate the biomass sulphur. More work is needed to improve the biochemical determination of microbial biomass sulphur.

2.9.2 Microbial sulphur in soils

Sulphur held in the microbial biomass has been tagged as an extremely labile form of sulphur which holds the key to sulphur turnover in soils (Biederbeck, 1978). An attempt to assess microbial sulphur in soils has been made by Kowalenko (1978) who used the values for fungal and bacterial C and N estimated by Clark and Paul (1970). Assuming the ratio between biomass N:S is 10:1, Kowalenko (1978) calculated that about 1.3% of the total organic sulphur was held in bacterial and fungi biomass residing in grassland soils. His calculation ignores the microbial sulphur which is being held in other microbes such as actinomycetes, protozoa and microfauna which could contribute a significant proportion of organic sulphur. Thus this assessment underestimates the amount of microbial biomass sulphur (B_S). In a closed incubation study, McLaren *et al.*, (1985) found between 3 and 6% of the organic sulphur actively involved in the sulphur cycling and presumed that this fraction of sulphur was largely composed of microbial biomass sulphur (Table 2.5). In a field study, Haynes (1987) has reported less than 1% of the total sulphur as biomass-S. The variation in these reports is understandable because B_S values will depend on microbial population distributions and the nature of the microbial colonies growing in the soil system. Although some of these estimations (Kowalenko, 1978; McLaren *et al.*, 1985) are based on calculation rather than any direct determination, they still provide useful information about the size of B_S pool.

2.10 DETERMINATION OF SULPHUR IN PLANTS AND SOILS

The quest for a rapid and reliable analytical method to determine low levels of sulphur in soils and plants is still a priority in sulphur research. A range of methods and techniques have been developed over the last 50 to 60 years. Definite progress has been made since 1940. However, Johnson and Nishita's (1952) method to estimate micro fractions of sulphur in soil and plants is still commonly employed. The versatility and accuracy of this method is such that it is often used as a standard with which to compare the newer methods.

Methods for determining sulphur can be grouped into seven types: gravimetry, turbidimetry, titrimetry, colorimetry, X-ray flourescence, inductively coupled plasma (ICP) and ion chromatographic methods. The ICP and ion chromatographic method are relatively new, developed in the late seventies and early eighties. The advantages and disadvantages of these methods will be discussed in the following sections.

2.10.1 Gravimetric method

The gravimetric method is one of the oldest methods for determining sulphate S. The method is based on the low solubility of $BaSO_4$. Usually $BaCl_2$ is used to precipitate sulphur as $BaSO_4$. The precipitated material is then filtered and weighed and sulphur is determined by weight of the precipitate. It is a crude but effective method for measuring sulphur at higher concentrations. However the problems associated with this method are many, which limits its use. Obtaining the precipitate free from contaminants (co-precipitate) is extremely difficult. The presence of Ca especially causes serious problems because it strongly co-precipitates with Ba. Errors involved with poor filtration , weighing the precipitate and washing the precipitate give poor reproduciblity. The presence of Al and Fe ions also cause problems with precipitation and their removal with NH_4^+ tends to remove a proportion of the $BaSO_4$ (Olsen, 1917).

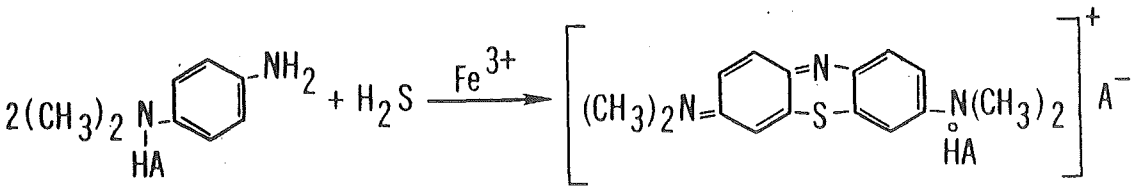
This method is also sensitive to organic sulphur. Extraction of soil sulphate is usually carried out by wide range of extractents (eg., LiCl, $Ca(H_2PO_4)_2$, CaCl), and depending on the nature of the extractant, there could be some organic sulphur present in the

extracts (Hesse, 1957). There are methods available to remove organic materials (Freney 1958; Little 1958; Azeem 1967) but they tend to be complicated and rather unsatisfactory. In conclusion, this method is tedious, time consuming and reproducibility is often very poor. Use of this method is not advisable in samples containing concentration below 10 μg S ml^{-1} (Beaton *et al.*, 1968). For the above reasons use of gravimetric methods became less common in the sixties, and today it is seldom used. In fact, as early as the 1950's emphasis was directed towards searching for alternative methods to improve the sensitivity and accuracy of sulphur determinations.

2.10.2 Sulphate reduction method

This method is also known as Johnson and Nishita's methylene blue method named after the workers who published the procedure in 1952. The method involves the reduction of SO_4^{2-} to H_2S by a reducing mixture containing hydriodic acid, formic acid and hypophosphorous acid. The reduced H_2S is absorbed in a solution of $Zn(OAc)_2$ and NaOAc, and subsequently treated with p-amino dimethylaniline sulphate and ferric ammonium sulphate which developes a blue colour. The intensity of the colour is measured colorimetrically at 670 nm wavelength. The blue colour is developed when H_2S in the absorbing solution reacts with the colour reagent in the presence of Fe^{3+} ions. The reaction can be represented as;

$$SO_4^{2-} + \text{Reducing mixture} \xrightarrow{\Delta} H_2S$$



The method has made it's impact on sulphur research due to its precision of determination over a wide range of sulphur levels in soils, plants, rain-water, sludges and animal wastes. Its accuracy in determining low levels of sulphur (0.50 μg S g^{-1} soil) is useful for comparing with the sensitivity of other modern methods. Since its publication in 1952, the method has been changed very little. One modification was suggested by

Archer (1956) who preferred NaOH solution for absorbing the H_2S gas rather than the mixture of $Zn(OAc)_2$ and NaOAc . The choice of using NaOH is preferable because unlike the original absorbing solution NaOH does not precipitate when stored and is also cheaper and easier to prepare. Another suggested modification was made by Dean (1966) who replaced the p-aminodimethylaniline sulphate reagent with a mixture containing bismuth nitrate, gelatine and glacial acetic acid. This mixture, when reacted with H_2S forms bismuth sulphide which gives a yellow colour. However, the sensitivity of Dean's method at low levels of sulphur is poor (Tabatabai, 1982).

In an attempt to speed up the analysis, Keay *et al.*, (1972) automated the whole reduction method of determining sulphur. It uses the reduction procedures involved in Johnson and Nishita (1952) method, combined with the Dean (1966) finish. It was a successful attempt in increasing the rate of analysis from 12 to 30 samples per hour. Despite the good results, the use of this automated method has been limited, mainly due to the special apparatus involved in the automation.

The reducing mixture is non-selective, it reduces all forms of sulphates (Tabatabai 1982), sulphide, sulphite, thiosulphate and most of the non carbon-bonded sulphur compounds (Freney, 1958; Bird and Fountain, 1970). Thus it will tend to overestimate the sulphate-S content. In the case of soil extracts this could be avoided with the choice of the correct extractant (Steinbergs, 1958). Johnson and Nishita (1952) showed that the reducing mixture did not reduce any sulphur from carbon-bonded sulphur compounds; for example cystine, cysteine, methionine, taurine and glutathione. This led to the chemical fractionation of soil organic sulphur into an HI-reducible fraction (HI-S) (Freney, 1958) and a non-reducible carbon-bonded fraction (see section 2.4.3 and 2.4.4). In conclusion, the reduction method is accurate and reproduceable at both low and high levels (0.5-300 μg S) of sulphur. It can be used as a check for other methods of sulphur analysis. It suffers from the drawback of being slow and the HI acid used for the reduction of sulphate is expensive and highly corrosive, posing problems in handling.

2.10.3 Turbidimetric methods

This method, like the gravimetric method, also involves the precipitation of sulphate with Ba^{2+} ions, but in this case the measurement of the precipitate is carried out in suspension using a spectrophotometer. When a beam of light passes through the suspended solution, some of the light is absorbed by the suspended particles in the solution. The greater the amount of suspended material, the lower is the transmission of light. Application of this method was seen as an improvement over the gravimetric method. Chesnin and Yien (1950) were the first to use this method in soil sulphur analysis. They extracted sulphate from soils with a mixture of acetic acid and sodium acetate buffer solution. This method soon became popular because of its simplicity and reproducibility. Hesse (1957) criticized the method after failing to find the same accuracy and suggested that the choice of the extractant made by Chesnin and Yien (1950) was not appropriate as it brought significant amounts of organic colloids into the suspension. He also suggested that the organic colloids were affecting the determination by dual action: at lower concentrations (0-10 μg S ml^{-1}) they shielded the sulphate from being precipitated (which gave lower values) and at higher sulphate concentrations colloidal organic matter coprecipitated with barium sulphate, increasing the turbidity of the solution, resulting in an overestimation of sulphur. Hesse (1957) suggested treatment with ferric hydroxide to coprecipitate the colloidal organic matter, which was subsequently discarded before the $BaSO_4$ precipitation. This tended to increase the accuracy of the method. Despite the improvement from the previously described method, Hesse's approach to determine sulphur was time consuming and complicated. To overcome this problem and to make this method more feasible , NaOAc extractant was replaced with a slightly acidic extractant so that soil organic matter did not dissolve in the extract. Williams and Steinbergs (1959) showed that the use of 0.15% $CaCl_2$ solution as extractant was effective in achieving organic matter free extracts which removed the need for further treatments.

Butters and Chenery (1959) conducted a study to evaluate the Chesnin and Yien (1950) method for determining total sulphur in soils and plants. They showed that turbidimetric measurements were influenced by a number of operational and external variables

including crystal size of $BaCl_2$, type and concentration of acid used for digestion, standing time of suspension, cell size, choice of optical filters and presence of certain cations (Ca^{2+}, Fe^{3+}, Mn^{2+}) and anions (Cl^-, SiO_3^{2-}). Dodgson (1961) also reported variation in sensitivity of the turbidimetric analysis which was dependent on the state of suspension, cell size and the amount of acid used. These two cases clearly showed that the results obtained by others using different reagents and operating conditions could not be compared with each other. Thus in the 1960's workers started standardizing the methods and impetus turned toward improving the sensitivity especially in the range below 20 μg S ml^{-1} (Rains, 1960; Dodgson, 1961; Massoumi and Cornfield, 1963; Garrido, 1964). In all these cases, sulphur was analysed manually which demanded continuous personal attention and consequently restricting the number of samples to be analysed.

Automation of the turbidimetric method started a new era in sulphur analysis. The concept of automation is based on continuous flow analysis (Ferrara *et al.*, 1965). Williams and Twine (1967) and Basson and Bohmer (1972) showed that with the exact repetition of reaction conditions provided by an auto analyzer system the $BaSO_4$ turbidimetric method can give reproducible SO_4^{2-} analysis. These methods have been applied to determine the total sulphur in plants which is much higher than extractable sulphate in soils. Measurement of extractable soil sulphur requires high sensitivity at lower concentrations (1-10 μg g^{-1}). When low sulphur containing samples are determined by automated turbidimetric procedures the detection limit is often very poor due to high background noise. That is why analysts (Mottershed, 1971; Sinclair, 1973; Sansum and Robinson, 1974) started introducing a known amount of sulphur into the flow to maintain a constant detectable level of sulphur in the system, so that even low amounts of sulphur can be easily detected with accuracy. Sinclair (1973) developed a method for determining extractable soil sulphate-S which has been adopted by the New Zealand Ministry of Agriculture laboratories for routine plant available-S analysis for fertilizer recommendations. In this method, soils are extracted with 0.4 M $Ca(H_2PO_4)_2$ solution in 1:2.5 soil:extractant ratio for 30 minutes. An acid mixture is added to the filtered extracts and organic colloids are removed with charcoal treatment. Sulphate-S is then determined in these extracts with the auto analyzer. This method can measure up to 40 samples hr^{-1} and it has given satisfactory results when compared with the Johnson and Nishita (1952) reduction/distillation method. The method uses a fast flow rate which

transports the suspended material quickly thus avoiding the problem of $BaSO_4$ adherence to the photocells, which causes drift in the base line, as was noticed by Basson and Bohmer (1972). However use of charcoal as suggested by Sinclair (1973) to remove organic colloids from the extracts some times has been less effective which tend to seriously underestimate S content in extracts. This problem can be avoided by using a dialyser in the automated system (Ogner and Haugen, 1977), which eliminates soluble organic matter. Again use of this device may be restricted, as it often removes a small proportion of soluble sulphur. Walls *et al.*, (1980) introduced an efficient and accurate automated method of determining total sulphur in plant materials. Plant tissues are acid digested (HNO_3 and $HClO_4$) at 150 °C to convert the total sulphur into SO_4-S . This method is also sensitive enough to measure extractable soil sulphates in the 0-15 μg S g^{-1} range. In conclusion, automated turbidimetric methods are relatively quick and also sensitive enough to be used for soil and plant tests. The determination is somewhat dependant on skill of the operator, operating conditions and the pretreatment to the samples prior to the $BaSO_4$ suspension.

2.10.4 X-ray fluorescence spectroscopy (XRF)

Use of X-ray technology is becoming a common feature in plant and soil analysis. The simple sample preparation, especially for plant materials, combined with less interference from other elements provides a very good method for multiple elemental analysis (Evans 1970; Norrish and Hutton, 1976). When samples are bombarded with high energy X-rays, the subshell electrons of atoms in the sample are excited and momentarily shift from their orbital positions. When they return to their original atomic configuration, they emit low energy X-ray radiation (fluorescence). The emitted X-ray fluorescence is characteristic for each element and its intensity is proportional to the amount of element present. XRF is commonly used to analyse the total sulphur in plants (Kubota and Lazar, 1971; McLaren and Swift, 1977). The sample preparation involves grinding the dried material to a particular size and pressing it at certain pressure to form pellets, which are then inserted into the X-ray spectrometer for analysis. This simple approach avoids the pretreatment of plant materials to convert organic S to sulphate using methods such as wet digestion with potentially dangerous chemicals, which often

gives unsatisfactory digestion (Randall and Spencer, 1980), or the high temperature technique employed by Steinbergs *et al.*, (1962).

Roberts and Koehler (1968) showed that extractable soil sulphate can be determined by a specialized XRF technique. The soil extracts are dried in a gelatinous solution to form a firm matrix to determine sulphur. They reported a strong agreement between the sulphur determined by using XRF and the Johnson and Nishita (1952) reduction method. The method is accurate and sensitive enough to measure levels of soil sulphate as low as 0.5-14 μg S g^{-1} soil. However adaptation to this method is perhaps not convenient due to the complicated sample preparation. Although this method is as good as the reduction method, it can analyse only 40 samples day^{-1}. Brown and Kanaris-Sotirio (1969) and Darmody *et al.*, (1977) analysed total sulphur in soils by matrix correction, standard addition and correction techniques respectively. They showed satisfactory agreement with chemical methods. Use of wax for pelletising has also been reasonably successful (Bergseth and Kristiansen, 1978). In general, determination of total sulphur in soils is complicated by the presence of large amounts of aluminium, silicon and organic matter (Darmody *et al.*, 1977). Soil particle size, shape and distribution vary from soil to soil hence the matrix effects are of great importance and somewhat restrict the application of the X-ray fluorescence method to total sulphur determination in soils.

2.10.5 Inductively coupled plasma-atomic emission spectrometry (ICP-AES)

The use of ICP has increased the range of elements which can be analysed by atomic emission spectroscopy in the UV range (Kirkbright *et al.*, 1972). It uses very high temperatures (6000-10000 °K) to atomise elements dissolved in solutions which can then be measured at a particular wavelength on a AES attachment. It can measure sulphur in soil extracts, rain water, total sulphur in digested soil and plant materials. Because ICP-AES uses very high temperatures which can atomise organic and inorganic compounds, incomplete digestion of total sulphur in plant material does not effect quantitative analysis by this method (Novozamsky *et al.*, 1986). These authors also found that there were no interference from added elements such as; Al, As, Ba, Cd, Cr,Cu, Fe, K, Mg, Mn, Na, Ni, P, Sr, Ti, B and Mo. The sensitivity of ICP-AES methods is limited to 2 ppm S concentration. Beside the determinations of sulphur, other elements can also be

determined in the same extract and this is a major advantage of using the ICP-AES method.

2.10.6 Ion exchange chromatographic (IC) method

Ion chromatography is the latest addition to techniques which can determine a range of ions in a single extract. Ions are separated by their differential migration on ion exchange columns, which are filled with different types of exchange resins: anion exchange, cation exchange and chelating resins (Gjerde and Fritz, 1987).

Anions e.g. (F^-, Cl^-, NO_3^-, SO_4^{2-} and PO_4^{3-}) can be separated on anion-exchange resins by elution with basic eluents such as $NaHCO_3/Na_2CO_3$ solution. The eluate flows through the suppressor unit which allows the conductometric determination of separated ion species (Small *et al.*, 1975). The suppressor unit contains a strongly acidic, cation exchange membrane in the hydronium form. The eluent $NaHCO_3/Na_2CO_3$ is neutralized and its conductivity is suppressed. At the same time, sample anions with low pk_a values (>7) are converted into ionised and highly conductive acidic forms. These reactions allow the sensitive conductrometric detection of analyte anions. Dick and Tabatabai (1979) analysed SO_4^{2-} and NO_3^- simultaneously in the same extractant and found that the $SO_4$2- values were similar to the reduction method values. High concentration of the extractant did not interfere with the SO_4^{2-} determination. The analytical value closely agreed with other standard methods. In another report, Tabatabai and Dick (1983) reported that the IC method was efficient in analysing several anions at the same time and the values were closely related to the respective chemical methods. Being very sensitive at lower concentration of ions, the IC method allows use wide range of extractants for determination anions. Kalbasi and Tabatabai (1986) were able to measure NO_3^-, Cl^-, SO_4^{2-} and PO_4^{3-} in water extracts of plant materials. The ion exchange chromatographic method is particularly attractive in the light of the special and varied techniques that would be required to measure these anions individually. It is particularly convenient for the combined analysis of sulphate and nitrate, which is often required to characterize the mineralisation of these two nutrients in soils.

The suppressed ion chromatography system described above, having two columns, adds complexity to the instrumentation and restricts the type of eluent that can be used, and to some extent the separating ability of the method. In 1979 Gjerde and co-workers (Gjerde *et al.* 1979) developed a single-column ion chromatography (SCIC) method to separate a number of anions. Using single- column chromatography anions are separated on an anion exchange column and subsequently measured by a conductivity detector connected directly to the separator column. This is made possible by an anion exchange resin of very low capacity (0.007 to 0.04 meq/g) and by choosing an eluent that has low conductance. Eluent should have lower conductance than the chloride, nitrate, sulphate and other common anions which are going to be analysed. Maynard *et al.*, (1987) have used single column chromatography technique on soil extracts where sulphate-S was extracted by $Ca(H_2PO_4)_2$. Using an anion exchange column and borate gluconate buffer as an eluent, these workers were able to measure very low levels of SO_4-S (0.25 mg l^{-1}) in solution. Extracting solutions having high concentrations of phosphate may interfere with sulphate detection. Nieto and Frakenberger, (1985) suggested that use of phthalic acid (4 mM, pH 4.5) as an eluent may eliminate phosphate interference. There is very limited information available on the use of ion chromatography for soil analysis, however, it appears to be a sensitive, accurate and rapid method for multi-ion analysis.

There are a number of other methods for determining sulphur such as the Ba-133 method (Kao *et al.*, 1971), residual lead method (Little *et al.*, 1969), uncombined Ba^{2+} ion method (McSwain and Walrous, 1974) which have not been included in the review because they have been rarely used since they were originally published.

2.11 DETERMINATIONS OF PLANT AVAILABLE SULPHUR

Extractable sulphate in surface soils has been generally regarded as sulphur available for plant growth. There have been many studies conducted to relate extractable sulphate with plant yield/S uptake. The most commonly used extractants are $Ca(H_2PO_4)_2$ and KH_2PO_4 which contain different amounts of P ml^{-1} solution. These extractant can extract soluble plus adsorbed sulphate. The levels of SO_4^{2-} measured by using These extractants have given good correlation with plant yield, both in pot trials (Lee and Speir, 1979;

Scott, 1981) and field studies (Hoeft *et al.*, 1973; Westmerman, 1974). Some workers have suggested the use of cold water or low concentrations of Cl salts such as LiCl and $CaCl_2$ to extract available sulphur (Walker and Doornenbal, 1972) but these extractants are ineffective in extracting adsorbed sulphate (Fox *et al.*, 1964). The use of extractable sulphate values determined as described above for estimating plant available sulphur has been criticized by many workers (eg. Spencer and Glendinning, 1980; Tabatabai, 1982; Jones *et al.*, 1983).

In New Zealand, the Ministry of Agriculture, uses 0.01 M $Ca(H_2PO_4)_2$ solution to extract sulphate-S. The soil:extractant ratio is maintained at 1:5 and samples are shaken on an end-over-end shaker for 30 minutes. The amount of sulphate-S determined by this procedure is regarded as plant available sulphur. Depending on the level of sulphate-S measure by the soil test and by using the balance equation as suggested by Sinclare and Saunders (1982), a sulphur fertilizer recommendation is made (see section 2.12). The equation takes into account factors such as how long the soil has been under pasture, the stocking rate and an approximation of sulphate leaching losses. Using the soil test as an indication of the soils ability to supply and support plant growth has been debated by many workers. The sulphate-S measured at any given period of time would be affected by the sampling errors and time of sampling (Tabatabai, 1982), therefore it is likely to introduce errors in fertilizer recommendations. These soil tests do not measure the amount of sulphate-S which may be released by mineralisation of organic sulphur during the ensuing season.

2.12 FERTILIZER MODEL

In the past, fertilizer recommendations have been determined by conducting field trials and glasshouse experiments in which herbage production has been related to fertilizer application rates. In the present economic climate, the time and cost involved in carrying out these experiment is a non-viable option. An alternative approach to minimize the expenses is to model the sulphur cycling in soil systems whereby total inputs and outputs can be calculated and the deficit can be supplemented by adding sulphur fertilizers. Sinclair and Saunders (1984) adopted this approach and suggested a sulphur

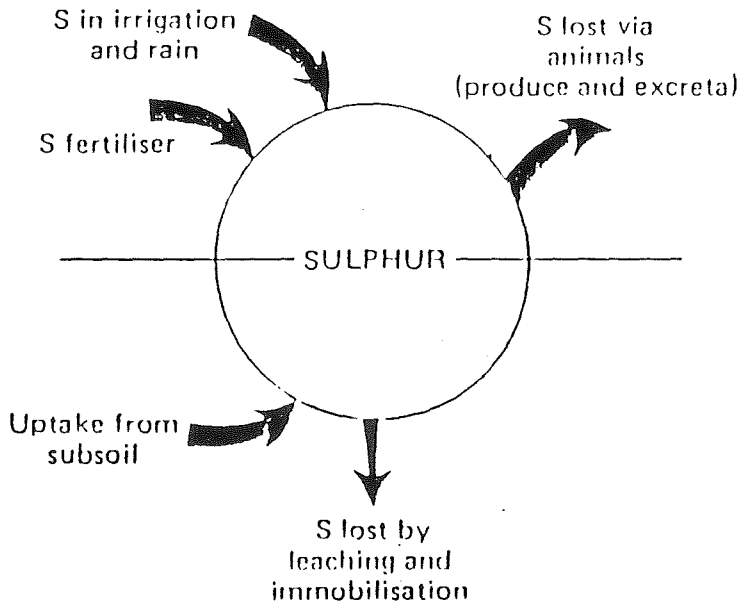


Figure 2.6 Simplified model of sulphur cycling in soil.
(Sinclair and Saunders, 1984)

cycling model in soil. These workers proposed the model by pooling the available information from field experiments and by making some gross assumptions.

Currently, the New Zealand Ministry of Agriculture and Fisheries, (M.A.F) uses the sulphur cycling model suggested by Sinclair and Saunders (1984) for it's Computerised Fertilizer Advisory Service (CFAS). This model is essentially an external model of sulphur cycling (Fig. 2.6), it takes little account of various transformational processes occurring within the soil other than the level of sulphate present in soils, which is usually measured yearly in the spring. On the basis of pasture development and intensity of utilisation, an amount of sulphur is added to maintain the sufficiency of sulphur in the soil system. Due to a lack of information about the internal cycling of sulphur in soils particularly the mineralisation and immobilisation of sulphur in soils, there are some inconsistent assumptions being made in this model. For example, the process of immobilisation has been defined as a function of allophane content which has high adsorption capacity. In fact, it is a **chemical fixation** of sulphate ions caused by high electropotentials in allophanic soils. There is no consideration given to the input of sulphur for plant growth from mineralisation. Most New Zealand agricultural soils contain more than 95% of the total sulphur in organic forms therefore even a small fraction of this sulphur can contribute substantial amounts of sulphur for plant uptake. It is assumed that on non-allophanic soils, if under pasture for more than 20 years, the rates of mineralisation and immobilisation will have reached an equilibrium. However, studies by Sorn-Srivichai (1980) show that these assumptions are not necessarily valid. Pasture sites on the Tokomaru silt loam soils which had similar pasture development indices showed large difference in the organic sulphur fractions which suggests that the rate of sulphur transformations in this soil type varied from field to field. Hence, the notion of equilibrium state of sulphur mineralisation and immobilisation need further investigation. Currently, there is no suitable method available to measure mineralisable sulphur in soils hence release of sulphur from organic sulphur can not be quantified. The study reported here has attempted to develop methods for determining the amount of mineralisable sulphur in soils. The values could then be integrated in the present M.A.F model in an attempt to improve the sulphur recommendation scheme. It has been recognised that sulphur minerlisation and immobilisation is also controlled by the availability of other nutrients (McGill and Cole, 1981) and other soil properties. The microbial interactions with these

factors are largely unknown. Most of the work in this thesis has examined such interactions which are thought to be essential for understanding the processes of mineralisation and immobilisation of sulphur in soils.

CHAPTER THREE

MATERIALS AND METHODS

3.1 SOILS

Soils were collected from a variety of sites in order to obtain samples with a wide range of soil properties that might be expected to affect the processes of sulphur mineralisation and immobilisation. A total of 18 soils were used in these studies, and were sampled mainly from pasture paddocks. Two samples were collected from the Port Hills area, representing the Rapaki and Summit soil series. These samples were from non-cultivated areas which were covered with scrub vegetation. One of the samples from the Templeton series (Templeton C) was collected from a farm which has been cropped for more than 10 years. The samples collected from the Horotiu and Te Kowhai soil series were from the Waikato region (North Island) and the rest of the samples were from Central and South Canterbury and Otago regions (South Island) of New Zealand. Chemical properties of the soils, listed in Table 3.2, show a wide range of organic carbon and total nitrogen. Soil pH also varies considerably. These properties are discussed in detail in section 3.3. Soil samples were collected from both S responsive sites such as the Meyer soil (Nuttal, 1988) and from non-responsive sites such as the Horotiu soil (Saunders, personal communication). The amounts of different forms of sulphur present in the soils are listed in Table 3.4, and details are discussed in section 3.6 . Samples were collected from the surface soil (0-10 cm depth) and were air-dried, screened through a 2 mm sieve and stored at 20 °C until required. Some basic information about the samples is listed in Table 3.1 .

3.2 GENERAL SOIL ANALYTICAL METHODS

Soils were analysed for organic carbon and total nitrogen and total sulphur contents. The ratio of nitrogen and carbon to sulphur in soils has been considered to influence the level of sulphur mineralisation and immobilisation (Walker, 1959; Stevenson, 1982).

Table 3.1 General information on experimental soils.

Soil series	Soil Classification (New Zealand)	Soil Texture	Agricultural land use	Parent material
Teviot (limed)	Podzolised yellow-brown earth	Sandy clay loam	Pasture	Schist and schist loess
Teviot	Podzolised yellow-brown earth	Sandy clay loam	Pasture	Schist and schist loess
Meyer	Yellow-brown soil associated with dry-subhygrous yellow-grey earth	Sandy clay loam	Pasture	Greywacke loess and alluvium
Wairaki	Lowland yellow-brown earth	Sandy clay loam	Pasture	Tuffacious greywacke alluvium
Te Houka	Dry-hygrous yellow-grey earth	Silt loam	Pasture	Schist loess overlying greywacke

To be continued ☞

Table 3.1/continued

Soil series	Soil Classification (New Zealand)	Soil Texture	Agricultural land use	Parent material
Temuka	Gley	Clay loam	Pasture	Greywacke alluvium
Lismore	Yellow-brown shallow and stoney soil associated with yellow-grey earth	Silt loam	Pasture	Greywacke loess on gravels
Rapaki Hill soil	Brown granular loam	Clay loam	Scrub	Basaltic tuff and ash
Summit	Upland yellow-brown earth	Silt loam	Scrub	Greywacke loess overlying basalt
Templeton (P)	Recent/yellow-grey earth	Silt loam	Pasture	Greywacke alluvium
Templeton (C)	Recent/yellow-grey earth	Silt loam	Cereals	Greywacke alluvium
Selwyn	Recent soil	Sandy loam	Pasture	Greywacke alluvium

To be continued ☞

Table 3.1/continued

Soil series	Soil Classification (New Zealand)	Soil Texture	Agricultural land use	Parent material
Mokotua	Lowland yellow-brown earth	Silt loam	Pasture	Gleyed alluvium
Takahe	Yellow-grey earth	Silt loam	Pasture	Loess overlying basalt
Wakanui	Recent yellow-grey earth/ gley	Silt loam	Pasture	Greywacke alluvium
Waimakariri	Recent soil	Sandy loam	Pasture	Greywacke loess and fine alluvium
Te Kowhai	Gley soil	Silt loam	Pasture	Rhyolitic alluvium
Horotiu	Yellow-brown loam	Silt loam	Pasture	Rhyolitic alluvium

Amounts of sulphur present in different forms were also determined using the methods described in section 3.3. Soil pH, which plays an important role in determining the microbial population growing in a particular soil, was also measured.

3.2.1 Soil chemical properties

3.2.1.1 *Soil pH*

The pH of air-dried, sieved soils was determined in a suspension of 1:2.5 soil and distilled water. The soil and water suspension was allowed to equilibrate for 4 hours. The pH was measured by using a combined glass and reference electrode saturated with KCl.

3.2.1.2 *Organic carbon in soils*

The amount of organic carbon present in soil samples was determined by the Walkley and Black (1934) titrimetric method. Finely ground (0.2 g, three replicates) samples were oxidised with $K_2Cr_2O_7$ and H_2SO_4. The unreacted $Cr_2O_7^{2-}$ was titrated aginst ferrous ammonium sulphate. The percentage of organic carbon was calculated by using the empirical correction factor of 1.3, as suggested by Kalembasa and Jenkinson (1973).

3.2.1.3 *Total nitrogen in soils*

The total nitrogen content of soils was determined by a semi-micro Kjeldahl method using 0.5 g samples of finely ground soil. The samples were digested in H_2SO_4 and a mixture of K_2SO_4, $CuSO_4.5H_2O$ and Se added as a catalyst to speed up the digestion (Bremner and Bundy, 1970). The digest was made up to 50 ml with distilled water in a volumetric flask. NH_4^+-N was determined in the diluted digests by autoanalyser using the method of Weatherburn (1971), in which sodium nitroprusside was used as a catalyst to develop pink colour. The colour intensity was measured at 625 nm wavelength and the total nitrogen was calculated by comparing the sample peak heights with known standards.

Table 3.2 Chemical characteristics of experimental soils.

Soil series	%Organic Carbon (W/W)	%Total Nitrogen (W/W)	Total sulphur ($\mu g\ g^{-1}$ soil)	pH	C:S	C:N	N:S
Teviot (limed)	5.85	0.345	421	5.0	138.95	16.95	8.19
Teviot	5.01	0.320	410	4.4	122.19	15.65	7.80
Meyer	1.90	0.195	196	6.2	96.94	9.74	9.95
Wairaki	2.03	0.365	370	4.5	54.86	5.56	9.86
Te Houka	3.39	0.425	470	4.3	72.19	7.98	9.04
Temuka	2.70	0.235	350	5.7	77.14	11.48	6.71
Lismore	4.29	0.375	280	5.5	153.21	11.40	13.39
Rapaki	3.96	0.365	510	5.5	77.64	10.84	7.16
Summit	4.00	0.335	385	5.3	103.90	11.94	8.70
Templeton(P)	3.31	0.230	364	5.7	90.93	14.39	6.31
Templeton(C)	3.10	0.200	390	5.9	79.48	15.50	5.12
Selwyn	2.35	0.262	302	5.9	73.43	8.96	8.67
Mokotua	8.19	0.295	580	4.8	141.20	27.76	5.08
Takahe	2.44	0.212	255	4.9	101.66	11.50	8.83
Te Kowhai	2.74	0.210	615	4.9	44.55	13.04	3.41
Horotiu	6.63	0.230	930	4.0	71.29	28.82	2.47
Waimakariri	1.45	0.160	185	6.1	78.37	9.06	8.64
Wakanui	2.60	0.182	250	5.0	104.00	14.28	7.28

3.2.2 General chemical characteristics of experimental soils

The distribution of organic carbon ranged from 1.90% for the Meyer soil to 8.19% for the Mokotua soil (Table 3.2). Total nitrogen contents in soils ranged from 0.16% for the Waimakariri soil to 0.425% for the Te Houka soil. There was a very poor correlation between the organic carbon and the total nitrogen content of soils (Table 3.4). Carbon:nitrogen ratios in some soils were higher than generally found in pasture soils. This could possibly be due to excessive mineralisation of organic nitrogen and its faster utilisation by plants and microfauna, combined with leaching from the surface soil. The amount of nitrogen was particularly low in the Horotiu and Mokotua soils, resulting in C:N ratios of 28.8 and 27.7 respectively. These values are exceptionally high considering that the samples were collected from pasture paddocks. The pH of the soils varied from fairly acid (pH 4.0) in the Horotiu soil to 6.2 in the Meyer soil.

3.3 WATER HOLDING CAPACITY OF SOILS (WHC)

The determination of field capacity moisture content was carried out to determine the amount of moisture needed to maintain soils at 75% field capacity moisture content during incubation. Coarsely sieved (2 mm) samples of soil were packed into small columns (2 cm x 4cm x 2 cm) and saturated with distilled water for 24 hours. The samples were then placed on pressure plates under 0.1 bar suction for 24 hours. Thereafter these samples were removed from the pressure plate, weighed, oven dried at 105 °C for 24 hours and then reweighed. The moisture held in the soil samples at 0.1 bar (regarded as field capacity) was then calculated.

3.4 SULPHUR ANALYSIS

3.4.1 Reduction method

Sulphur contents in soil and plant samples were analysed using the Johnson and Nishita (1952) reduction method. The method involves the reduction of SO_4-S to H_2S by reducing solution which is a 2:1:1 mixture of hydriodic acid (55%), hypophosphorous acid and formic acid (90%). The reduced H_2S is absorbed in a solution of $Zn(OAc)_2$ and

NaOAc and subsequently treated with p-amino dimethylaniline sulphate and ferric ammonium sulphate which developes a blue colour. The intensity of colour is measured spectrophotometrically. Before adopting this method, comparative studies were conducted to examine the following aspects of the analysis;

(a) reaction times; samples were reacted with 4.5 ml of reducing solution for 5,10,15,20,30 and 40 minutes at 120±3 °C. Known amounts of added sulphur were recovered fully between 15-20 minutes of reaction time. A prolonged distillation (30 and 40 minutes) did not improve the recovery.

(b) the H_2S absorbing ability of $Zn(OAc)_2$ and NaOAc solution originally used by Johnson and Nishita (1952) and use of M NaOH for absorbing the evolved H_2S were evaluated. It was found that both of these solutions were equally good in recovering the added sulphur.

(c) the sensitivity of Dean's (Dean, 1966) colour finish (using a mixture of gelatine and bismuth nitrate) and methylene blue (using p-aminodimethylaniline) was compared. It was found that Dean's method was less sensitive than the methylene blue method. Dean's method could not measure any sulphur if the amount of S was below 5 μg while the methylene blue method was sensitive enough to measure amounts as low as 2 μg sulphur.

On the basis of the above findings, the method used in this study as follows: soil extracts containing SO_4-S were dried in an oven at 110 °C and reacted with reducing solution for 20 minutes at 120±3 °C and the reduced sulphur was absorbed in a mixture of 10 ml M NaOH and 70 to 80 ml distilled water. *p*-aminodimethylaniline was used to develop the blue colour. The intensity of the colour increases with the amount of S in the solution. Colour intensity was then measured by a Schimadzu double-beam spectrophotometer (UV-140-02) at 670 nm wavelength. There was a linear relationship between the S concentration in solution and optical absorbance over the concentration range 2 to 80 μg sulphur ml^{-1} (Fig. 3.1).

3.4.2 Ion exchange chromatography to determine sulphate

Sulphate extracted from soil by the method described in section 3.5.1 was, in some cases, analysed by ion exchange chromatography using a Waters ion exchange chromatograph. Before analysis, the samples were filtered through a Sep-Pack C18

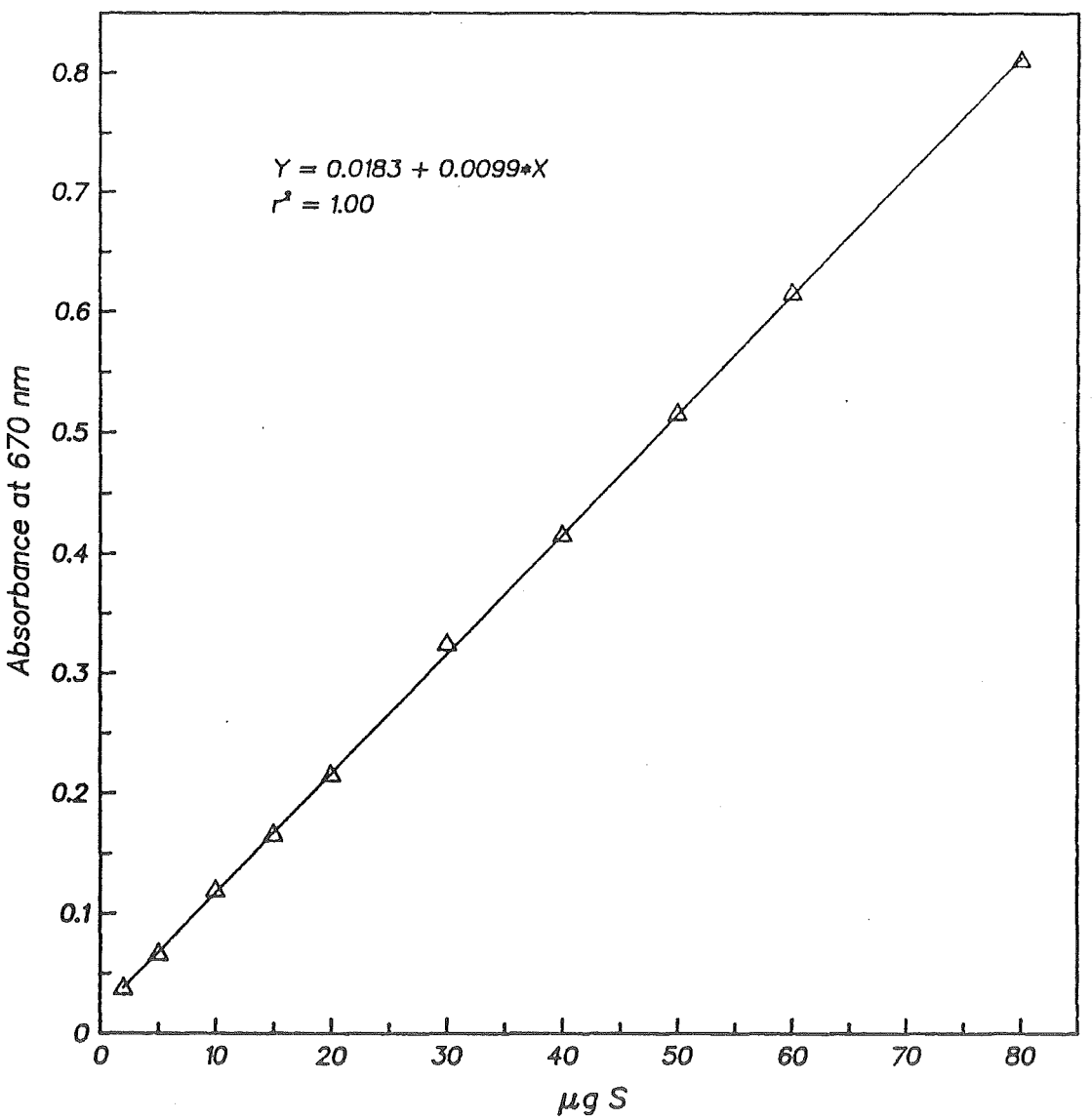


Figure 3.1 Standard curve for sulphur determined by the reduction method.

cartridge (Water Associates) to remove any soluble organic matter from the solution. The removal of soluble organic matter is essential to attain high performance of the instrument. The ion chromatograph was equipped with a non-suppressed column and a conductivity detector. The instrument settings were ;

Injection size	100 μl
Detector	430 Conductivity detector
Column	Waters IC-PAK anion column (non-suppressed)
Eluent	Borate/Gluconate buffer
Eluent flow rate	1.2 ml ml^{-1}
Retention time	12 min.
Temperature	30 °C

The sulphur concentration in samples was calculated by comparing the peak area with those of known standards. There was a linear relationship between the the sulphate-S concentration in solution and the peak area recorded on the chart.

3.5 DETERMINATION OF DIFFERENT FORMS OF SOIL SULPHUR

3.5.1 Determination of water-soluble sulphate (W-SO_4^{2-})

Water-soluble sulphate was determined in both air-dry and field-moist soil samples. Air-dry, sieved soil (5.0 g, four replicates) was weighed into 40 ml polyproplyene centrifuge tubes. These samples were extracted with 25 ml distilled water by shaking on an end-over-end shaker for two hours. The samples were then centrifuged at 10,000 rpm for 10 minutes before filtering through a Whatman No. 42 filter paper. A ten (10) ml aliquot of the filtrate was dried at 105 °C for 24 hours in an oven and the sulphate content was determined by the Johnson and Nishita (1952) method. In some cases the filtrate was analysed directly for sulphate-S by ion exchange chromatography (see section 3.4.2) .

3.5.2 Determination of phosphate-extractable sulphate (P-SO_4^{2-})

Phosphate has the ability to displace sulphate sulphur from the adsorption sites in the soil (Barrow, 1969). A solution of KH_2PO_4 containing 500 μg P ml^{-1} was used to extract the adsorbed sulphate. The soil and extractant ratio was the same as used for W-SO_4^{2-} determinations but these samples were extracted for a longer period (4 hours) on an

end-over-end shaker. Extracted sulphate was measured using the reduction method described in 3.4.1.

3.5.3 Determination of hydriodic acid-reducible sulphur (HI-S)

The amount of HI-reducible sulphur was determined in soils which had been pre-extracted with KH_2PO_4 to remove any inorganic sulphate sulphur. Finely ground (125 micron sieved), 0.10 g soil samples were reacted with 5 ml HI-reducing solution (Freney, *et al.*, 1969). The HI-reducing solution was a mixture of 2:1:1 hydriodic acid (55%), formic acid (90%) and hypophosphorous acid (50%) respectively. Soil samples and HI-reducing mixture were reacted for 1 hour on a distillation unit maintained at 120 °C temperature throughout the reaction. The H_2S produced during the reaction was entrapped in 10 ml of 1 M NaOH solution. Measurement of reduced sulphur was carried out as described in section 3.4.1.

3.5.4 Determination of total sulphur (T.S)

The method of Steinbergs *et al.*, (1962) was used to determine total sulphur in soils, using a mixture of $NaHCO_3$ and AgO_2 (mixed in a 25:1 ratio on weight basis) as the oxidising agent. Finely ground soil samples (0.10 g) were weighed into porcelain cups and mixed with 0.10 g of oxidising mixture. An additional layer of 0.10 g oxidising mixture was spread on top of soil + oxidising mixture. This additional layer is used to entrap any SO_2 escaping when samples are combusted in a muffle furnace at 550 °C for 6 hours. These oxidised samples were then extracted with 20 ml KH_2PO_4 solution (containing 500 μg P ml^{-1}), and sulphur determined in the extracts by the Johnson and Nishita method as described in section 3.4.1.

3.5.5 Carbon-bonded sulphur (C-S)

Carbon-bonded sulphur was calculated by subtracting the phosphate extractable sulphur and the HI-reducible sulphur from the total sulphur (Freney *et al.*, 1971).

3.5.6 Total organic sulphur (T.O.S)

Total organic sulphur was calculated by subtracting the phosphate extractable sulphur from the total sulphur.

3.6 FORMS OF SULPHUR IN EXPERIMENTAL SOILS

The distribution of sulphur between forms varied from soil to soil. Most soils contained less than 5% of the total sulphur as inorganic sulphate. This distribution is typical of surface soils from temperate countries (Tabatabai, 1982). Sulphate-S exists either as water soluble sulphate ($W\text{-}SO_4^{2-}$) or adsorbed sulphate ($P\text{-}SO_4^{2-}$ - $W\text{-}SO_4^{2-}$). About 1-3% of the total sulphur was present as water-soluble SO_4^{2-} and 0.5-2% of the S was present as adsorbed-S. The Rapaki and Horotiu soils contained considerable amounts of sulphate-S in the adsorbed form, approximately 60 and 50% of the total inorganic-S in these soils was present as adsorbed SO_4^{2-}. The Teviot and Summit soils also contained substantial proportions of sulphate as adsorbed sulphate-S. Soils like the Meyers, Temuka, Lismore and Waimakariri contained most of their sulphate in water-soluble form.

The total organic sulphur constituted more than 95% of the total sulphur in the soils which is within the range as described by Tabatabai (1982) for agricultural surface soils. The distribution of organic sulphur between HI-reducible sulphur and C-bonded sulphur forms varied from soil to soil. HI-reducible S was the dominant form of organic sulphur in eight of the eighteen soils (Table 3.3). The highest proportion of HI-S was found in the Selwyn soils, which contained 70% of the organic sulphur in HI-S forms and the lowest proportion of HI-reducible S was measured in the Meyer soil (36%). These variations are within the range which has been found by other workers in other parts of the world (Williams and Steinbergs, 1959; Bettany *et al.*, 1973 and Neptune *et al.*, 1975). The total sulphur varied from a minimum of 185 $\mu g\ g^{-1}$ soil in the Waimakariri soil to a maximum of 930 in the Horotiu soil. Forms of sulphur in soils were highly correlated with each other (Table 3.4). Total sulphur was positively correlated with the organic carbon status of the soils, giving a correlation coefficient (r) value of 0.77 which is highly significant at $P \leq 0.01$ (Table 3.4). Although the amount of total sulphur showed no relationship with total nitrogen, N:S ratios were less variable in the soils compared with C:N or C:S ratios. N:S ratios ranged

Table 3.3 The distribution of different forms of sulphur in soils.

Soil series	W-SO_4*	P-SO_4*	Adsorbed-SO_4*	HI-S*	C-S*	T.O.S*	HI-S	C-S
	(μg S g^{-1} soil)						% of T.O.S	
Teviot (limed)	11.4±0.3	14.6±0.4	3.2±0.7	170±3.6	236.4±6.0	406.4±2.8	41.83	58.17
Teviot	9.2±0.2	17.6±0.4	8.4±0.6	162±4.1	230.4±6.4	392.4±2.2	41.28	58.77
Meyer	1.2±0.0	1.5±0.0	0.3±0.0	70±2.4	124.5±3.0	194.5±0.6	35.89	64.11
Wairaki	6.5±0.0	10.0±0.0	3.5±0.1	230±4.8	130.0±5.7	360.0±0.9	63.88	36.12
Te Houka	16.9±0.4	23.8±0.9	6.9±1.3	250±4.9	196.2±7.2	446.2±3.2	56.05	43.95
Temuka	9.2±0.4	9.2±0.2	0.0±0.6	138±3.9	202.8±7.0	340.8±3.3	40.58	59.42
Lismore	6.8±0.0	7.0±0.2	0.2±0.2	125±3.2	148.0±4.8	273.0±1.8	45.78	54.22
Rapaki	4.6±0.0	11.4±0.4	6.8±0.4	255±2.7	243.6±4.7	498.6±2.5	51.14	48.86
Summit	4.9±0.0	8.5±0.2	3.6±0.2	180±1.9	196.5±3.4	376.5±1.7	47.80	52.20

* mean ± s.e of four replicates

To be continued ☞

Table 3.3/continued

Soil series	W-SO_4*	P-SO_4*	Adsorbed-SO_4*	HI-S*	C-S*	T.O.S*	HI-S	C-S
	(μg S g^{-1} soil)						% of T.O.S	
Templeton(P)	8.9±0.0	11.0±0.2	2.1±0.2	134±1.5	219.0±3.1	353.0±1.8	37.96	62.04
Templeton(C)	10.3±0.4	13.0±0.2	2.6±0.5	155±1.5	222.0±3.1	377.0±1.8	41.11	58.89
Selwyn	4.0±0.1	6.4±0.1	2.4±0.2	208±1.8	89.0±3.4	297.0±1.7	70.03	29.97
Mokotua	19.4±0.1	23.6±1.0	4.2±0.1	254±5.1	302.0±7.8	556.0±3.7	45.68	54.32
Takahe	4.9±0.0	6.1±0.0	1.2±0.0	105±1.8	138.9±3.2	243.8±1.4	43.05	56.92
Te Kowhai	12.7±0.1	17.5±0.0	4.8±0.2	360±4.0	237.5±6.8	597.5±2.8	60.30	39.70
Horotiu	16.4±0.3	32.2±1.0	15.8±0.3	530±6.7	367.8±10.0	897.8±4.8	59.08	40.92
Waimakariri	1.7±0.0	1.8±0.0	0.1±0.0	100±2.8	83.2±3.5	183.2±0.7	54.64	45.36
Wakanui	4.6±0.0	4.8±0.0	0.2±0.0	150±2.2	95.2±3.2	245.2±1.0	61.22	38.78

* mean ± s.e of four replicates

Table 3.4 A matrix of correlation coefficients (r) between soil properties in soils

Soil Properties	T.O.S	N:S	C:N	C:S	pH	C-S	HI-S	P-SO_4	W-SO_4	T.S	T.N	O.C
Total organic sulphur	1.00	-0.73***	0.70**	0.22	-0.65**	0.89***	0.95***	0.89***	0.80***	0.99***	0.17	0.66**
N:S ratio		1.00	0.67**	0.17	0.30	-0.69**	-0.65**	-0.61**	-0.58*	-0.72***	0.42	-0.36
C:N ratio			1.00	0.34	-0.41	0.79***	0.55*	0.72***	0.69**	0.69**	-0.16	0.85***
C:S ratio				1.00	-0.02	0.05	-0.38	-0.02	-0.02	-0.22	0.25	0.54*
Soil pH					1.00	-0.53*	-0.65**	-0.77***	-0.71***	-0.64**	-0.41	-0.51*
C-bonded sulphur						1.00	0.70**	0.87***	0.80***	0.89***	0.20	0.80***
HI-reducible sulphur							1.00	0.79***	0.70**	0.95***	0.13	0.49*
Phosphate-extractable SO_4								1.00	0.95***	0.90***	0.31	0.77***
Water-soluble SO_4									1.00	0.81***	0.27	0.67**
Total sulphur										1.00	0.18	0.67**
Total Nitrogen											1.00	0.35
Organic Carbon												1.00

* significant at $P < 0.01$

** significant at $P < 0.01$

*** significant at $P < 0.001$

from 7-12, which is in close agreement with the figures summarised by Freney and Williams (1983) for a number of soils from different parts of the world. The variations in C:N:S ratios are understandable because samples were collected from different climatic regions, and consisted of several different soil types. Although, most of the samples were collected from pasture sites, this does not mean that the nutrient status of these soil would be similar. Fertilizer application and the age of the pasture would significantly affect C:N:S ratios of soils (Walker *et al.*, 1959).

3.7 BIOMASS SULPHUR (B_S)

Four sub-samples (5.0 g) of moist soil were taken and two of these samples were extracted with KH_2PO_4 solution as described in section 3.5.2, the remaining two samples were fumigated for 24 hours in an air tight desiccator to cause cell lysis of the living micro-organisms in the soil. Methanol-free chloroform was used as a fumigant liquid (Saggar *et al.*, 1981a). Fumigated soils were then extracted twice with 25 ml KH_2PO_4 solution containing 500 μg P ml^{-1}. The amount of HI-reducible S in the extracts was determined as described in section 3.4.1. Biomass sulphur was calculated as follows;

$$\text{Biomass S} = \frac{\text{HI-S extracted after fumigation} - \text{HI-S extracted without fumigation}}{K_S}$$

Where K_S is the fraction of biomass-S released following the fumigation. A value of K_S=0.40 was used in calculation of biomass-S which approximates to the values determined by Saggar *et al.*, (1981a) and Strick and Nakas (1984). However, since only HI-reducible rather than total S was determined, the method used in this thesis for measuring biomass-S is believed to have underestimated the actual biomass-S. Any C-bonded S released by fumigation would not have been determined. In effect the K_S value used would have been an overestimate of the proportion of biomass-S recovered by the above procedure. It is therefore suggested that the biomass-^{32}S determined in this thesis should be regarded as relative biomass-S estimates. However, in case of

sulphur-35 studies, since total ^{35}S was determined in the soil extracts, biomass-^{35}S determinations are not subject to this same error.

3.8 MICROBIAL ACTIVITY IN SOILS

The microbial activity in soils during incubation was determined by examining the microbial respiration rate i.e. evolution of CO_2 from the incubated soils on a per day basis. The concentration of CO_2 evolved from the soils was measured on a Varian aerograph gas liquid chromatograph, series 2800. The instrumental settings used to determine the CO_2 were as follows ;

Sample size	1 ml
Detector	Thermal conductivity
Detector temperature	25° C
Filament current	150 mA
Gas flow	40 ml/minute
Carrier gas	Helium
Column packing	Propak Q
Column size	3 m x 3.12 mm
Retention time	10 minutes
Detection limit for CO_2	0.02% V/V
Valve actuator	Carle micro volume valve (1 ml sample loop), equipped to back flush through the detector between the samples.

The Carle micro volume valve helped in sampling the CO_2 evolved from the soils and gave greater precision than was achieved through manual sample injections to the column. Following passage of the CO_2 peak the backflush valve was turned on to clean the column before the new sample was injected by turning on the injection valve. The peak heights of the CO_2 in the samples were compared with the CO_2 standards. There was a linear relationship between the CO_2 concentration and the peak height over the concentration range of 0.3% to 20% CO_2/air (Fig. 3.2). Measurements of CO_2 evolution were carried out in both systems of incubation i.e. closed and open systems. Measurments of respiration rates were relatively easy in the closed compared to the open system. Soils were placed in respiration vessels which were capped with a rubber septum (Plate 3.1). Concentrations of CO_2 in the air space above the soil were

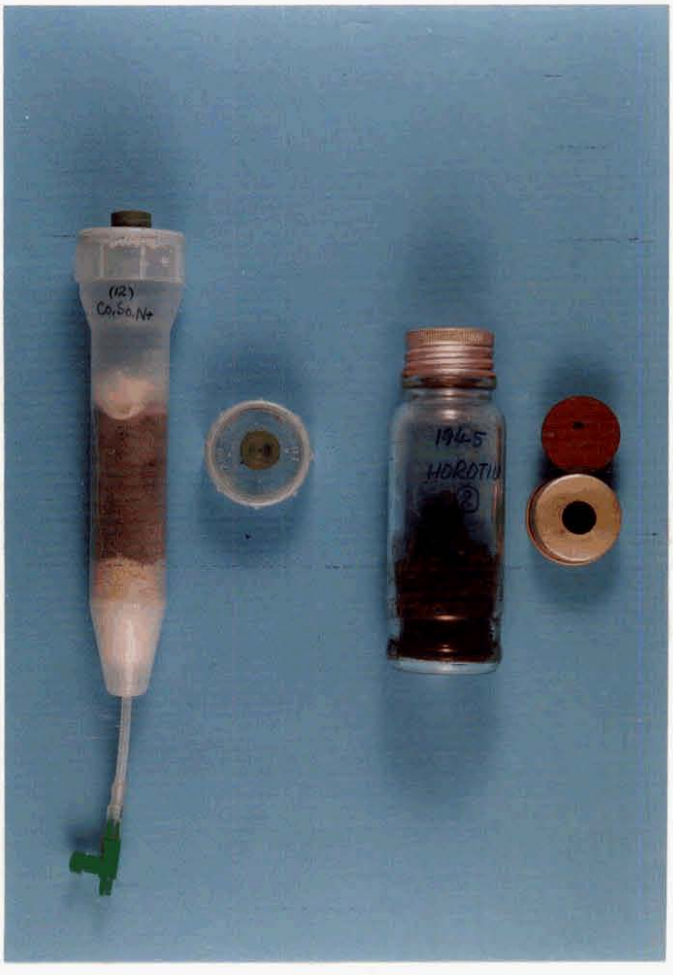


Plate 3.1 Respiration vessels (a) showing the open leaching column equipped with a specially designed cap, (b) closed respiration vessel with the rubber cap through which CO_2 gas was sampled.

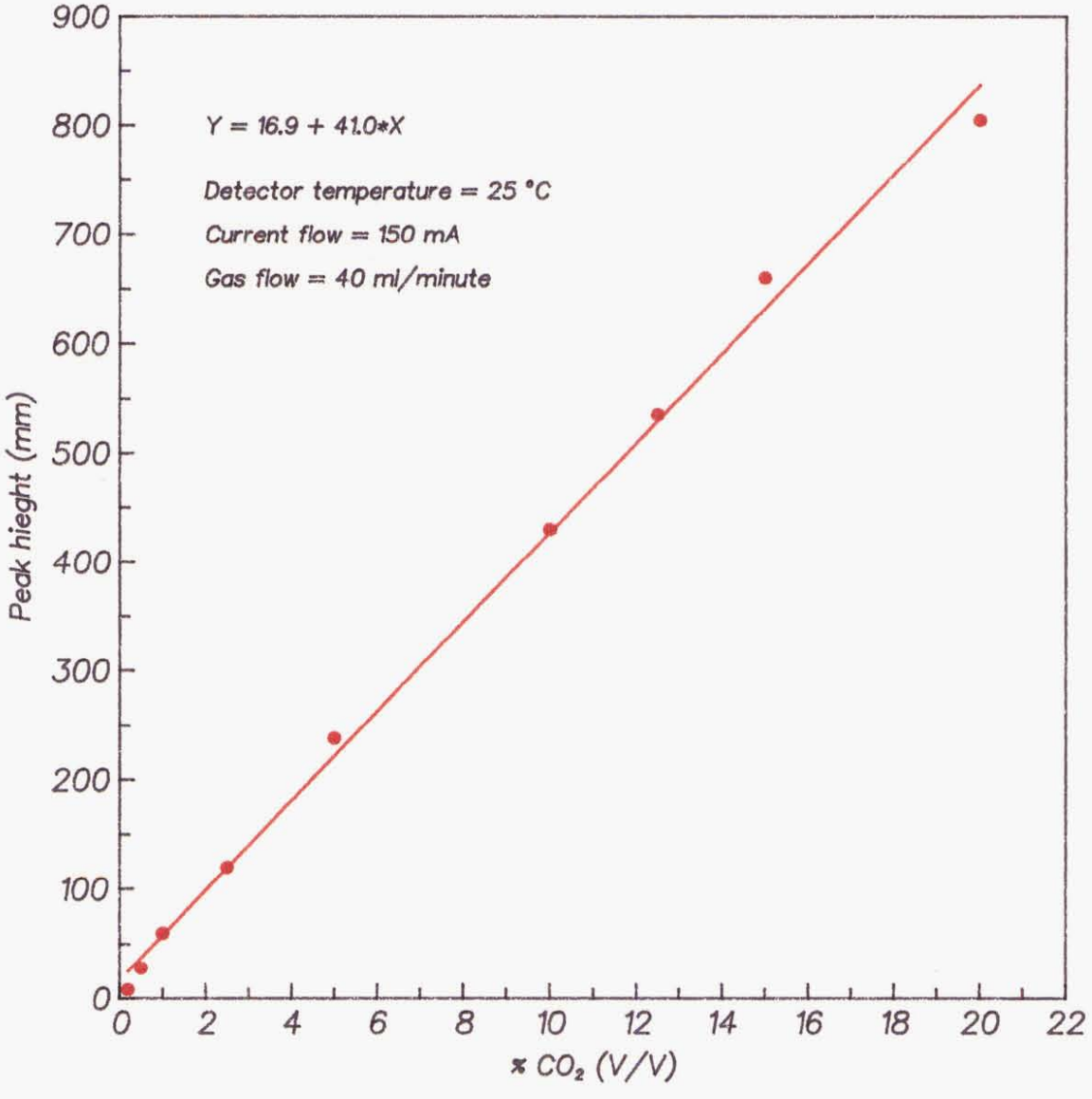


Figure 3.2 The standard curve of CO_2 detection on Varian aerograph gas liquid chromatograph series 2800, fitted with thermal conductivity detector.

measured by syringing gas samples through the septa and directly injecting then into the GLC. Open incubations were carried out in polypropylene leaching columns. To measure CO_2 evolution in these columns, they were capped tightly for 12 hours. The caps used to seal off these leaching columns to prevent any loss of respiratory CO_2, were equipped with a rubber septum which allowed easy sampling of the CO_2 produced by the microbes (Plate 3.1). Gas samples were syringed and injected into the GLC in the same way as described above. The respiratory CO_2-C evolved was calculated as μg C produced g^{-1} soil day^{-1} by using the gas equation (3.2).

$$PV = nRT \qquad \text{.....} \qquad (3.2)$$

where P = pressure in atmosphere, V = volume of the gas (litres), n = number of moles of gas, R = universal gas constant, T = temperature in degrees Kelvin.

Assuming that pressure inside the respiratory vessels is 1 atmosphere and the universal gas constant is 0.08205, then CO_2 concentration can be calculated by knowing the volume of the gas inside the respiratory vessel.

3.9 DETERMINATION OF TOTAL SULPHUR IN PLANT TISSUES

Plant samples were dried at 60 °C and finely ground using a hammer mill (Glen Creston). Samples were initially analysed using one of three methods;

(i) Oxygen combustion/ion exchange chromatography
(ii) Steinbergs method
(iii) X-ray fluorescence spectroscopy

3.9.1 Oxygen combustion/ion exchange chromatography

Total sulphur in plant samples was determined by the conventional oxygen flask method (Ismaa, 1959). Distilled water was used as absorbing solution rather than 1M KOH solution to suit the ion chromatographic detection of sulphate. Finely ground plant samples (0.05 g, in duplicate) were weighed and folded into 2 cm squares of filter paper, which were

placed into the platinum holder. The samples were then combusted in oxygen flushed Erlenmeyer flasks using 20 ml distilled water mixed with 2 drops of H_2O_2 to absorb the products of combustion. The absorbing mixture was swirled three to four times around the walls of the flask. After 30 minutes, sulphur (as sulphate) was analysed in the solution using the Waters ion chromatograph as described in section 3.4.2.

3.9.2 Oxidation/combustion method

This method has been described in section 3.5.4 for soils. The only difference between the soil and plant analysis was the initial weight of sample, for plants only 0.05 g of the finely ground material was used to determine the sulphur content.

3.9.3 X-ray fluorescence spectroscopic method

The x-ray fluorescence method of Norrish and Hutton (1977) was used to determine total sulphur in some plant samples. The amount of sulphur obtained by this method was used to compare with the total sulphur content in plant samples determined by either the Steinbergs oxidation/combustion method or the oxygen combustion-ion exchange chromatogrphy method. Finely ground samples (3-5 g) were pelleted at 4000 PSI pressure. Samples were analysed by a Phillips model PW 1400 instrument. The settings used to determine total S were:

X-ray source	Cr x-ray tube, 1.5 KW
kV	40
mA	30
Gas	Argon P-10
Counter	Flow-proportional
Preset counting time	50 seconds
Determination time	10 minutes

A series of herbage samples from different plant species were used as standards (as determined by CSIRO, Australia) to calibrate the results.

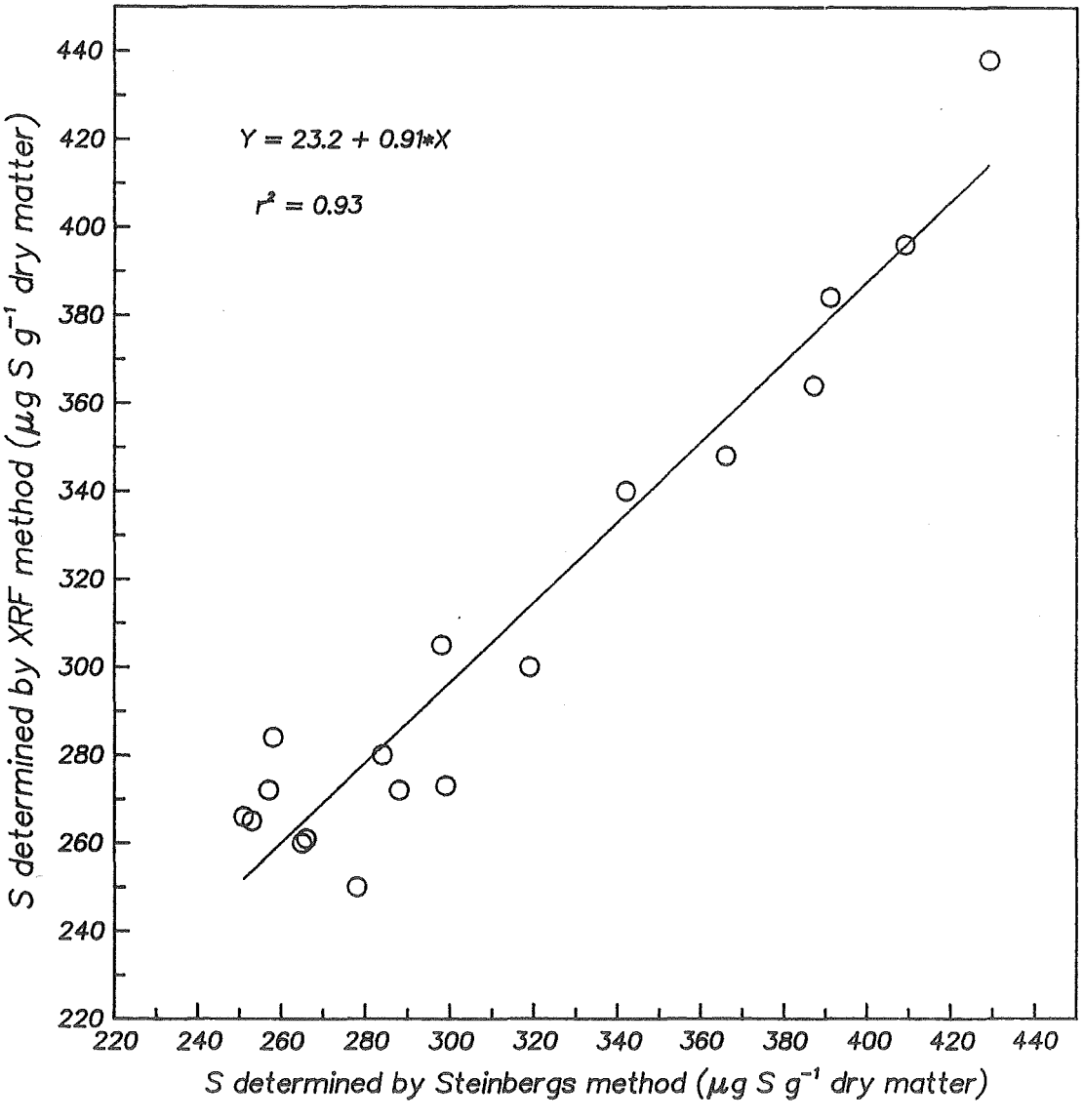


Figure 3.3 The relationship between the amount of sulphur measured in plant tisues by X-ray fluorescence spectroscopy and the Steinbergs oxidation methods.

3.10 COMPARISON OF METHODS USED TO DETERMINE PLANT SULPHUR

A comparative study of plant sulphur analysis carried out by these three methods showed that the oxygen flask combustion method recovered the least amount of total sulphur from the plant materials. In comparision to sulphur determined by the XRF method, the oxygen combustion method recovered between 79-89% of total sulphur in plants. Although, this method is simple and quick, due to poor recovery, it was not considered for any further for plant analysis. The Steinbergs *et al.*, (1962) method of oxidation and combustion was also compared with the XRF method (Figure 3.3). The results obtained by the Steinbergs *et al.* (1962) method were comparable with the XRF method (94-103% recovery). Since the Steinbergs *et al.* (1962) method was more economic and accessable than the XRF method, all the sulphur determinations in plant samples were carried out using the Steinbergs *et al.* (1962) method.

CHAPTER FOUR

MEASUREMENTS OF SULPHUR MINERALISATION IN NEW ZEALAND SOILS

4.1 INTRODUCTION

There are two basic methods of incubation which can be used to study sulphur mineralisation and immobilisation in soils: closed and open systems. In the closed incubation system, soils are incubated at a controlled moisture level and at a constant temperature within a container such as a conical flask. The mineralised sulphate is allowed to accumulate in the soil until the end of the incubation period. The concentration of the sulphate after the incubation is compared with the initial concentration present in the soil. The net increase/decrease in the sulphate content indicates the level of sulphur mineralisation/immobilisation in the soil. Up until 1980, the closed system of incubation was essentially the only method used to study sulphur mineralisation/immobilisation in soils (e.g. Williams, 1967; Kowalenko and Lowe, 1975a) . The net mineralisation measured in such studies is usually relatively small, often less than 1% of the total soil sulphur.

The open system of incubation in sulphur mineralisation studies was first used by Tabatabai and Al-Khafaji (1980), and has since been adopted by other workers e.g. Maynard *et al.*, (1983); Pirela and Tabatabai, (1988). Usually soils are packed in a leaching column and incubated at a fixed moisture and temperature. The mineralised-SO_4^{2-} is removed at regular intervals. This removal of sulphate from the soil simulates the field effect in an actively growing crop where sulphate is either utilised by plants or lost by leaching. This method of incubation has given significantly higher levels of mineralisation compared with the closed system (Maynard *et al.*, 1983). Tabatabai and Al-Khafaji (1980) reported as much as 35% mineralisation of sulphur from soil organic sulphur pools over a 28 week incubation period. One point of concern with the open incubation system is that the regular leaching used to remove the mineralised-SO_4^{2-},

could also remove nutrients other than sulphur, which might affect microbial activity and consequently reduce the rate of sulphur mineralisation. A series of preliminary studies was carried out to examine various aspects of the open incubation system.

4.2 PRELIMINARY STUDIES

The aims of the preliminary studies were to develop an open incubation system to study the mineralisation of sulphur from New Zealand soils and in particular to;

(a) compare the amount of sulphur mineralised from such a system with the amounts mineralised from a traditional closed incubation system

(b) assess the possible effects of continuous nutrient leaching in the open system on the levels of sulphur mineralisation

(c) examine the effect of temperature on sulphur mineralisation in the open system and

(d) study the effects of prolonged incubation on mineralisation.

4.2.1 Materials and Methods

4.2.1.1 *Soils*

Nine soils were used in these preliminary experiments - Teviot (limed), Teviot, Meyer, Summit, Templeton (P), Rapaki, Temuka, and Lismore . The soils were air-dried, sieved (2 mm) and stored at 20 °C temperature prior to the incubation.

4.2.1.2 *Closed incubation*

Soil samples (20 g) were weighed into 100 cm^3 conical flasks. Appropriate amounts of distilled water were added to each flask to bring the moisture content of the soil to 75% field capacity. The flasks were plugged with cotton wool and then incubated at 20 °C in darkness for a 10 week period. Moisture levels were maintained by adding distilled water to a known weight on every alternate day. After 1, 2, 3, 4, 6, 8 and 10 weeks, three flasks were removed from the incubator for determining net sulphur mineralisation or immobilisation. Each flask was sub-sampled in duplicate (5 g air-dry wt.). These samples

were extracted with 25 ml KH_2PO_4 containing 500 μg P ml^{-1} and sulphate-S was determined by the reduction method described in section 3.4.1. To determine the mineralisation/immobilisation of sulphur during the incubation, soil sulphate-S levels were compared to the amounts of sulphate-S prior to incubation.

4.2.1.3 *Open incubation*

Samples of soil (25 g.) were incubated in polypropylene columns. There were four replicates for each soil. The columns were packed at the base with a plug of glass wool plus a layer of coarse-textured antibumping granules, and the soil, mixed with 15 g of inert glass beads (2.5-2.8 mm diameter), was placed on top of that layer. The mixing of the glass beads was carried out to avoid packing of soils in the column during the leaching, to enhance leaching and to maintain the aeration. The upper soil surface was protected by a thin layer of glass wool (Fig. 4.1). The rate of out-flow during the leaching was controlled by a valve attached at the base of the column. Soil samples used in these experiments were preconditioned for 2 weeks at 75% field capacity and 20 °C. This was carried out in order to stabilise the soil microbial population to avoid the sudden flush of sulphur mineralisation which can occur when dried soil samples are rewetted (Williams, 1967). After placing the soils in the columns, samples were leached with 100 ml KH_2PO_4 solution, containing 500 μg P ml^{-1} to remove all the inorganic sulphate (W-SO_4^{2-} and adsorbed-SO_4^{2-}) The columns were further leached with 100 ml distilled water to ensure the removal of any KH_2PO_4 extractable sulphate from the columns. Before incubating, excess moisture was removed from the columns by applying a suction of 670 mm Hg through the flow control valve. The columns were incubated at 20 °C for 10 weeks and the mineralised sulphate was removed slowly from the columns at biweekly intervals. This was achieved by leaching the columns with 100 ml of either 0.01 M KCl or 0.01 M $CaCl_2$ solution at a rate of approximately 1 ml min^{-1}. The leachates were analysed for SO_4-S. After each leaching, excess moisture was removed form the columns by applying suction as described above. At the end of the incubation, the last leaching with 0.01M KCl/$CaCl_2$ was followed by a further 100 ml KH_2PO_4 to ensure the removal of any mineralised-SO_4^{2-} which may have been adsorbed by the soil.

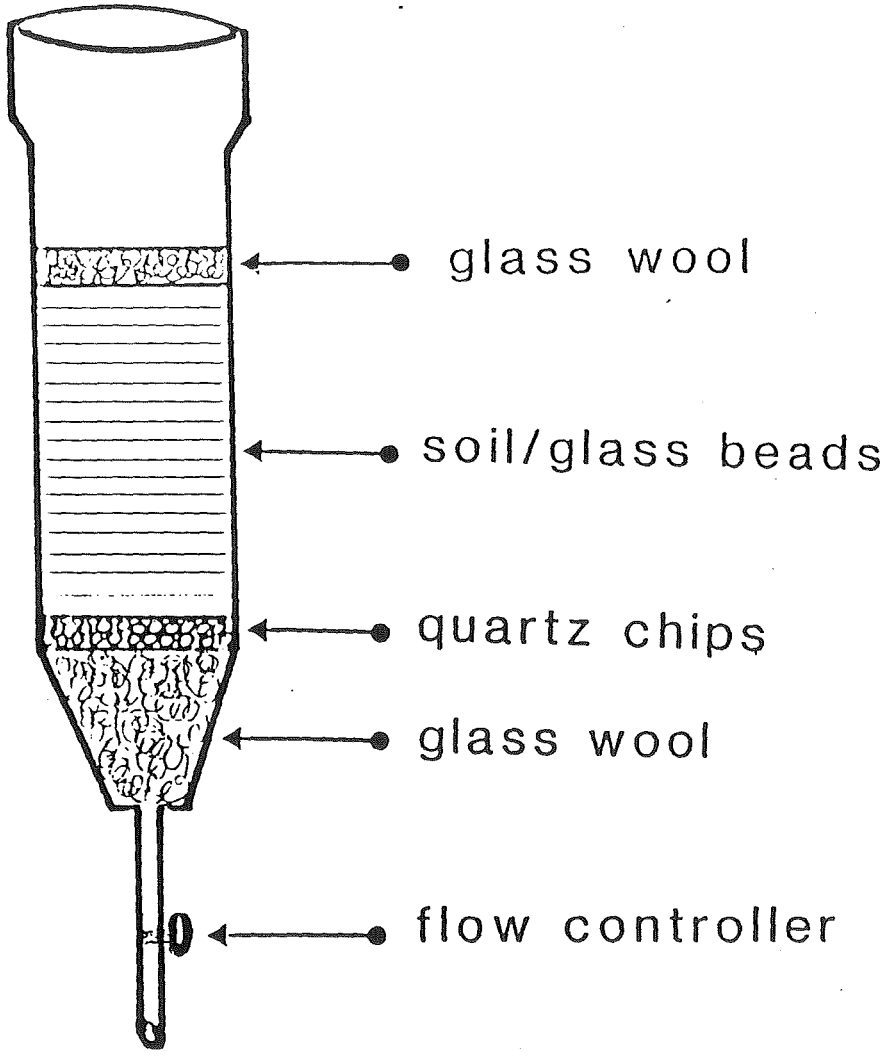


Fig. 4.1 A cross-sectional view of the soil column used in the open incubation system.

4.2.1.4 *Addition of nutrients*

The second part of the preliminary studies was conducted using the open incubation system in which one set of columns was leached with 100 ml 0.01 M KCl as described above, and another set of columns leached with 75 ml KCl followed by 25 ml of KCl containing added nutrients (a mixture of 0.002 M $Ca(NO_3)_2$, 0.002 M $Mg(NO_3)_2$ and 0.005 M $Ca(H_2PO_4)_2$). The leaching with nutrient solution was to ensure an adequate supply of other nutrients which might otherwise limit microbial activity and hence sulphur mineralisation. Both sets of columns were leached every two weeks and the amount of sulphate-S was determined in the leachates.

4.2.1.5 *Effect of temperature*

The effect of temperature on the level of sulphur mineralisation in soils was examined using the open incubation system. Soils were incubated at 10, 20 and 30 °C for a period of 18 weeks.

4.3 RESULTS AND DISCUSSION

4.3.1 Comparison between closed and open incubation systems

The pattern of sulphur mineralisation and the amount of sulphur mineralised were considerably different in the two systems of incubation. The closed system of incubation gave irregular patterns of sulphur mineralisation (Figure 4.2). For example, the Temuka and Lismore soils released a significant amount of sulphur as sulphate within the first week of the incubation, i.e. showing net mineralisation of sulphur. After this initial flush of mineralisation, these soils also showed a net immobilisation of the sulphate sulphur. In the Rapaki and Teviot soils some sulphate-S was immobilised after the initial mineralisation but the concentration of sulphate in these soils always remained higher than the preincubation concentration. The only consistency which was observed was the flush of mineralisation in all the soils in the first two weeks of incubation, as shown in Fig. 4.2. This could be due to the way this incubation was carried out. The addition of moisture to an

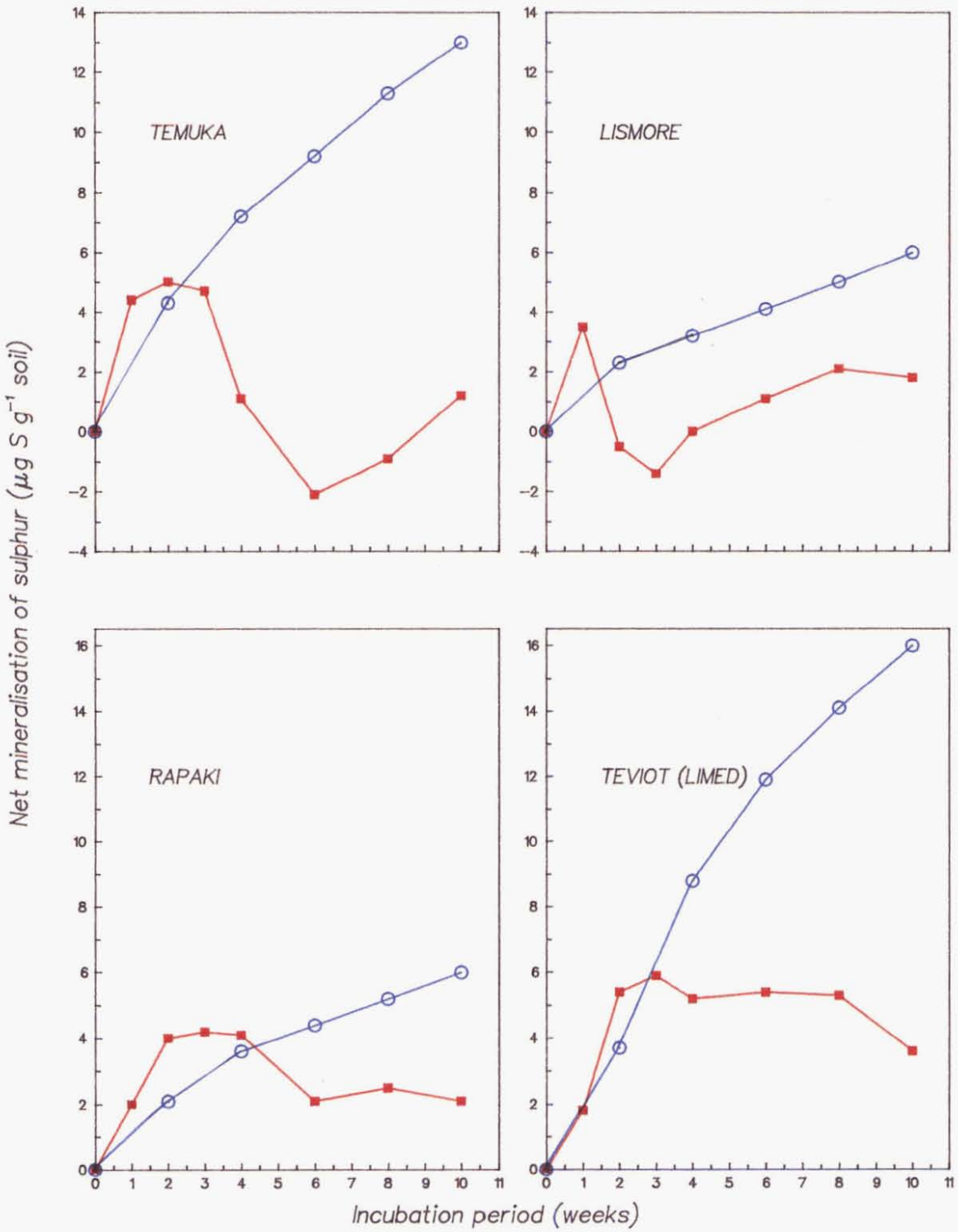


Figure 4.2 A comparision between the amount of sulphate–S mineralised in the closed (▪ — ▪) and the open (∘ — ∘) systems of incubation.

air-dry soil and favourable temperature would have suddenly activated the soil microbes resulting in a high release of sulphur in the first few days of incubation. When microbial activity decreased at the later stages of the incubation, the release of sulphur from organic sources decreased. A similar pattern of mineralisation has been reported by Williams (1967). In some cases (Temuka and Lismore) the microbial demand for sulphur clearly exceeded the amount of sulphur released and resulted in a net immobilisation of soil sulphate. After the initial fluctuations, the rate of mineralisation decreased substantially and the amount of sulphur mineralised remained fairly constant (particularly in the Rapaki and Teviot soils). The open system of incubation, in which mineralised-SO_4^{2-} was removed every two weeks, showed a continuous mineralisation of sulphur throughout the incubation period. A considerably higher amount of sulphur was mineralised in this type of incubation than the closed incubation system (Table 4.1). The Temuka soil showed the maximum difference in the rate of sulphur mineralisation between the two systems, where approximately 11 times more sulphur was mineralised in the open than in the closed incubation system. These results are in agreement with the results of Maynard *et al.*, (1983) who also recorded a similar difference in the level of mineralisation using these two incubation systems.

A direct comparison of these two methods of incubation may not be entirely appropriate because there is a fundamental difference between the closed and open incubation systems. In the closed system of incubation, mineralised-SO_4^{2-} remains within the soil and almost certainly re-cycled back to organic forms of sulphur. Thus the results obtained from this incubation involve both processes of sulphur transformation in soils i.e. mineralisation of sulphur from organic forms of sulphur and also incorporation of sulphate into organic sulphur (immobilisation). The reactions in the closed system are reversible and can be summerised in equation 4.1. Difference in the rates of mineralisation and immobilisation are often quite small, hence for any given period of time, the net mineralisation of sulphur is likely to be a small fraction of the total sulphur.

$$\text{Organic sulphur} \underset{\text{Immobilisation}}{\overset{\text{Mineralisation}}{\rightleftharpoons}} SO_4^{2-} \qquad ..(4.1)$$

Table 4.1 Sulphur mineralised during 10 weeks incubation in the closed and open incubation systems.

Soils	Amount of sulphur mineralisation (μg S g^{-1} soil)		Ratio (open:closed)
	(open)	(closed)	
Temuka	12.0	1.2	10.83
Lismore	5.0	1.8	2.77
Rapaki	4.8	2.1	2.57
Teviot (limed)	17.9	3.6	4.97

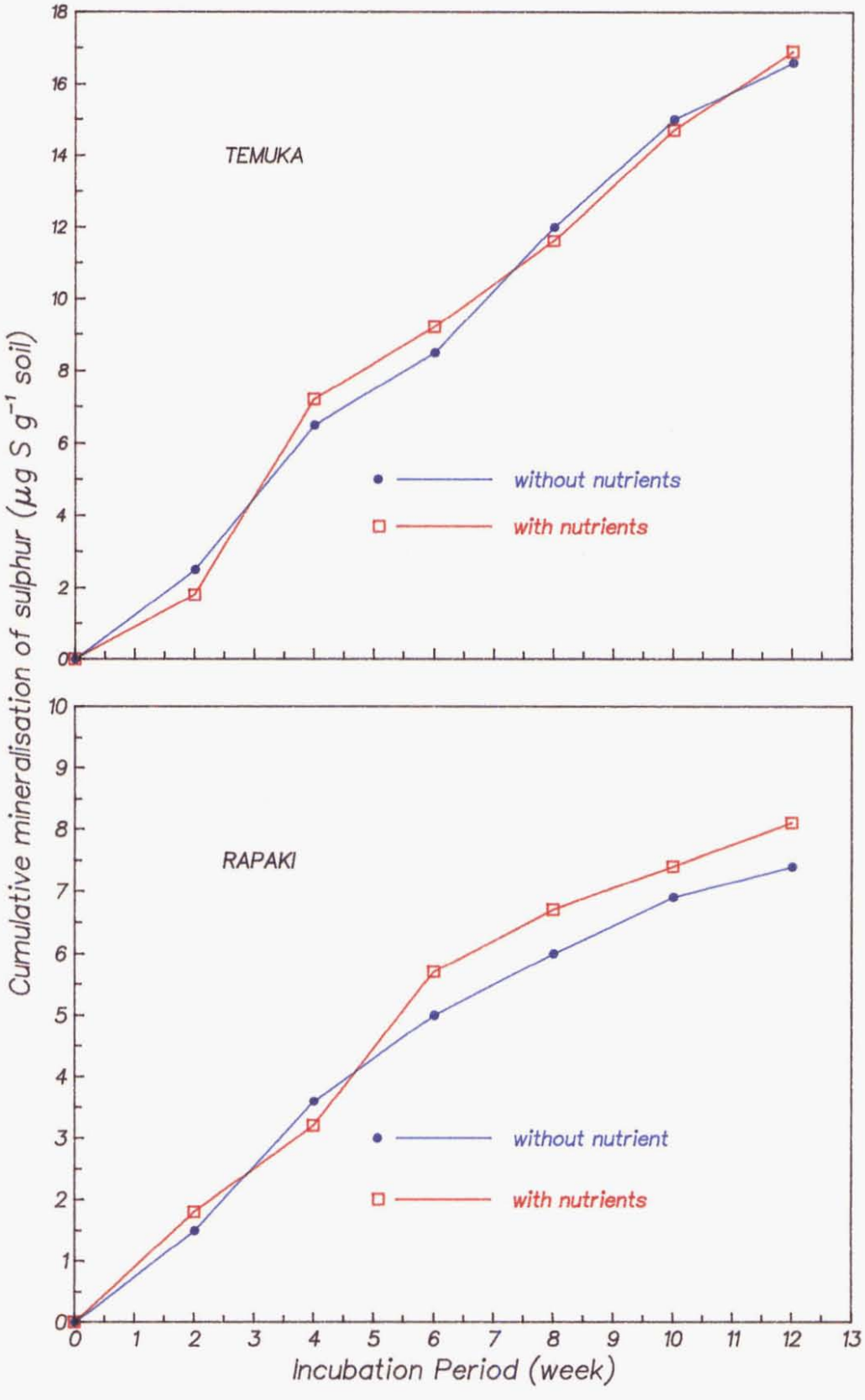


Figure 4.3 Sulphur mineralisation measured in the presence and absence of added nutrients.

On the other hand, the open system measures predominantly the release of sulphur as sulphate (mineralisation) and restricts the opportunity for immobilisation of to take place. The removal of sulphate-S at frequent intervals prevents its re-incubation into soil organic matter, thus the reaction is essentially non-reversible (equation 4.2).

$$\text{Organic sulphur} \xrightarrow{\text{Mineralisation}} SO_4^{2-} \qquad \text{.....(4.2)}$$

Unlike the closed system where mineralised-SO_4^{2-} is allowed to stay in the soil system, and thus has little relevance to the field situation, the amount of sulphur mineralised using the open system more closely resembles field conditions, where mineralised sulphate-S is continuously lost either by plant uptake or by leaching down the profile. Thus assessments of potentially mineralisable sulphur in soils carried out using an open system of incubation should be more appropriate than those obtained in the closed system.

4.3.2 Comparison between leaching with and without added nutrients

There were no significant differences in the rates of sulphur mineralisation between samples leached solely with 0.01 M KCl and samples to which nutrients were added at the end of each leaching (Fig. 4.3). The rate of sulphur mineralisation did not appear to be affected by the continual leaching with 0.01 M KCl. This was apparent in both soils used in this study (Temuka and Rapaki). When nutrients nitrate, phosphate, calcium and magnesium were added to soils to ensure an adequate supply of these nutrients to microbes, there was no increase in the rate of sulphur mineralisation. Therefore it can be concluded from this experiment that the nutrients nitrate, phosphate, calcium and magnesium are unlikely to limit the mineralisation of sulphur over a 10-12 week period. However over longer periods of leaching, nutrient deficiencies may occur which affect microbial activity and consequently the mineralisation of sulphur.

4.3.3 Mineralisation of sulphur at 10, 20 and 30 °C

The level of mineralisation was poor at 10 °C in all soils, resulting in an average of only 2.44±0.25% mineralisation from the total organic sulphur during an 18 week incubation

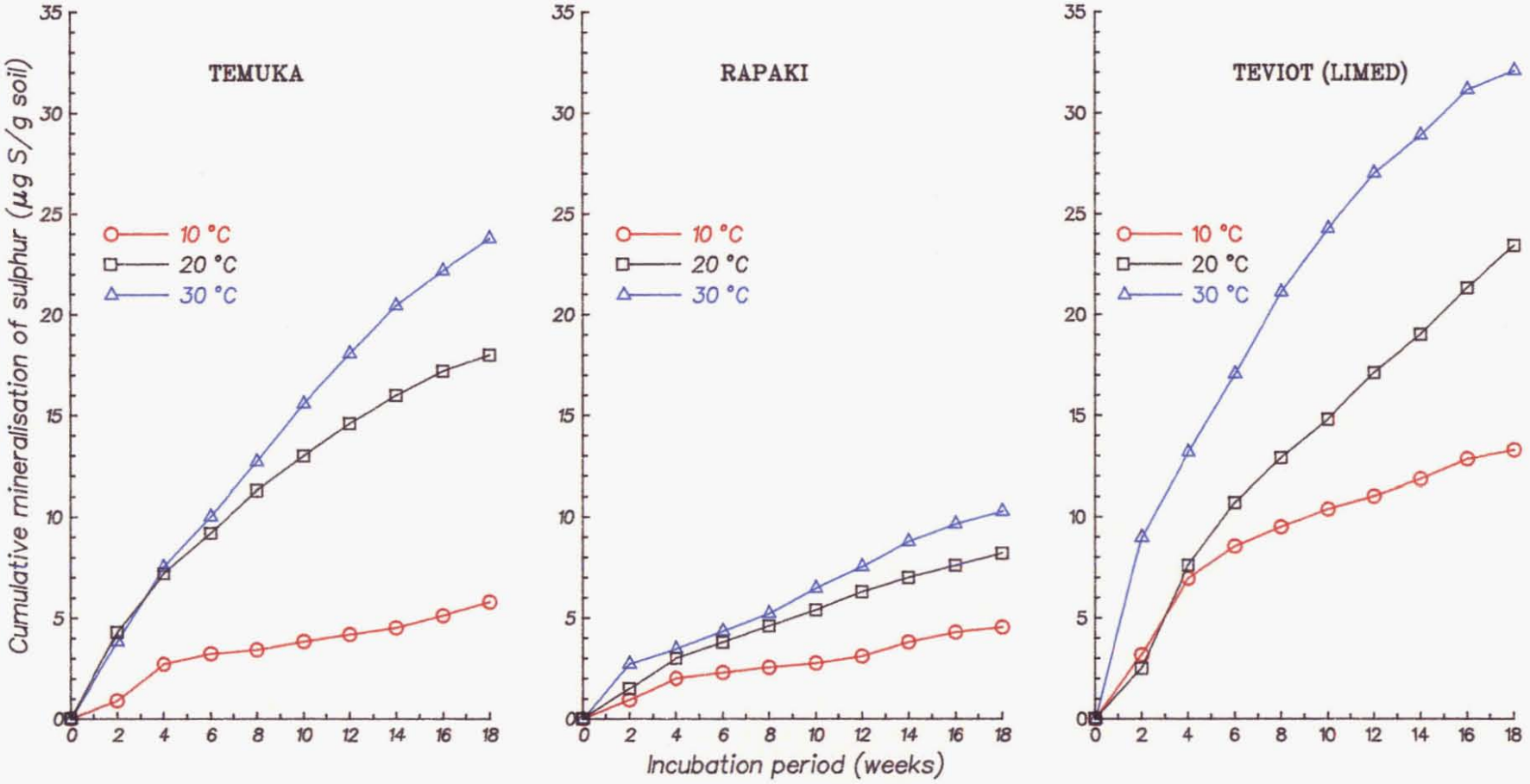


Figure 4.4 Mineralisation of sulphur at 10 20 and 30 °C in the Temuka, Rapaki and Teviot (limed) soils.

period (Table 4.2). The Rapaki and Temuka soils showed the lowest levels of sulphur release at this temperature, only 1.76 and 1.78% of the total organic sulphur (TOS) respectively were mineralised in these soils (Table 4.2) (0.48 and 0.36 μg sulphur g^{-1} soil $week^{-1}$ respectively). In most soils the level of mineralisation was highest during the first 4 weeks of incubation and thereafter declined with every subsequent leaching. About 55-60% of the mineralised sulphate-S was produced during the first 4 weeks of incubation (Fig. 4.4) and only 40-45% over the next 14 weeks.

Soils incubated at 20 °C showed an almost 2 fold increase in the level of sulphur mineralisation compared with the 10 °C incubation (Table 4.2). Again, the Rapaki soil showed the lowest level of mineralisation amongst the four soils which were incubated at this temperature. The release of sulphate was certainly faster in the first four weeks and then slowed down. However, unlike the 10 °C incubation, the mineralisation of sulphur was continuing at appreciable rates, especially in the Teviot (limed) soil when the experiment was stopped.

Soils incubated at 30 °C mineralised the highest percentage of TOS in all soils. On average 6.53±0.58% of the TOS was mineralised at this temperature. The rate of sulphur mineralisation was relatively high in the first 4-8 weeks. Thereafter a decrease in the rate of mineralisation was noticeable in most soils. Generally an increase in the incubation temperature has raised the amount of mineralised sulphur. Such an increase in sulphur mineralisation has also been found by Tabatabai and Al-Khafaji (1980) and Pirela and Tabatabai (1988). This increase undoubtedly related to an increased microbial activity in the soil at higher temperatures.

Results from these studies (10,20 and 30 °C incubation) indicate that an increase or decrease in the temperature would give significantly different rate of SO_4-S production from organic forms of soil sulphur. Thus it is necessary that incubation studies for assessing mineralisable sulphur should be carried out at a constant temperature. The effect of temperature may be more prominent in some soils than others. For example, in the Temuka soil the mineralisation of sulphur from the organic fractions increased more than four times when the incubation temperature was increased from 10 to 30 °C (Table 4.2).

Table 4.2 The effect of incubation temperature on the level of sulphur mineralisation.

Soils	Incubation temperatures		
	10°C	20°C	30°C
	Percent of Total organic sulphur mineralised*		
Teviot (limed)	3.78	6.27	8.51
Teviot	2.47	N.D	4.35
Meyer	1.82	N.D	7.25
Rapaki	1.76	2.93	4.15
Summit	2.30	N.D	7.18
Takahe	3.00	N.D	7.85
Temuka	1.78	5.54	7.42
Templeton(P)	2.62	N.D	7.73
Lismore	N.D	3.35	4.30
Mean	2.44	4.52	6.53
S.E.	0.25	0.82	0.58

* The adsorbed S was included in the amount of S mineralised during the 18 weeks of incubation at 10, 20 and 30 °C, N.D = not determined

Table 4.3 Maximum mineralisable S in soils measured over a 28 week incubation period using the open incubation system.

Soils	Mineralised-SO_4^{2-}	
	[1] $\mu g\ S\ g^{-1}$ soil	[2] $kg\ ha^{-1}$
Teviot (limed)	41.6±3.6	49.9±4.3
Teviot	20.1±2.1	24.1±2.5
Meyer	16.3±1.8	19.6±2.2
Templeton (P)	33.4±3.6	40.1±4.3
Rapaki	28.8±3.1	34.6±3.7
Takahe	25.4±1.9	30.5±2.3
Temuka	31.8±2.1	38.2±2.5
Summit	33.4±3.7	40.1±4.4

1. mean ± s.e (four replicates), it also included the mineralised-SO_4^{2-} recovered as adsorbed sulphates during the last leaching with KH_2PO_4.
2. Calculated for the top 10 cm surface soil, assuming the bulk density of soil as 1.20 $g\ cm^{-3}$.

On the other hand in the Teviot soils the effect of temperature was less significant, and showed less than a two fold increase with a similar increase in the temperature.

4.4 MINERALISABLE SULPHUR IN SOILS

Soils were incubated for a longer period of time at 30 °C to measure the maximum mineralisable sulphur in soils. The incubation period was extended to 28 weeks, and mineralised sulphate was removed biweekly by leaching with 100 ml 0.01 M $CaCl_2$. By the end of the 28th week of the incubation, the amount of mineralised-SO_4^{2-} was approaching the minimum detection limit of the method used to measure the SO_4^{2-}-S, thus the experiment was stopped at that stage.

There were large differences in the amount of sulphur mineralised in soils during the prolonged incubation (Table 4.3). The lowest of 16.3 $\mu g\ g^{-1}$ soil was mineralised in the Meyer soil and the highest amount of mineralised-SO_4^{2-} (41.6 $\mu g\ g^{-1}$ soil) was measured in the Teviot (limed) soil. The mineralised-SO_4^{2-} calculated as kg ha^{-1} showed a substantial contribution to the available sulphur pool. For example, sulphur mineralised in the Teviot (limed), Templeton and Summit soils ranged between 50 and 40 kg ha^{-1} which would provide sufficient sulphur for pasture growth. The rate of sulphur mineralisation dropped after first 12-14 weeks of incubation in most soils (Fig. 4.7), indicating a slower rate of mineralisation during the later stages of incubation. The reduction in the rate of mineralisation varied from soil to soil. For example, in the Teviot soil the rate of sulphur mineralisation declined rapidly after the 4th week of incubation; the Meyer soil showed this decline in the 18th week of incubation. More than 70% of the total sulphate released by the soils was mineralised during the first 14 weeks of the incubation, the next 14 weeks of incubation resulting in less than 30% of the total mineralised-SO_4^{2-}. There were significant differences between this study and the studies reported by Tabatabai and co-workers (Tabatabai and Al-Khafaji, 1980; Pirela and Tabatabai, 1988), showed a linear release of SO_4-S from soil organic sulphur, even when mineralisation was carried on for more than six months (28 weeks). This would tend to suggest that the rate of breakdown of the soil organic matter was constant during the entire period of incubation and that microbial activity also remained at a constant level. It would seem unlikely that microbial activity would remain at a constant level for such a long period of time. Since the release

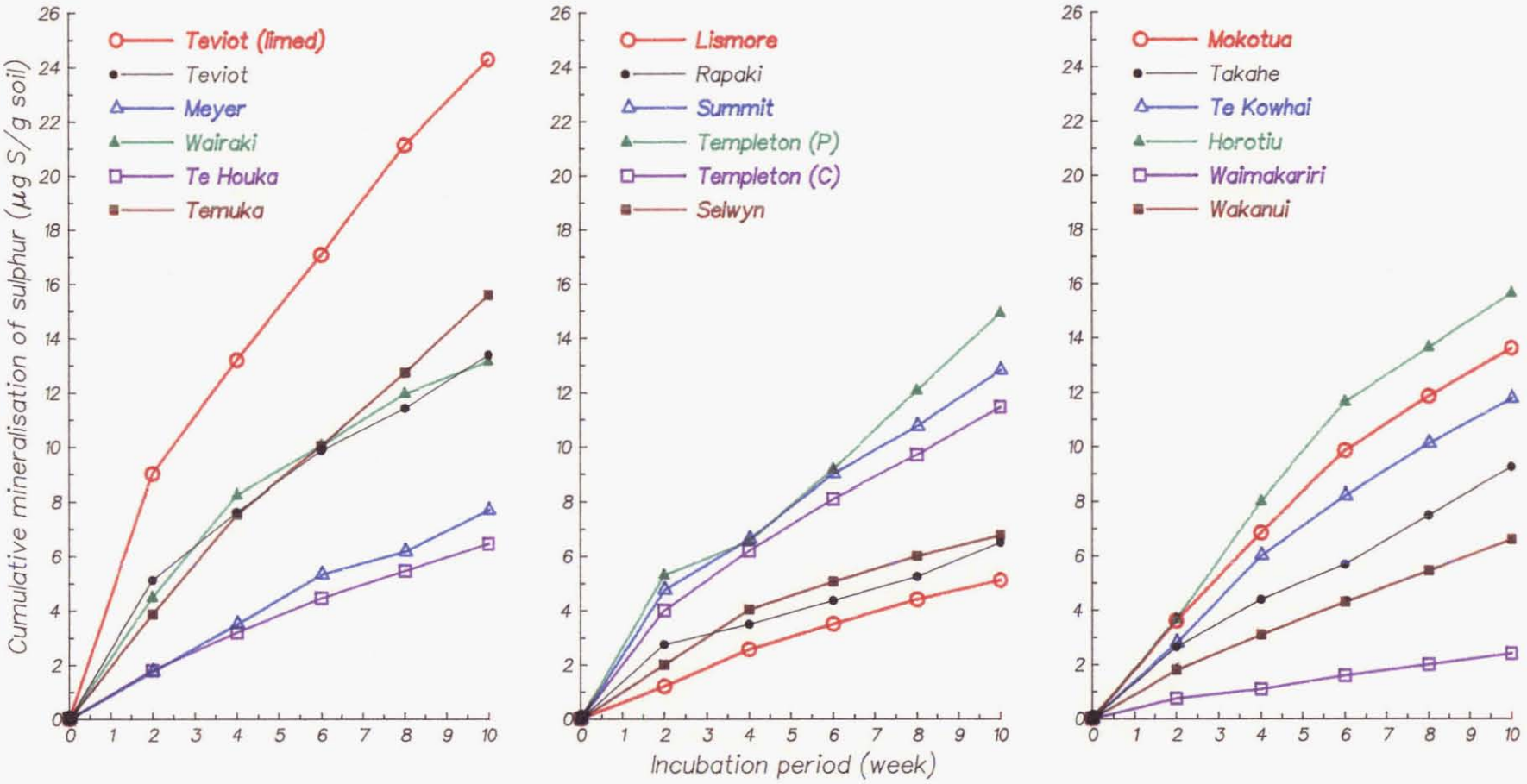


Figure 4.5 Cumulative mineralisation of sulphur in New Zealand soils following the open system of incubation at 30 °C.

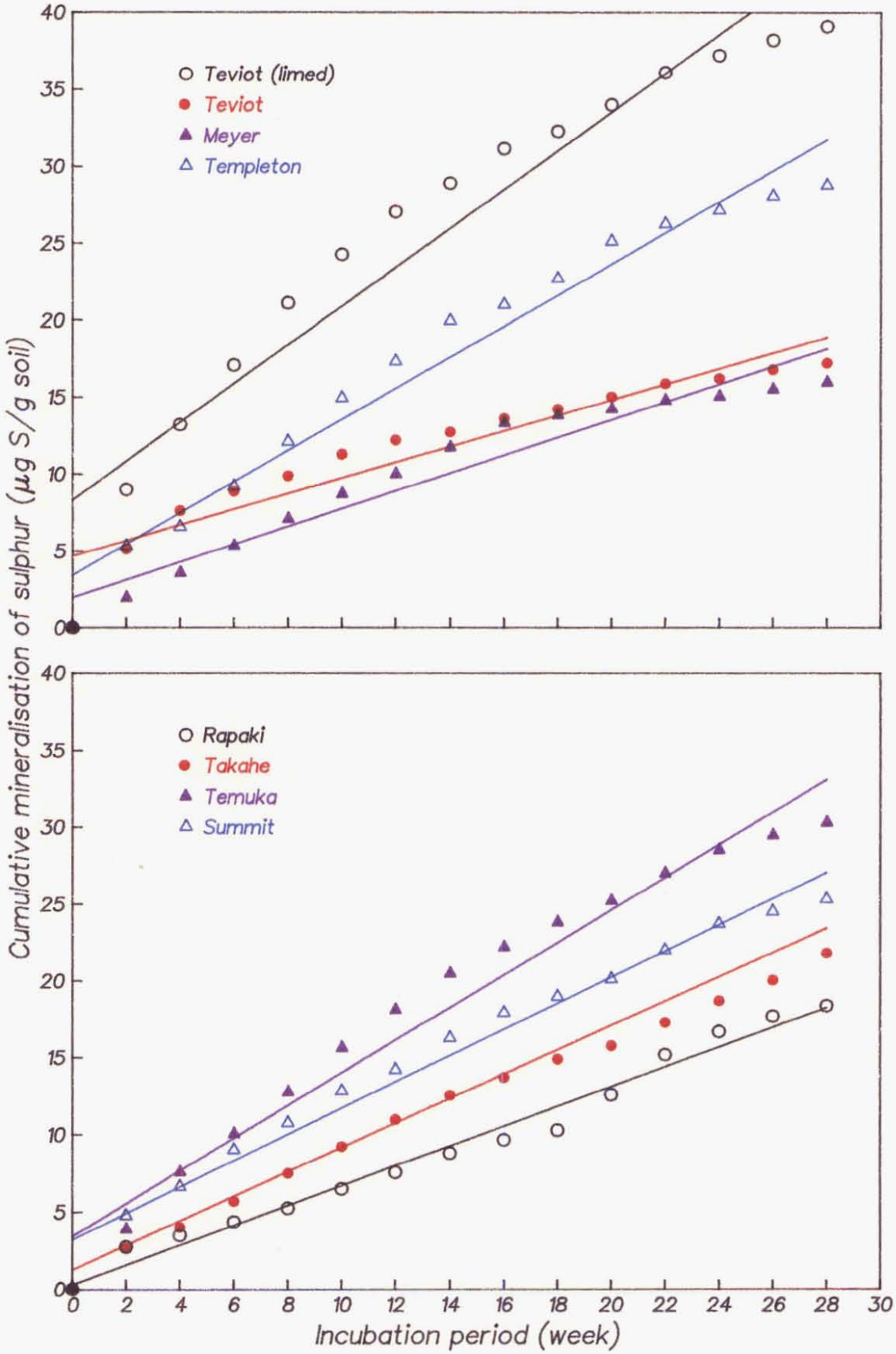


Fig. 4.6 Linear fit lines on the amounts of sulphur mineralised from the soils incubated at 30 °C for 28 weeks.

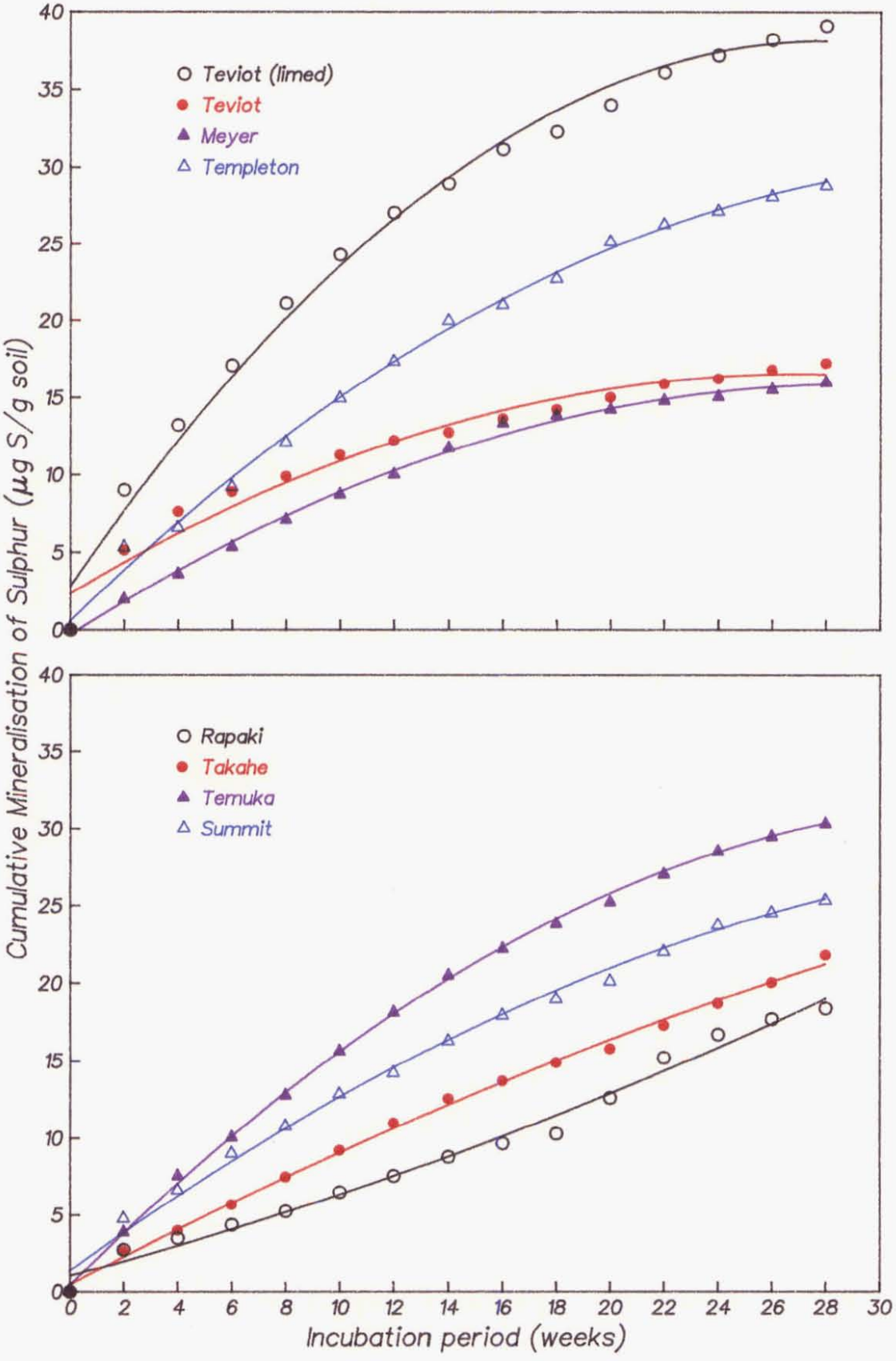


Fig. 4.7 Lines fitted using the quadratic equations. Coordinates are taken from table 4.4.

Table 4.4 The intercepts, slopes and correlation coefficients of the linear fitted lines and quadratic fitted lines to the observed mineralised-SO_4^{2-} in soils.

Soils	linear fits			quadratic fits			
	intercept	slope	r^2	intercept	b	c	r^2
Teviot (limed)	8.32	1.260	0.92	2.81	2.53	-0.0453	0.99
Teviot	4.67	0.507	0.89	2.31	1.05	-0.0194	0.97
Meyer	1.98	0.577	0.94	-0.33	1.11	-0.0191	0.99
Templeton (P)	3.43	1.010	0.97	0.56	1.68	-0.0237	0.99
Rapaki	0.29	0.640	0.98	1.04	0.47	0.0061	0.99
Takahe	1.26	0.790	0.99	0.43	0.93	-0.0067	0.99
Temuka	3.42	1.060	0.96	0.38	1.77	-0.0250	0.99
Summit	3.20	0.852	0.97	1.35	1.28	-0.0152	0.99

All the correlation coefficients (r^2) presented in this table from linear and the quadratic fits are significant at 0.001% level of significance.

of SO_4-S depends on microbial activity, thus fluctuations in their activity will affect the rate of sulphur mineralisation in soils. In this present study, the rate of sulphur mineralisation decreased with an increase in the incubation period. With the exception of the Rapaki soil, none of the soils showed a linear relationship between the mineralised-SO_4^{2-} and the incubation period as has been reported by Tabatabai and Al-Khafaji (1980). A linear model (linear regression) fitted to the mineralisation of sulphur with time gave high correlation coefficients (Table 4.4) but did not explain the true nature of the curves (Fig 4.7). For most soils, the linear model predicted significant amounts of mineralisation at time zero (large intercept values, see Table 4.4). Fitting a non-linear model (quadratic), showed much better agreement between the observed and calculated values (Fig 4.7). Intercept values were much lower than with the linear model (Table 4.4). The decline in the rate of sulphur mineralisation with increasing incubation period could possibly be the result of a reduction in microbial activity caused by the depletion of easily metabolisable source of carbon. In the early periods of incubation, micro-organisms would have utilised easily metabolisable source of carbon rapidly and as a result of that more sulphur was mineralised in the first four weeks of incubation. As the time of incubation increased, the amount of easily metabolisable source of carbon in soil system decreased and hence the rate of sulphur mineralisation also decreased.

4.5 SULPHUR MINERALISATION IN NEW ZEALAND SOILS

4.5.1 Method of comparing sulphur mineralisation in soils

The open system of incubation as described in section 4.2.4 was used to measure mineralisable sulphur in soils. The open incubation system was selected for three main reasons:

(a) the results obtained by this system were reproducible and consistent

(b) the method when used in the preliminary studies showed significant differences in the amounts of sulphur mineralised by different soils and

(c) the method more closely simulates field conditions where mineralised sulphur is removed frequently by plants or lost by leaching.

Soils were preconditioned at 20 °C and 75% FC moisture level for two weeks before incubation. This was to avoid a sudden flush of mineralisation in the early stages of incubation which has been found in the closed system incubation. Soil columns were incubated at 30 °C for 10 weeks and mineralised-sulphate was removed as described in section 4.2.1.3 The duration of the incubation and the temperature were selected because mineralisation of sulphur up to 10 weeks was approximately linear and thus easier to compare between soils, and at 30 °C levels of sulphur mineralisation are significantly higher than at lower temperatures and therefore easier to determine. A 10 week period of incubation was also preferred because it was known that other nutrients such as Ca^{2+},Mg^{2+} and K^{+} did not limit sulphur mineralisation during this period (see section 4.3.2).

4.5.2 Sulphur mineralisation in soils

A total of eighteen soils were used to compare the sulphur mineralisation in New Zealand soils. There was considerable variation in the rates of sulphur mineralisation measured for the different soils. Teviot (limed) soil showed the highest level of mineralisation during the 10 week incubation period and the lowest level of mineralisation was recorded in the Waimakariri soil. The amount of mineralised-SO_4^{-2} in these soils were 26.78 and 2.86 μg sulphur g^{-1} soil respectively (Table 4.5), showing a ten-fold difference between the maximum and the minimum. After the first two weeks, the cumulative mineralised-SO_4^{2-} increased approximately at a linear rate (Fig. 4.5). In some soils such as the Rapaki, Selwyn and Wairaki the rate of sulphur mineralisation was higher in the first two weeks than at the later stages of the incubation. Some soils e.g. Horotiu and Mokotua showed a fall off in the mineralisation after 6 weeks (Fig. 4.5). The cumulative mineralisation of sulphur showed highly significant correlation coefficient values ($P \leq 0.001$ level of significance) when regressed linearly with the incubation period.

In the Rapaki, Summit and Horotiu soils, the amounts of mineralised sulphur retained as adsorbed sulphate were considerably higher than in the other soils. To estimate the total sulphur mineralisation, the amount of adsorbed-S was included in the mineralised-sulphur pool. In the Rapaki soil, the amounts of adsorbed sulphate was higher than measured in the $CaCl_2$ leachates (Table 4.5). If only the leachate values, presented in Table 4.5 were

Table 4.5 Sulphur mineralisation in soils during the 10 week incubation at 30 °C.

Soil series	Mineralised sulphur	Adsorbed sulphur	Total S mineralised
	(μg S g^{-1} soil)		
Teviot (limed)	24.28	2.50	26.78
Teviot	13.40	2.86	16.26
Meyer	7.69	0.31	8.00
Wairaki	13.16	1.96	15.12
Te Houka	6.46	5.13	11.59
Temuka	15.61	1.48	17.09
Lismore	5.10	1.16	6.26
Rapaki	6.50	10.37	16.87
Summit	12.81	8.09	20.99
Templeton(P)	14.92	4.62	19.54
Templeton(C)	11.46	3.10	14.56
Selwyn	6.77	1.58	8.35
Mokotua	13.60	3.00	16.60
Takahe	9.24	3.50	12.74
Te Kowhai	11.75	4.16	15.91
Horotiu	15.64	9.36	24.90
Waimakariri	2.40	0.46	2.86
Wakanui	6.60	1.40	8.00

Table 4.6 Amount of sulphur mineralised in soils during the 10 weeks of open system of incubation.

Soil Series	Sulphur mineralised	
	% of T.O.S[1]	(kg ha^{-1})*
Teviot (limed)	6.58	32.16
Teviot	3.50	19.51
Meyer	4.30	9.60
Wairaki	4.14	18.14
Te Houka	2.33	13.90
Temuka	5.57	20.50
Lismore	3.12	7.51
Rapaki	3.38	20.24
Summit	5.57	25.18
Templeton (P)	5.53	23.44
Templeton (C)	3.87	17.47
Selwyn	2.49	10.02
Mokotua	2.68	19.92
Takahe	5.44	15.28
Te Kowhai	2.45	19.09
Horotiu	2.53	29.88
Waimakariri	1.13	3.43
Wakanui	3.42	9.60

1. % of total organic sulphur,

* calculated for the top 10 cm surface soil, assuming the bulk density of soils as 1.20 g cm^{-3}.

recorded, total mineralised sulphur would have been underestimated in the high sulphate-adsorbing soils. Spencer and Freney (1960) have reported that KH_2PO_4 extractable sulphur is a good indicator of plant available sulphur. Thus it seems appropriate to complete the final leaching with KH_2PO_4 solution for the determination of mineralised-SO_4^{2-} which represents plant available sulphur. The mineralisation of sulphur from organic sulphur fractions (T.O.S) was highest in the Teviot (limed) soil which mineralised 6.58% of the T.O.S. The Waimakariri soil showed the lowest amount of mineralisation, only 1.13% of the total organic sulphur being mineralised. Mineralised-S calculated as kg sulphur released ha^{-1} showed that significant amounts of SO_4-S are potentially available for plant uptake in these soils (Table 4.6).

4.6 PREDICTION OF POTENTIALLY MINERALISABLE SULPHUR IN SOIL

In a recent study, Pirela and Tabatabai (1988) reported a method of estimating the potentially mineralisable pool (S_0) of sulphur in soils. On the basis of the cumulative sulphate mineralised over a 14 weeks period, these workers calculated S_0 and also K_f (time taken to mineralise 50% of the S_0) values by using a reciprocal-plot technique. The equation used by Pirela and Tabatabai (1988) to determine these values is as follows;

$$1/S_c = 1/S_0 + K_f/S_0 . 1/t \qquad .....(4.3)$$

where S_c is cumulative mineralised-S at time t, K_f is a constant (K_f= the time required to mineralise 50% of the S_0), when the results are plotted as $1/S_c$ vs 1/t, the intercept on the Y axis gives $1/S_0$ and the slope is equal to K_f/S_0.

In another attempt to calculate S_0, Pirela and Tabatabai (1988) used an exponential equation (4.4). The observed values of mineralised-SO_4^{2-} (S_m) and incubation time (t) are fitted to this equation using an alternative procedure to determine the values of k (a first order rate constant).

$$S_m = S_0 (1 - e^{-kt}) \qquad .....(4.4)$$

Table 4.7 Comparison between calculated values for potentially mineralisable sulphur (S_0) and k_t (time in weeks needed to mineralise 50% of the S_0) obtained using the reciprocal-plot and exponential fit techniques (10 weeks incubation).

Soil series	S_{m10}*	Reciprocal-plot technique		Exponential equation		
		S_0∇	K_tΔ	S_0	k¶	K_t
Teviot (limed)	26.78	36.23	6.2	31.69	0.1388	5.0
Teviot	16.26	19.97	5.8	16.10	0.1648	4.2
Meyer	8.00	67.11	74.5	18.65	0.0525	13.2
Wairaki	15.12	27.24	10.0	16.04	0.1705	4.1
Te Houka	11.59	17.24	17.2	11.40	0.0826	8.4
Temuka	17.09	59.52	28.6	35.62	0.5601	1.2
Lismore	6.26	48.07	76.9	10.23	0.0826	8.4
Rapaki	16.87	14.51	4.7	15.64	0.1207	5.7
Summit	20.99	18.18	5.9	17.12	0.1296	5.3
Templeton (P)	19.54	18.69	54.6	29.55	0.0673	10.3
Templeton (C)	14.56	18.52	7.4	15.27	0.1317	5.3
Selwyn	8.35	19.88	17.5	8.99	0.1393	4.9
Mokotua	16.60	52.35	27.0	23.42	0.0878	7.9
Takahe	12.74	18.21	11.9	20.18	0.0594	11.7
Te Kowhai	15.91	102.04	69.8	22.04	0.0766	9.1
Horotiu	24.90	273.97	144.4	27.40	0.0863	8.0
Waimakariri	2.86	4.16	9.4	4.54	0.0738	9.4
Wakanui	8.00	16.42	16.4	14.22	0.0614	11.3

* total mineralised-SO_4^{2-} (μg S g-1 soil) during the 10 week incubation period.

∇ amount of potentially mineralisable sulphur ($\mu g\ g^{-1}$ soil).

Δ time required (weeks) to mineralise 50% of the S_0.

¶ sulphur mineralisation rate constant.

Although Pirela and Tabatabai did not attempt to calculate K_t for the S_0 values calculated from the exponential equation (4.4), it can be calculated from the value of K equation 4.5 (for derivation of equation 4.5 see Appendix 1).

$$K_t = \frac{0.69315}{k} \qquad \text{.....(4.5)}$$

The use of equations 4.3 and 4.4 for predicting potentially mineralisable sulphur was examined for the soils in this present study. Data for both the 10 week incubation (Fig. 4.8) and 28 week incubation (Fig. 4.7) periods were used.

Calculated values for S_0 and K_t are shown in table 4.7. The S_0 values calculated from the 10 week incubation data by using the reciprocal-plot technique showed a wide variation in the amount of potentially mineralisable sulphur present in the soils. The minimum amount of S_0 (4.16 $\mu g\ g^{-1}$ soil) was calculated for the Waimakariri soil and the maximum amount of S_0 (273.97 $\mu g\ g^{-1}$ soil) was calculated for the Horotiu soil (Table 4.7). The time required to mineralise 50% of the calculated S_0 in the soils ranged from 4.7 in the Rapaki soil to 144.4 weeks in the Horotiu soil.

Using the exponential equation (4.4), the calculated S_0 values were generally lower than those calculated by the reciprocal-plot technique, equation (4.3). These differences were particularly obvious in the Meyer, Te Kowhai and Horotiu soils where the use of the reciprocal-plot technique estimated S_0 as 67.11, 102.04 and 273.97 μg sulphur g^{-1} soil respectively. In contrast, use of the exponential equation gave S_0 values of 18.65, 22.04 and 27.40 μg sulphur g^{-1} soil respectively. Also the K_t values calculated by equation 4.5 were relatively lower than those calculated by the reciprocal-plot technique, the highest value being 13.2 weeks. Most soil showed that 50% of the S_0 could be mineralised within 13 weeks. The k values calculated for different soils reported in Table 4.7 refer to the rate constant in equation 4.4. The higher the k value the higher is the rate of sulphur mineralisation.

4.6.1 Effect of incubation period and incubation temperature on S_0 and K_t

The quantitative values of potentially mineralisable sulphur and the rate constant as calculated amount of sulphur calculated by the Pirela and Tabatabai (1988) method could provide valuable information for estimating fertilizer sulphur requirements, possibly by incorporating these values into the fertilizer model. However, if the S_0 value is true measure of the potentially mineralisable sulphur in the soil, it should be independent of the incubation conditions used for obtaining the data from which it is calculated. If calculated values for S_0 change significantly by altering the temperature or period of incubation then the usefulness of such an estimation can be questioned.

Use of the reciprocal-plot technique equation and the exponential equation on the cumulative mineralisation observed during a 28 week incubation (section 4.4.2) gave significantly different S_0 and the K_t values than those calculated from 10 week incubation data (Table 4.8a and 4.8b). For some soils S_0 values calculated from the 28 weeks data were significantly higher and in other soils significantly lower than those calculated using the 10 week data. For example, S_0 for the Meyer soil was calculated to be 67.11 using 10 week data and only 47.87 $\mu g\ g^{-1}$ soil using 28 week data. In the Takahe soil calculated S_0 values increased from 18.21 to 33.33 $\mu g\ g^{-1}$ soil by prolonging the incubation period. Similar changes were recorded in the K_t values (Table 4.8a and 4.8b).

Potentially mineralisable sulphur and K_t were calculated using the reciprocal-plot technique and half-life equations (4.3,4.4 and 4.5) on the cumulative mineralisation data obtained at 10, 20 and 30 °C (data shown in Fig. 4.4). Calculations were carried out using only the cumulative mineralisation of sulphur occurring during the first 10 week of incubation. Calculated S_0 values at different temperature are shown in Table 4.9a. There is a considerable variation in the calculated values for both as potentially mineralisable sulphur (S_0) and also the time required (K_t) to mineralise 50% of the S_0. Some of the results calculated by reciprocal-plot technique have given negative S_0 values. For example, in the Temuka and Teviot (limed) soils, the calculated S_0 values are -48.78 and -25.44 μg sulphur g^{-1} soil respectively which would suggest that at 10 °C, these soil would show immobilisation, which is not true. These results show the shortcomings of the Pirela and

Table 4.8a Effect of incubation period on the calculation of S_0 (μg S g^{-1} soil) and K_t (week) by the reciprocal plot technique.

Soils	period of incubation			
	$S_0\nabla$	$K_t\Delta$	$S_0\nabla$	$K_t\Delta$
	10 weeks		**28 weeks**	
Teviot (limed)	36.23	6.2	47.62	9.1
Teviot	19.97	5.8	18.52	5.5
Mayer	67.11	74.5	47.84	47.4
Templeton (p)	18.69	54.6	35.84	13.1
Takahe	18.21	11.9	33.33	24.7
Temuka	59.52	78.6	65.36	31.7
Summit	18.18	5.9	29.41	13.3

∇ amount of potentially mineralisable sulphur (μg S g-1 soil).

Δ time required (weeks) to mineralised 50% of the S_0.

Table 4.8b Effect of incubation period on the calculation of S_0 (μg S g^{-1} soil) and K_t (weeks) by the exponential equation.

Soils	period of incubation					
	S_0∇	k¶	K_tΔ	S_0∇	k¶	K_tΔ
	10 weeks			28 weeks		
Teviot (limed)	31.69	0.1380	6.2	42.14	0.0864	8.1
Teviot	16.10	0.1648	4.2	17.04	0.1139	6.1
Mayer	18.65	0.0525	13.2	20.64	0.0564	12.3
Templeton (P)	29.55	0.0673	10.3	39.60	0.0479	14.5
Takahe	20.18	0.0594	11.7	44.95	0.0226	30.5
Temuka	35.62	0.561	1.2	41.45	0.04759	14.6
Summit	17.12	0.1296	5.3	42.4	0.0469	14.8

∇ potentially mineralisable sulphur.

Δ time required to mineralise 50% of the S_0.

¶ is rate constant for sulphur mineralisation.

Table 4.9a Effect of incubation temperature on the calculation of S_0 (μg S g^{-1} soil) and K_t (weeks) by the reciprocal plot technique.

Soils	Incubation temperature					
	10 °C		20 °C		30 °C	
	$S_0\nabla$	$K_t\Delta$	$S_0\nabla$	$K_t\Delta$	$S_0\nabla$	$K_t\Delta$
Temuka	-48.78	103.9	24.21	9.3	59.52	7.8
Lismore	12.61	17.7	105.82	137.9	48.07	76.9
Rapaki	6.71	11.7	16.67	29.0	7.52	3.7
Teviot (limed)	-25.44	21.5	34.48	18.9	36.23	6.2

∇ amount of potentially mineralisable sulphur (μg S g^{-1} soil).

Δ time require (weeks) to mineralised 50% of the S_0.

Table 4.9b Effect of incubation temperature on the calculation of S_0 (μg S g^{-1} soil) and K_t (weeks) by the exponential equation.

Soils	Incubation temperature								
	10 °C			20 °C			30 °C		
	S_0	k	K_t	S_0∇	k¶	K_tΔ	S_0∇	k¶	K_tΔ
Temuka	4.68	0.1778	3.90	17.75	0.1280	5.40	35.63	0.0566	12.23
Lismore	15.72	0.0375	18.48	27.91	0.0285	24.32	10.23	0.0696	9.96
Rapaki	3.11	0.2254	3.07	8.04	0.1091	6.35	7.64	0.1600	4.33
Teviot (limed)	12.45	0.1840	3.76	32.49	0.0625	11.09	31.69	0.1388	4.99

∇ amount of potentially mineralisable sulphur (μg S g-1 soil).

Δ time required to mineralise 50% of the S_0.

¶ rate contant for sulphur mineralisation.

Tabatabai (1988) methods of assessing S_0. Results from this study have shown that an increase in temperature increases the sulphur mineralisation in soil (Fig. 4.4). Therefore an increase in temperature should reduce the time required to mineralise 50% of the potentially mineralisable sulphur. Use of reciprocal-plot technique to calculate K_t often showed a reverse trend (Table 4.9a).

Use of exponential equation to calculate S_0 and K_t values showed similar variation between temperatures as use of reciprocal-plot technique. However, use of this equation (4.4) did not produce any negative S_0 values (Table 4.9b). Results from this study shows that potentially mineralisable sulphur and K_t values calculated by equations 4.3, 4.4 and 4.5 give variable estimates depending on the duration and the temperature of incubation used to obtain the data from which calculations are carried out. Therefore, such an approach to estimate the S_0 is likely to give inconsistent results in the assessment of mineralisable sulphur in soil and hence it would be inappropriate to incorporate such figures in a fertilizer recommendation scheme. On the basis of the above comparisons of S_0 and K_t, it is suggested that the prediction of the potentially mineralisable sulphur and the time taken to mineralise 50% of S_0 by either of the procedures suggested by Pirela and Tabatabai (1988) is unsatisfactory.

4.7 THE EFFECT OF SOIL CHEMICAL PROPERTIES ON SULPHUR MINERALISATION

The variation in the level of sulphur mineralisation between soils could be due to several reasons;

(a) lack of organic sulphur in soils which limits the availability of organic sulphur which could be mineralised,

(b) presence of sulphur in organic forms not susceptible to microbial degradation,

(c) variation in soil physical conditions may influence microbial activity of soils and

(d) variations in the relative availability of other nutrients (including C) which influence the microbial activity.

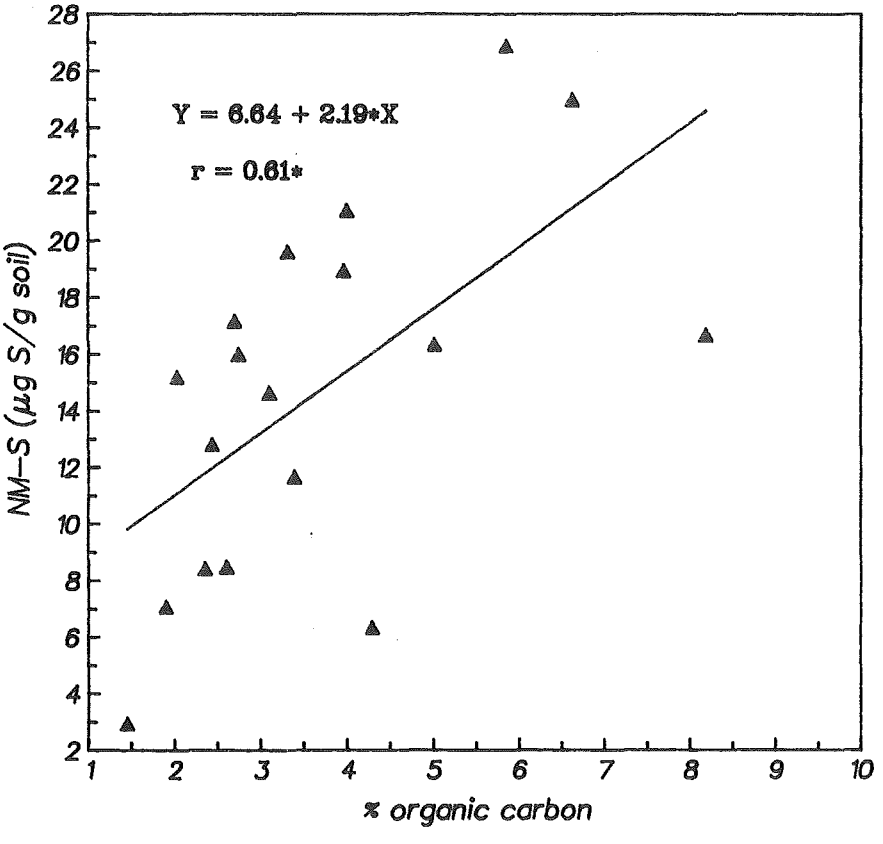
In these incubation studies, physical factors such as moisture, temperature and aeration were maintained at a constant level throughout the incubation period for all soils. Thus,

the variation in mineralised-SO_4 observed in this study would have been caused mainly by the soil chemical properties which varied from soil to soil (Table 3.2 and 3.3).

Therefore, possible relationships between the net mineralised-S (NM-S) and various individual soil chemical properties were examined by means of statistical correlation (Table 4.10). The purpose of carrying out such statistical tests was to identify any soil properties which might be useful in predicting the likely mineralisation of sulphur in soil. Net mineralised-sulphate was poorly correlated with organic carbon, KH_2PO_4 extractable-sulphur (P-SO_4), water-soluble sulphur (W-SO_4), HI-reducible S, soil pH and the soil N:S ratio in soils (Figures 4.8, 4.9, 4.12a and 4.11). Slightly better correlation were obtained between NM-S and total sulphur or total organic sulphur contents in soils (Fig. 4.10). The best correlation was obtained between NM-S and C-bonded sulphur in soils (4.12b).

The correlation between the NM-S and soil organic carbon content are able to explain up to 36% of the variability. This relationship is largely due to the fact that organic carbon is related to the level of various types of sulphur in soils and particularly with organic sulphur from where sulphur in mineralised. The correlation coefficients between the NM-S and W-SO_4 and P-SO_4 were 0.59 and 0.55 respectively which accounted for between 30 and 34% of the variability in mineralised-sulphate between soils. Thus, these two factors are not good predictors for estimating mineralisable sulphur in soils. However, the amounts of sulphate held in these forms at any given time may give some indication about the balance between mineralisation and immobilisation of sulphur in the soil. High amounts of sulphate in soils would indicate that the process of mineralisation was more dominant.

A negative relationship between the NM-S and soil pH suggests that the amount of mineralisable-S decreases when the soil pH increases. A similar relationship has been reported by Tabatabai and Al-Khafaji (1980) and Pirela and Tabatabai (1988), who also using an open incubation system, found that the level of sulphur mineralisation in Iowa and Chilian soils was negatively related to soil pH. The reason for the significant negative correlation with pH in this present study is most likely due to the relationship between soil pH and organic carbon (Table 4.10), *viz.*, organic carbon (and organic sulphur) decreasing with an increase in soil pH. Thus more organic sulphur would have been available for mineralisation in low pH soils than in high pH soils. When NM-S was partially



Figur 4.8 The relationship between the net mineralised–S (NM–S) and organic carbon (O.C) in soils.

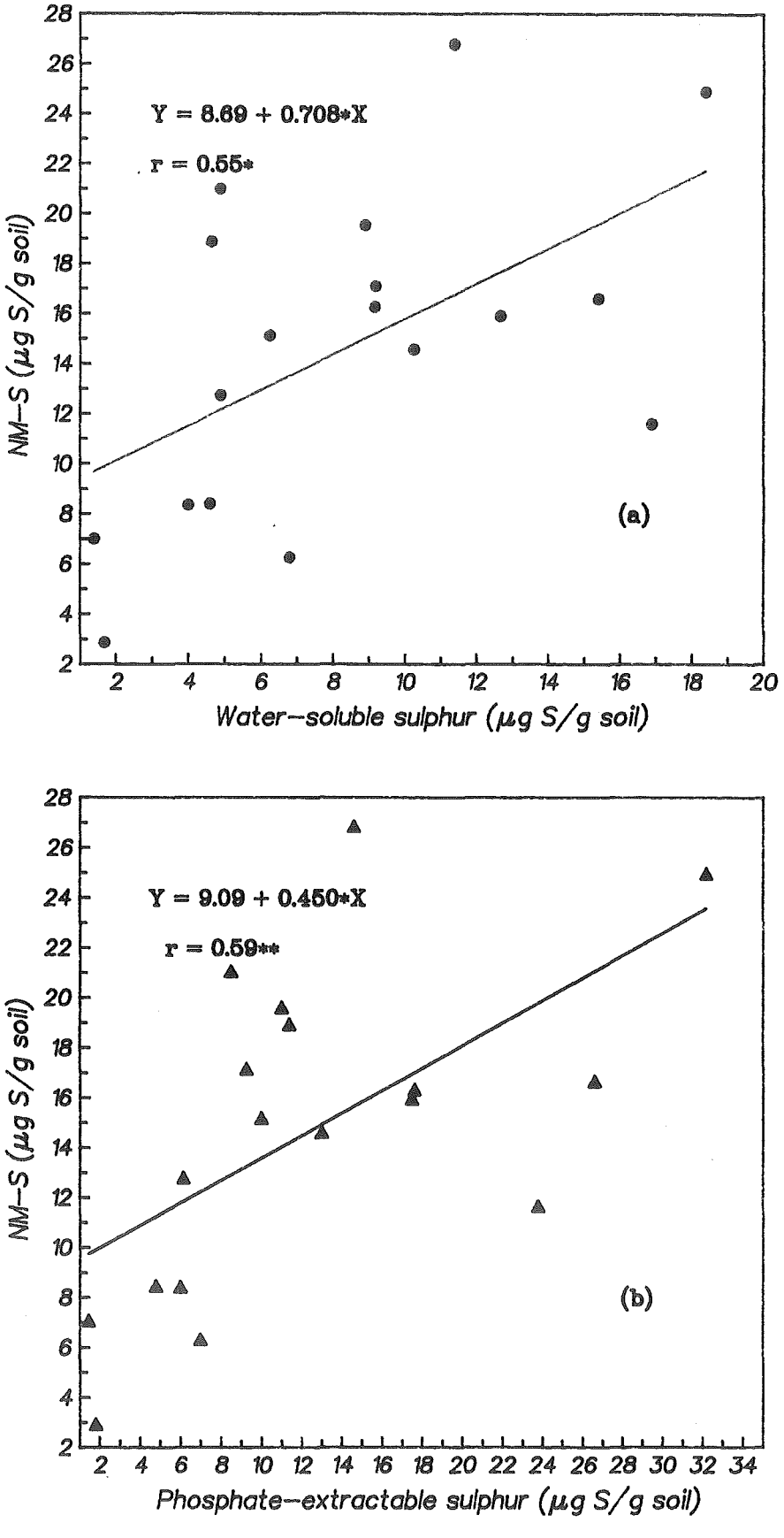


Fig. 4.9 The relationship between the net mineralised–S (NM–S) and (a) water soluble sulphur and (b) phosphate–extractable sulphur.

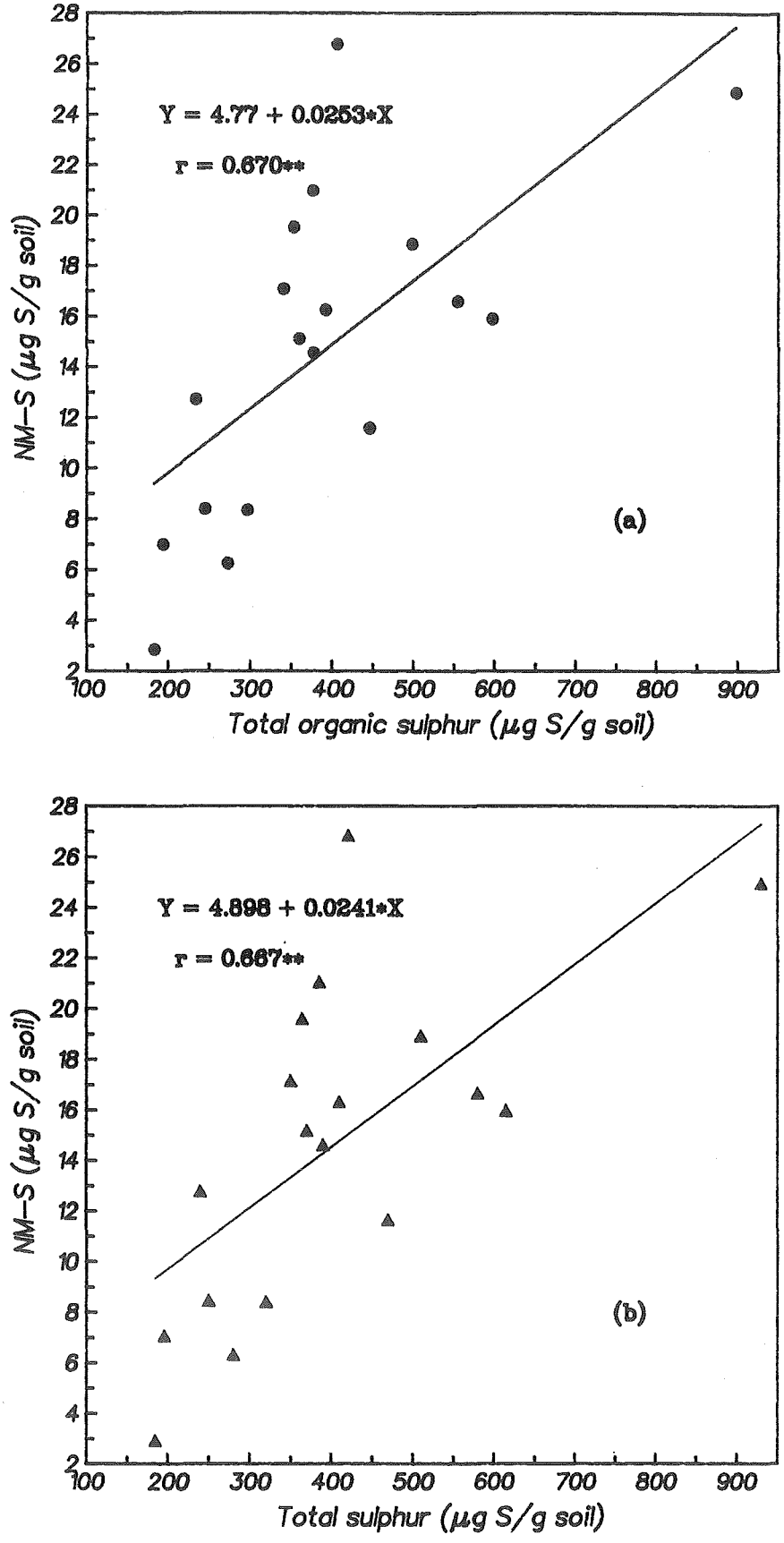


Fig. 4.10 The relationship between the net mineralised–S (NM–S) and (a) total organic sulphur and (b) total sulphur in soils.

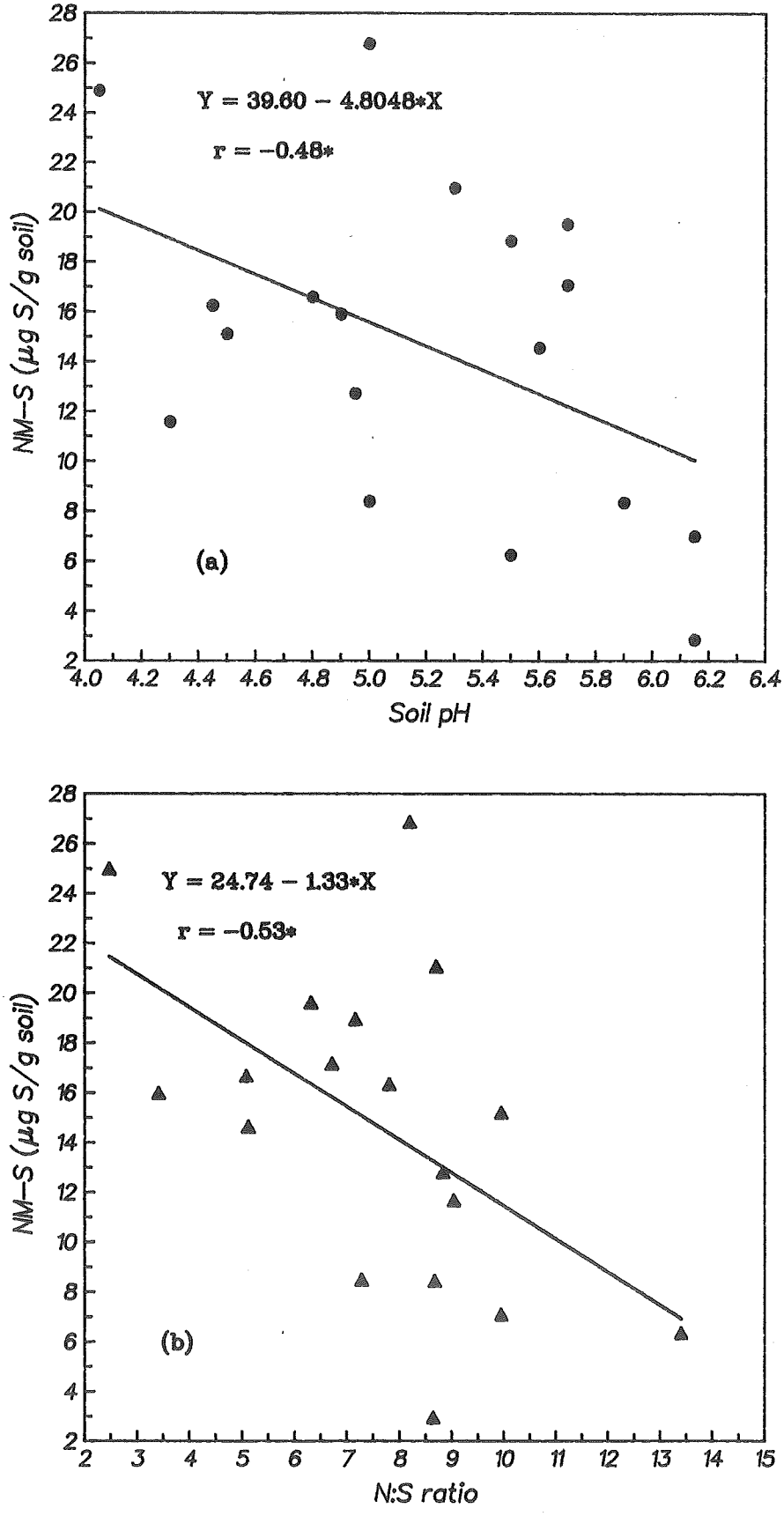


Fig 4.11 The relationship between the net mineralised–S (NM–S) and (a) soil pH , (b) N:S ratio in soils.

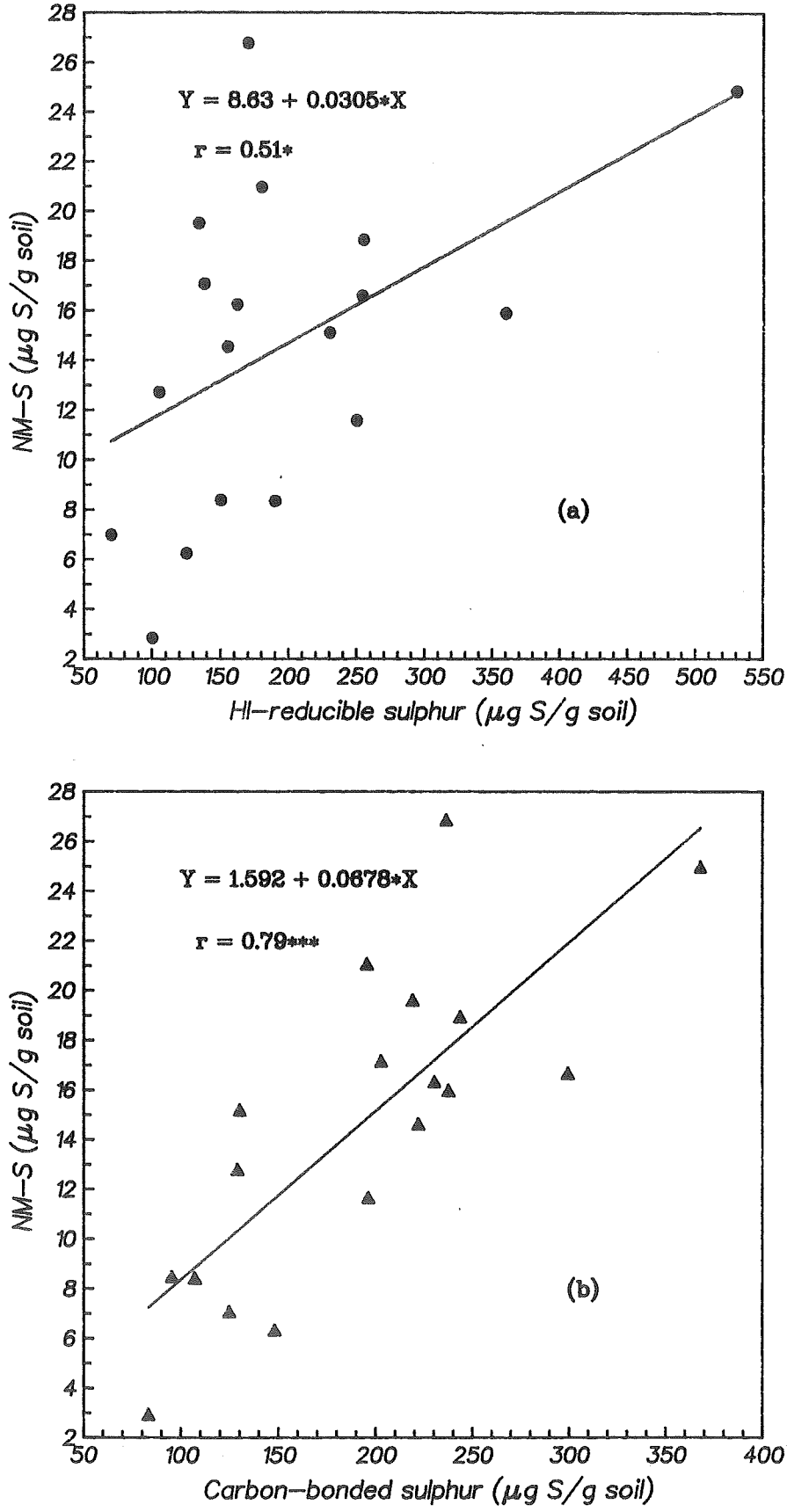


Fig 4.12 The relationship between the net mineralised–S (NM–S) and (a) HI–reducible sulphur, (b) carbon–bonded sulphur

Table 4.10 Correlation coefficients (r) between soil properties and the amount of net mineralised S in soils.

Soil Properties	NM-S	T.O.S	N:S	C:N	C:S	pH	C-S	HI-S	P-SO_4	W-SO_4	T.S	T.N	O.C
Net mineralised-SO_4	1.00	0.67**	-0.53*	0.47	-0.03	-0.48*	0.79***	0.51*	0.59**	0.55*	0.67**	0.28	0.60**
Total organic sulphur		1.00	-0.73***	0.70**	0.22	-0.65**	0.89***	0.95***	0.89***	0.80***	0.99***	0.17	0.66**
N:S ratio			1.00	0.67**	0.17	0.30	-0.69**	-0.65**	-0.61**	-0.58*	-0.72***	0.42	-0.36
C:N ratio				1.00	0.34	-0.41	0.79***	0.55*	0.72***	0.69**	0.69**	-0.16	0.85***
C:S ratio					1.00	-0.02	0.05	-0.38	-0.02	-0.02	-0.22	0.25	0.54*
Soil pH						1.00	-0.53*	-0.65**	-0.77***	-0.71***	-0.64**	-0.41	-0.51*
C-bonded sulphur							1.00	0.70**	0.87***	0.80***	0.89***	0.20	0.80***
HI-reducible sulphur								1.00	0.79***	0.70**	0.95***	0.13	0.49*
Phosphate-extractable SO_4									1.00	0.95***	0.90***	0.31	0.77***
Water-soluble SO_4										1.00	0.81***	0.27	0.67**
Total sulphur											1.000	0.18	0.67**
Total Nitrogen												1.00	0.35
Organic Carbon													1.00

* significant at $P < 0.05$

** significant at $P < 0.01$

*** significant at $P < 0.001$

correlated with soil pH maintaining the effect of organic carbon constant, the relationship was non-significant (r=0.301). This would tend to confirm that the negative effect of pH on NM-S is due to its correlation with organic carbon. It has been found that liming of acidic soil increases sulphate-S in the soil solution (Williams and Steinbergs, 1962; Williams, 1967). Certainly, this was evident for the Teviot soil where liming increased the sulphur mineralisation substantially (see Fig 4.11a).

The NM-S also showed a significant negative correlation with the N:S ratio at the 0.05% level of significance. This indicates that sulphur mineralisation decreases with an increase in N:S ratio. Kowalenko and Lowe (1975b) have also observed a decrease in the level of sulphur mineralisation with an increased N:S ratio of the soil. The presence of high N would encourage microbial growth which would immobilise sulphur for the synthesis of S-amino acids. Thus a large proportion of any mineralised sulphur may be reconsumed by the soil microorganisms leaving less sulphur as inorganic sulphate-S.

There are considerable disagreements as to whether the relative amounts of carbon, nitrogen and sulphur in the soil determine the level of sulphur mineralisation. In light of evidence presented by previous workers it is difficult to associate the level of sulphur mineralisation with the mineralisation of other nutrients (Swift , 1977; Maynard *et al.*, 1983). Therefore, its not surprising that soil properties such as % organic carbon, % total nitrogen, C:S and C:N ratios do not show any significant correlation with the level of sulphur mineralisation. In some studies, release of sulphur from organic fractions has been slower than the nitrogen (Haque and Walmsley, 1972; Kowalenko and Lowe 1975b, Swift, 1977) while other studies have found faster release of sulphur in comparison with nitrogen (Nelson, 1964; Tabatabai and Al-Khafaji, 1980). Saggar *et al.* (1981b) have reported a different observation: sulphur was immobilised at the same time as N was mineralised from the soil. This suggests that N and sulphur tend not to be associated with the same organic molecules (Maynard *et al.*, 1983). The results from this study and the cases reported above cast doubt on using the relative proportion of C,N and sulphur as a predictive parameters for sulphur mineralisation in soils.

The correlations between NM-S and total sulphur (T.S) and total organic sulphur (T.O.S) were significant at the 0.01 level of significance despite only accounting for approximately 45% of the variation in mineralised-sulphate between soils.

The mineralised-sulphate and the C-bonded sulphur fraction showed the strongest correlation (r 0.786, significant at $P<0.001$). In effect, the amount of C-bonded sulphur in soils can account for 62% of the variation in mineralised-sulphate between different soils. The regression equation for this relationship is (see Fig. 4.12b):

Mineralised-S = 1.592 + 0.0678 Carbon-bonded S ..(4.6)

The positive correlation between NM-S and C-bonded sulphur means soils containing high amounts of C-S are likely to mineralise more sulphur. Since this fraction of sulphur is bonded to carbon, it is highly correlated to the organic carbon status of the soil (Table 4.10). The consumption of organic carbon by soil microorganisms will lead to the release of sulphur, which is then oxidised to SO_4^{2-}-S. It has been shown in some glasshouse and field experiments that the mineralisation of carbon-bonded sulphur was greater than from sulphur present in HI-reducible forms (Freney *et al.*, 1975; McLaren and Swift, 1977; McLachlan and DeMarco, 1975). Freney *et al.* (1975) attempted to identify the mineralisable fraction of soil organic sulphur and found that plants consumed about 60% of recently formed organic sulphur from the carbon-bonded sulphur fraction and 40% from HI-reducible forms of sulphur. Similarly, pasture soils when continuously cultivated for cropping, showed a significant net loss in the amount of organic sulphur (McLaren and Swift, 1977). The loss was mainly from carbon-bonded forms of sulphur which accounted for 75% of the total loss while the remaining 25% loss was from HI-S. In conclusion, since C-bonded sulphur accounted for the highest amount of variability (over 60%) in mineralised-SO_4^{2-} between soils. It could be used as a a relatively good predictor for assessing the amount of sulphur mineralised in soils.

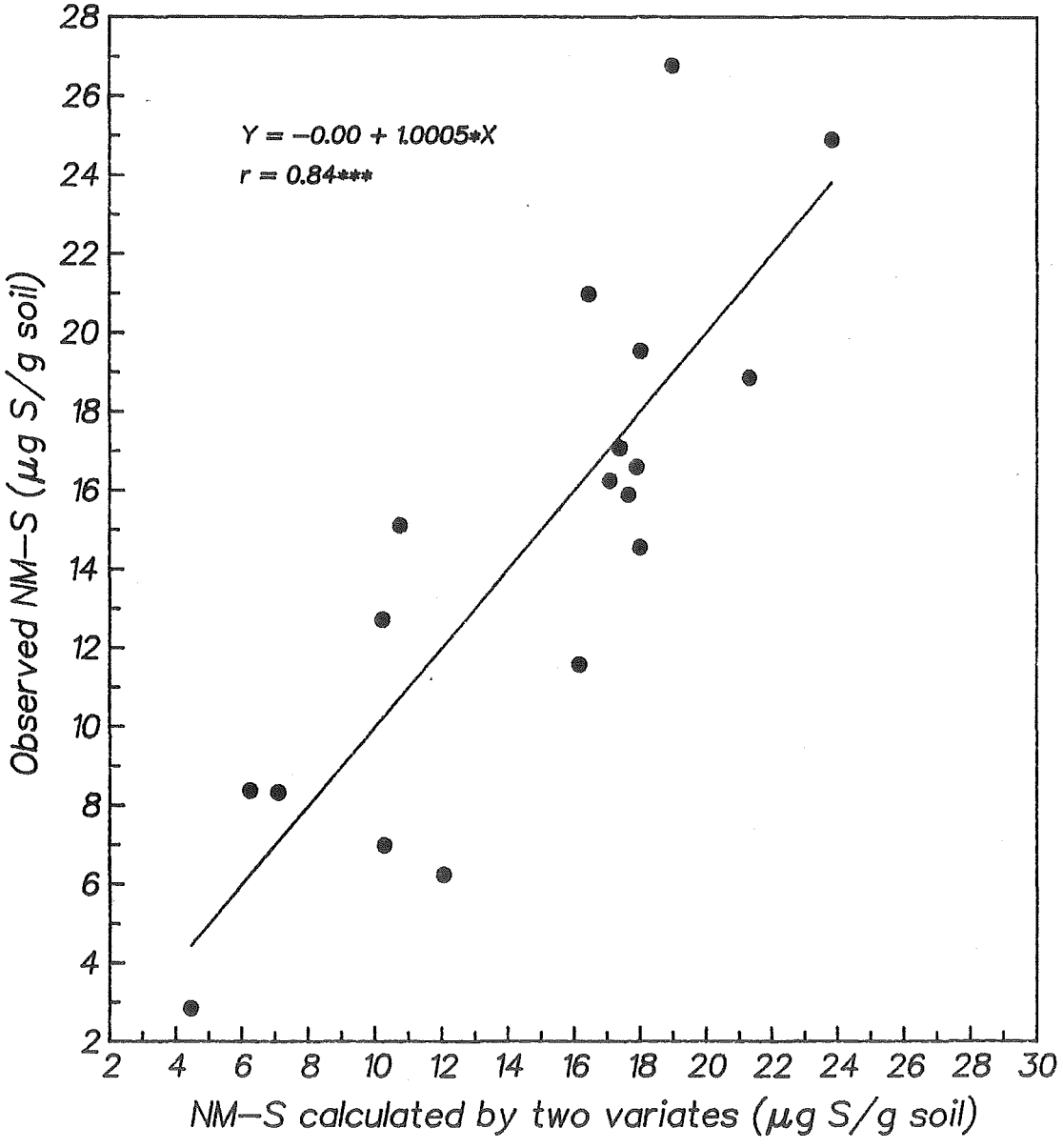


Fig. 4.13 The relationship between the observed net mineralised–S (NM–S) and the calculated mineralised–S by using the regression equation 4.7.

The single factor analysis has shown that a number of soil chemical properties could account for between 2-62% of the total variation in mineralised-SO_4^{2-} between soils. However, given the interrelated nature of these properties it seemed desirable to assess the combined effect of these factors on the level of sulphur mineralisation in soils. An attempt was made to see whether use of multivariate regression would provide an improved prediction of sulphur mineralisation. The only multiple regression to give an improved prediction was a combination of C-bonded sulphur and C:N ratio which accounted for 72% of the overall variation in the mineralised-sulphate (Fig. 4.13). However this is not much improvement over the variation accounted for by C-bonded sulphur alone. The multiple regression equation was ;

Mineralised-S = 1.07 - 0.272 C:N ratio + 0.0952 C-bonded S ...(4.7)

It is perhaps not surprising that even the combination of different factors could not account for more than 72% variation in the sulphur mineralisation in soils. Beside chemical variations in soils, there are other factors related to soil micro-organisms ; such as the population density of the micro-organism, type of micro-organisms which are difficult to quantify, which could affect the mineralisation of sulphur. Also the composition of the organic matter in soils would play an important role in the process of sulphur mineralisation in soils.

4.8 CONCLUSIONS

A comparison between the open and closed systems of incubation showed that the pattern of mineralisation in the open system was consistent and reproducible. Also, use of the open system of incubation measured higher levels of sulphur mineralisation compared with a closed system. The amount of mineralised-sulphate obtained by the open system is believed to be a more realistic estimation of the likely release of sulphur from organic fractions under field conditions. The frequent removal of mineralised-sulphate simulates the uptake of sulphate by plants or loss of this ion from surface soils by leaching (Tabatabai and Al-Khafaji, 1980). Therefore this method was adopted to examine the mineralisation of organic sulphur in New Zealand soils.

It is suggested that soils might be classified into different sulphur mineralising groups on the basis the amount of sulphur mineralised during a 10 week incubation period at 30 °C. The soils examined in this study have been provisionally classified into four mineralisation groups; high (>20 μg S g^{-1} soil), medium (15-20 μg S g^{-1} soil), low (10-15 μg S g^{-1} soil) and very low (<10 μg S g^{-1} soil) (Table 4.11). This approach of classification as it stands at present is arbitrary and would need to be tested under field conditions. However, by adopting catogries such as those mentioned above it may be possible to classify soils as high, medium, low and very low S mineralising soils.

There is an urgent need to compare the S mineralising ability of soils measured in the open system with response to plant growth and response to added S under field conditions. If this done then such a procedure would provide a means of classifying which would help in screening of soils which are able to meet the demand of sulphur for plant growth through mineralisational input and also isolate those soils which can not supply enough sulphate-S from the mineralisation process. This will have direct practical use in fertilizer recommendation schemes.

Studies reported in this chapter have shown that the release of sulphur increases proportionally with an increase in temperature from 10-30 °C. Thus measurement of mineralisable sulphur in soils has to be carried out at a constant temperature. The methods of estimating potentially mineralisable sulphur in soils suggested by Pirela and Tabatabai (1988) are not satisfactory because values calculated of S_0 vary depending on the temperature and duration of incubation used to obtain the data from which the calculations are made.

Table 4.11 Classification of sulphur mineralisation in New Zealand soils.

Classes of sulphur mineralisation in soils			
[1]High	[2]Medium	[3]Low	[4]Very Low
1.Teviot (limed)	1.Teviot	1.Te Houka	1.Lismore
2.Horotiu	2.Wairaki	2.Templeton (c)	2.Waimakariri
3.Summit	3.Temuka	3.Takahe	3.Selwyn
	4.Rapaki		4.Meyer
	5.Templeton (P)		5.Wakanui
	6.Mokotua		
	7.Te Kowhai		

1. release of sulphate-S is >20 μg g-1 soil
2. release of sulphate-S is 15-20 μg g-1 soil
3. release of sulphate-S is 10-15 μg g-1 soil
4. release of sulphate-S is 10 μg g^{-1} soil

CHAPTER FIVE

SOURCES OF MINERALISABLE SULPHUR IN SOILS

5.1 INTRODUCTION

The continuous removal of mineralised-SO_4^{2-} from the open incubated soils used to study the effect of temperature on mineralisation (4.2.3), and the soil incubated for a prolonged period at 30 °C (4.4), provided an opportunity to examine the extent to which the various fractions of organic sulphur which contributed to the release of sulphur. This part of the study examines the amount of organic sulphur remaining in HI-reducible and C-bonded forms of sulphur after the incubation. A comparison between the amounts of sulphur held in these fractions of organic sulphur before and after the incubation enables the determination of the sources of sulphur mineralised during the incubation.

5.2 MATERIALS AND METHODS

5.2.1 Separation of soil from soil+glass bead mixture

Following the removal of excess moisture from the soil columns after the last leaching (see section 4.2.1.3), the mixtures of soil+glass beads were removed from the leaching tubes (Plate 5.1a) and air-dried at 20±2 °C for 5-6 hours and then screened through a 2 mm sieve to separate the soil and glass beads (Plate 5.1b). Following the separation, soils were completely air-dried before the analysis.

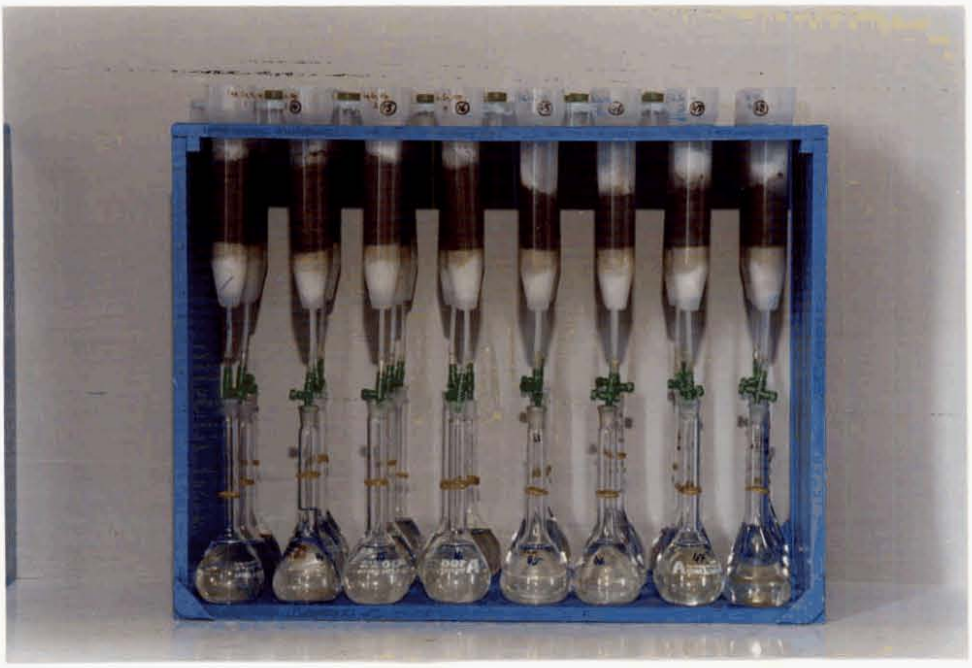


Plate 5.1a The leaching columns used in the open incubation leachates containing the mineralised-SO_4^{2-} were collected in 100 ml volumetric flasks.

Plate 5.1b The separation of soil and glassbeads from the soil columns used for measuring sulphur mineralisation.

5.2.2 Soil analysis

Soils removed from the columns were reanalysed for HI-reducible sulphur and total sulphur using the methods described in section 3.5 and 3.6 respectively. Carbon-bonded sulphur was calculated by subtracting the amount of HI-reducible sulphur from the total sulphur.

5.3 RESULTS AND DISCUSSION

5.3.1 Mineralisable organic sulphur in soils

Mineralised-SO_4^{2-} in the soils could have been derived either from HI-S or C-S alone, or from both forms of organic sulphur simultaneously. Soils incubated at 30 °C for 28 weeks were analysed for total organic sulphur (T.O.S) and HI-S after incubation showed significant losses in the original amounts of T.O.S. Obviously these losses were caused by mineralisation of organic sulphur during the incubation. Losses in T.O.S ranged between 1.9 and 13.5% (Table 5.1). The losses in T.O.S should be equal to the mineralised-sulphate. The differences between these values are due the errors involved in measuring the sulphur in these pools. Generally, the sum of the mineralised-SO_4^{2-} plus the amount of T.O.S after the incubation was close to the amount of T.O.S before the incubation (Table 5.1).

A comparison between the amounts of sulphur held in HI-reducible and C-bonded forms before and after the incubation showed interesting results. Apart from the Teviot (limed) soil, the amounts of sulphur present in HI-reducible forms did not change significantly during the incubation. In the Teviot (limed) soil, the amount of HI-reducible sulphur increased significantly during the incubation. Sulphur in C-bonded forms of sulphur decreased considerably during incubation. Losses in the C-bonded forms of sulphur were particularly large in the Teviot (limed), Rapaki, Temuka and Summit soils (Fig. 5.1).

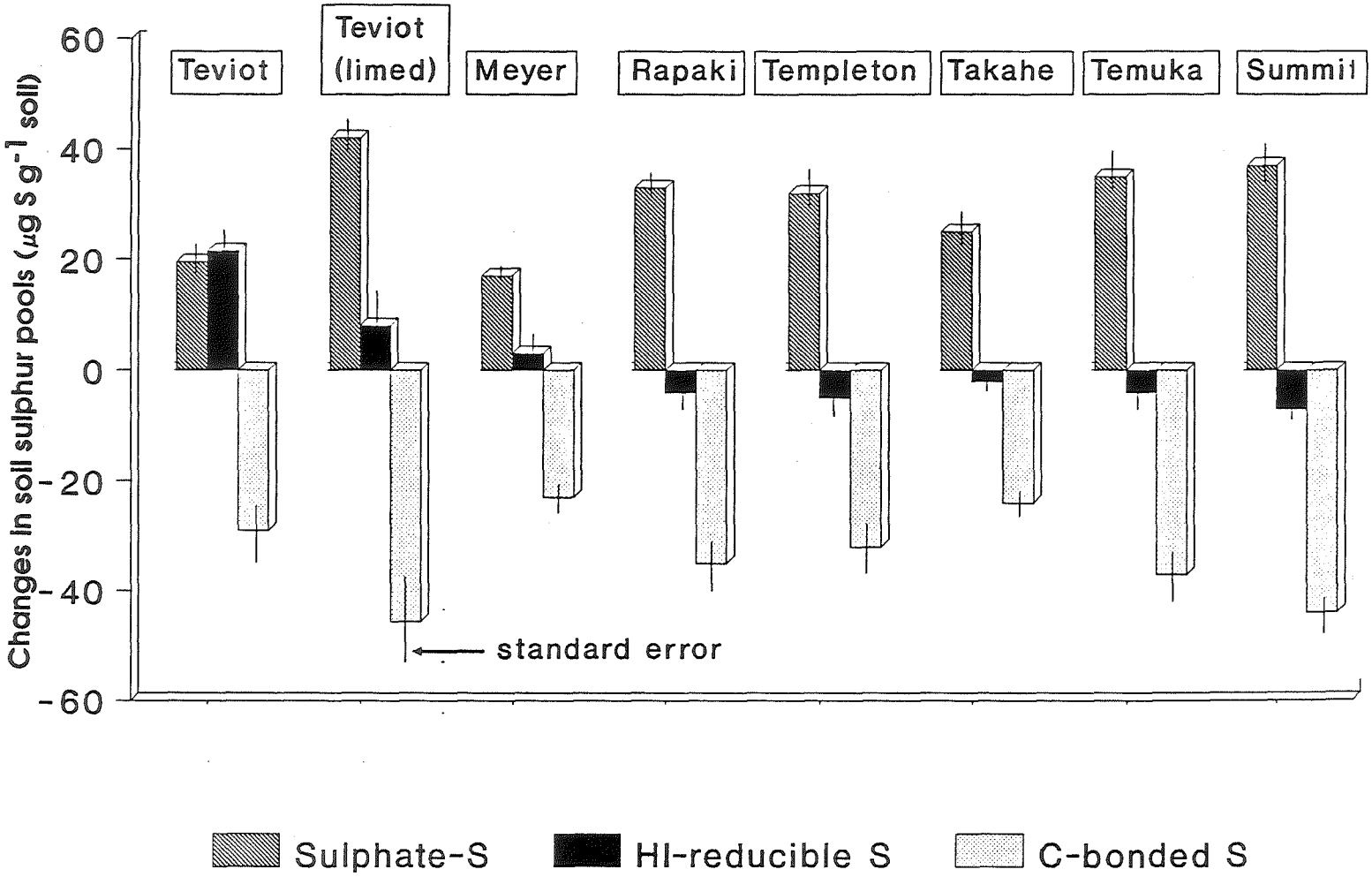


Figure 5.1 Changes in the individual sulphur fractions after 28 weeks of open incubation at 30 °C.

Table 5.1 Gains and losses of sulphur in different pools of sulphur in soils. The balance has been calculated from the sulphate-S, HI-reducible and C-bonded sulphur.

Soil type	Gains and losses in sulphur pools (μg S g^{-1} soil)				Loss in T.O.S	
	Mineralised-SO_4^{2-}	HI-S	C-S	Net balance	μg S g^{-1} soil	% of T.O.S*
Teviot	19.5	21.5	-29.0	12.0	7.5	1.9
Teviot (limed)	42.0	8.0	-45.6	4.4	37.6	9.2
Meyer	17.0	3.0	-23.0	-3.0	20.0	10.3
Rapaki	33.0	-4.0	-35.0	-6.0	39.0	7.8
Templeton	32.0	-5.0	-32.0	-5.0	37.0	10.5
Takahe	25.0	-2.0	-24.0	-2.0	26.0	10.7
Temuka	35.0	-4.0	-37.0	-6.0	41.0	12.0
Summit	37.0	-7	-44.0	-14.0	51.0	13.5

* % of the total organic sulphur reported in Table 3.3.

Clearly the major contribution to mineralised-SO_4^{2-} was from the C-bonded forms of sulphur because the losses from this form of sulphur were higher than from HI-reducible sulphur. Similar pattern of losses were also observed in soils incubated at 10 and 20 °C (shown in Appendix 2 a,b). However, errors involved in measuring different forms of sulphur were significantly larger than those shown in Fig. 5.1.

It is evident from the results that sulphur contained in C-bonded forms decreased during the incubation period. As the mineralisation from the organic forms of sulphur increased, i.e. with increasing temperature, the loss of sulphur in C-bonded forms also increased. The results appear to indicate that the carbon-bonded form of sulphur represent the major source of mineralisable organic sulphur. On average 12-25% of the C-bonded sulphur was lost during incubation (30 °C) but not all of that sulphur was released as sulphate-S; some was transformed into HI-reducible forms. The identification of C-bonded sulphur as the source of mineralisable sulphur agrees well with the earlier finding that it was the best single variate for predicting the likely mineralisation of sulphur in soils (see section 4.7.1). It is interesting to note that during the incubation, HI-reducible sulphur appeared to have made little net contribution towards the mineralised-SO_4^{2-}. However, it may still be possible that the C-bonded sulphur might have passed through HI-reducible forms of sulphur prior to its mineralisation as sulphate.

The results presented here show an unexpected pattern of mineralisation, especially the increase in the HI-reducible forms of sulphur during the incubation period. According to the hypothesis of McGill and Cole (1981), it might have been expected that most of the SO_4^{2-} mineralised from organic forms of sulphur would come from HI-reducible S. It has been suggested by McGill and Cole (1981) that the mineralisation of sulphur occurs by two different pathways, biological and biochemical. According to their hypothesis, biological mineralisation occurs when microbes degrade C-bonded sulphur to attain the C from these compounds and sulphur is released as a by-product of that process. Biochemical mineralisation takes place when the inorganic sulphate levels are too low to fulfill microbial demand for sulphur. The low sulphate levels either activate enzymes or stimulate soil micro-organisms to release extracellular/intracellular enzymes which degrade ester sulphates (HI-S). In this study the removal of inorganic sulphate prior to

incubation would have made the system deficient in free sulphate. If microorganisms needed any sulphur during the incubation period for their protein synthesis, it would seem likely that the enzymatic mechanism to degrade ester sulphates would have been activated. As a result of that, the amount of ester sulphates should have decreased. In contrast, in many instances an increase in HI-reducible sulphur was recorded which largely consists of ester sulphates. The reason for this contradictory result could be that the soil microorganisms were more in need of C than sulphur, and thus they mineralised C-bonded forms of sulphur. The sulphur released as a by-product due to consumption of carbon was sufficient to meet the microbial demands for sulphur, so microbes did not need to utilise HI-reducible forms of sulphur. However, this hypothesis still does not explain the increase in HI-reducible sulphur during the incubation. It is plausible that the original HI-reducible sulphur in soil was not mineralised and at the same time microorganisms utilised a certain proportion of the mineralised-SO_4^{2-} and transformed it to HI-reducible forms of sulphur giving rise to the final concentration of HI-reducible forms of sulphur.

5.3.2 Mineralisation pathways

Possible pathways of organic sulphur mineralisation in soils are presented in Figures (5.2 and 5.3). The thickness of the lines in the figures indicate the most likely transformations from one form of sulphur to another.

The first pathway (Fig. 5.2) suggests that C-bonded forms of sulphur are initially transformed into HI-reducible forms. The HI-reducible sulphur is then enzymatically hydrolysed and released as inorganic sulphate-S. However, there is a lack of evidence to support the direct transformation of C-bonded sulphur to HI-reducible sulphur. Mineralisation studies conducted with individual C-bonded sulphur compounds have not shown any direct transformation to HI-reducible forms of sulphur (Fitzgerald *et al.*, 1983; 1984). These studies, examining the short-term transformation of methionine in soil, found that most of the sulphur in this compound was mineralised to sulphate within 48 hours of incubation. Such findings would appear to support the second pathway of sulphur mineralisation (Fig. 5.3), which suggests that C-bonded sulphur is mineralised

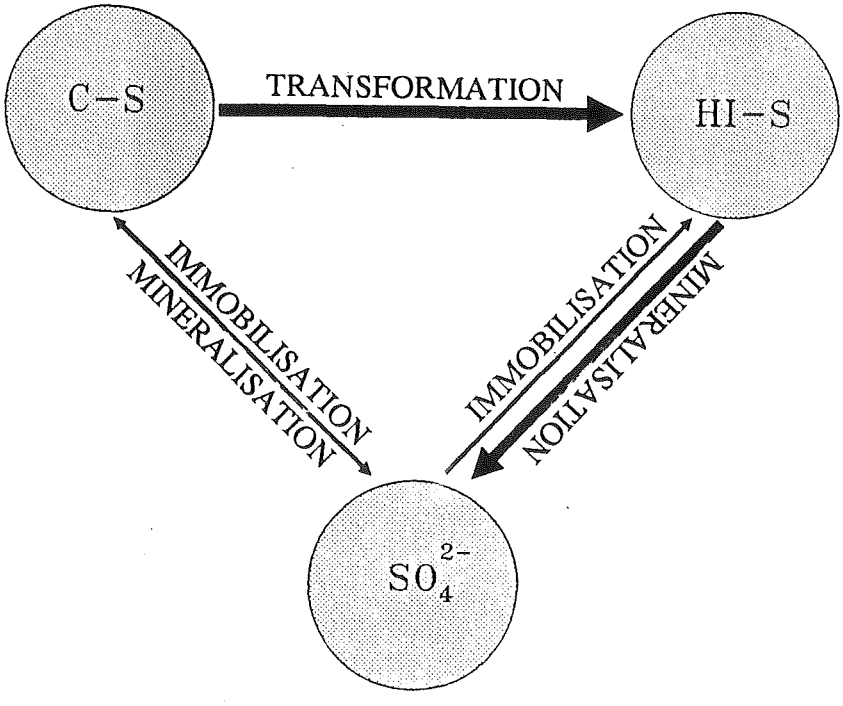


Figure 5.2 Proposed pathway of sulphur mineralisation during the open incubation of soils. The C-bonded forms of sulphur may have been transformed to HI-reducible forms of sulphur prior to being mineralised as sulphate-S..

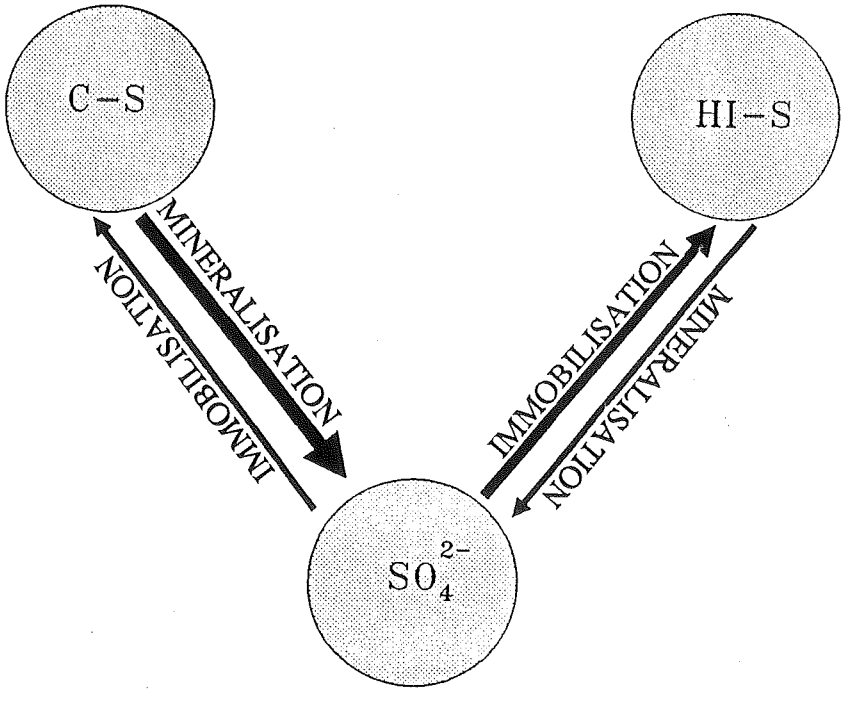


Figure 5.3 Proposed pathway of sulphur mineralisation during the open incubation of soils. The C-bonded forms of sulphur may have been mineralised directly to sulphate and some of the sulphate may have been incorporated into HI-reducible forms of sulphur.

directly to sulphate-S. Some of the mineralised sulphate-S is then utilised by soil microorganisms and converted to HI-S forms (or back in C-bonded forms). There appears to be sufficient evidence to support such a mechanism of sulphur transformation in soils (Freney *et al.*, 1971; 1975; McLaren *et al.*, 1985). These workers have demonstrated the incorporation of added $^{35}SO_4$-S into HI-reducible forms of organic sulphur.

5.4 CONCLUSIONS

It can be concluded from the results described above that the mineralised-sulphate measured during the incubation period was mainly derived from the degradation of C-bonded sulphur in soils. These findings support the studies reported in this thesis (Chapter 4) where C-bonded sulphur was found to be the best single predictor of the amount of sulphur mineralised in these soils.

CHAPTER SIX

EFFECT OF NUTRIENTS; CARBON, NITROGEN AND SULPHUR ON THE PROCESS OF SULPHUR MINERALISATION AND IMMOBILISATION

6.1 INTRODUCTION

The process of sulphur mineralisation is complex. It depends not only on the nature of the organic sulphur present in soils but also on the type of microbial population, population size, and the physiological state of the organisms. The involvement of soil micro-organisms in this process is essential. The activity of the micro-organisms depends on the physical and chemical state of soil, and the availability of other nutrients. Some previous studies (5.3.1) have shown that C-bonded forms of sulphur represents the most readily mineralisable source of organic sulphur. It was concluded in section 5.4.2 that the mineralisation of C-bonded sulphur was driven by microbial consumption of organic carbon. McGill and Cole (1981) have also suggested the same reason for the mineralisation of the C-bonded sulphur in soil. If this is true, then an adequate supply of readily metabolisable carbon in soils should slow or stop the breakdown of C-bonded S. Another suggestion made by McGill and Cole (1981) is that the mineralisation of HI-S takes place if soils are deficient in sulphate-S for microbial use. Therefore, soil incubated with no inorganic sulphate-S and oversupplied with easily oxidisable carbon should then force the micro-organisms to utilise the proposed biochemical mechanism to degrade HI-reducible forms of sulphur. Hence there should be a decrease in the concentration of HI-reducible S during the incubation. In addition to carbon, other nutrients including nitrogen and sulphur are essential for microbial growth, and therefore likely to affect sulphur mineralisation/immobilisation. Nitrogen is a major component of protein therefore its supply is essential. If nitrogen is limiting in soil systems then micro-organisms are likely to degrade soil nitrogenous organic compounds to acquire the N to form a variety of essential amino acids and proteins. Sulphur is another important element of protein and polysaccharides which are part of microbial biomass

tissues, therefore its limitation would also affect microbial growth and hence S mineralisation. Soils containing low sulphate-S have to meet microbial S requirement through mineralisation of organic sulphur. Presence of high amounts of nitrogen and carbon together are likely to cause net immobilisation of sulphate-S. As suggested earlier, addition of sulphate-S to soil might be expected to decrease the biochemical mineralisation of native sulphur.

The objectives of this present study were to examine the effects of carbon, nitrogen and sulphur on sulphur transformations, particularly mineralisation/immobilisation in the soil. By altering the amounts and availability of C,N and S in soils it was also hoped to test the dichotomous model of S cycling in soils as proposed by McGill and Cole (1981).

6.2 MATERIAL AND METHODS

6.2.1 Experimental design

A factorial experiment was set up using the open incubation system whereby soils were treated with and without additional C, S and N in all possible combinations. Each treatment was replicated four times. Treatments consisted of the following combinations;

1. C0,S0,N0	control
2. C0,S0,N+	only NO_3-N was added
3. C0,S+,N+	SO_4-S and NO_3-N were added
4. C0,S+,N0	only SO_4-S was added
5. C+,S0,N0	only glucose-carbon was added
6. C+,S+,N0	glucose-carbon and SO_4-S were added
7. C+,S0,N+	glucose-carbon and NO_3-N were added
8. C+,S+,N+	glucose-carbon, SO_4-S and NO_3-N were added

(Note 0 = no addition of nutrient, + = nutrient added)

The Teviot (limed) and the Takahe soils were selected to examine the effects of the above treatments on sulphur mineralisation. The Teviot and Takahe soils are relatively high and low S mineralising soils respectively (see Table 4.11). Prior to the incubation, soils were preconditioned at 20 °C and 75% F.C moisture for two weeks. Soil columns were

incubated at 30 °C in darkness for 14 weeks and mineralised-SO_4^{2-} was removed at two weekly intervals as described in section 4.2.1.3. The nutrient treatments were applied at the start of the incubation and after each two weekly leaching to remove mineralised sulphate. Details of adding the nutrients are described in the next section. Mineralised-SO_4^{2-} was measured in the leachates by the reduction method (see section 3.4.1).

6.2.2 Addition of nutrients

Immediately after removing sulphate-S from the columns, 10 ml of solution containing the combinations of nutrients described above, were applied to appropriate columns. Solutions containing glucose (C+) added 500 μg glucose-C g^{-1} soil. Solutions containing sulphate (S+) added 5.5 μg SO_4-S g^{-1} soil and solutions containing nitrate (N+) added 20 μg NO_3-N g^{-1} soil. Addition of the nutrient solution displaced moisture held in the soil. The displaced solution from each tube was collected and analysed for SO_4^{2-} and NO_3^- by ion exchange chromatography (section 3.4.2). This was necessary to check whether the applied nutrient had remained within the columns of soil. Generally between 87-98% of the applied nutrients remained within the columns. Only the amounts of sulphate S actually retained within the columns were taken into account during the final calculation of sulphur mineralisation and immobilisation.

6.2.3 CO_2 measurements

Microbial activities were examined by measuring the CO_2 evolved during the incubation period. The amount of CO_2 released by the micro-organisms during the 24 hours after the addition of nutrients was measured by using gas liquid chromatography following the method described in section 3.8.

6.2.4 Soil analysis

After the final leaching, soils were analysed for HI-reducible S, total organic sulphur and C-bonded S following the methods described in section 3.5.3, 3.5.4 and 3.5.5.

6.3 RESULTS AND DISCUSSION

6.3.1 Microbial activity

Microbial activity during the incubation of the control columns (Treatment 1) decreased after the first four weeks of incubation (Figure 6.1 and 6.2). Thereafter the rate of CO_2 evolution fluctuated a little but never reached the initial rate. The addition of SO_4^{2-} and NO_3^- individually or together showed little effect on the rate of CO_2 evolution in the Takahe soil. However, addition of these nutrients (Treatments 2,3 and 4) in the Teviot (limed) soil raised the CO_2 evolution significantly higher than the control soils. This was evident after the 4th week of incubation. The effect on the soil microbial activity of adding carbon (Treatments 5-8) was prominent in both soils. High availability of easily metabolisable C (added as glucose-D carbon) enhanced the growth of soil micro-organisms which increased CO_2 evolution sharply in both soils. Addition of NO_3^- and SO_4^{2-} with carbon (Treatments 6,7 and 8) had little influence on the microbial activity in the Takahe soil and would suggest that these nutrients were not limiting microbial activity. That is why there was no further increase in CO_2 evolution when these two nutrients were added to the soils along with carbon. However, addition of N with C and S (Treatment 8) showed a marked increase in the CO_2 evolution of the Teviot soil (Fig. 6.2). Such an increase in CO_2 evolution was possibly due to availability of C,S ans N together which encouraged relatively higher microbial activity.

6.3.1 Patterns of sulphur mineralisation and immobilisation

Sulphur mineralisation in the control treatments of both soils was fastest during the first 2-4 weeks of incubation and thereafter decreased with time (Figures 6.3 and 6.4). This was probably caused by the reduction in microbial activity with time, as observed by the CO_2 evolution, which also decreased after the first 2-4 weeks of incubation (Figures 6.1 and 6.2). Addition of nutrients (Treatments 2-8) showed some significant effects on sulphur mineralisation and immobilisation in both soils. However, there were more statistically significant effects with the Takahe soil compared with the Teviot soil. Application of NO_3^--N either with or without SO_4-N (Treatments 2 and 3) increased the amount of sulphur mineralisation in the Takahe soil (Fig. 6.3). In the Teviot soil, Treatment 2 (NO_3-N only)

showed an increase in sulphur mineralisation (Fig. 6.4) whereas Treatment 3 (NO_3-N plus SO_4-S) showed less mineralisation than the control. This tends to suggest that NO_3-N may have increased microbial degradation of organic sulphur in both soils hence increased the release of SO_4-S into the soil solution. In both soils, addition of SO_4-S with NO_3-N (Treatment 3) showed a slight decrease in sulphur mineralisation in comparison to addition of only NO_3-N (Treatment 2). Addition of SO_4^{2-} alone (Treatment 4) showed an even greater decrease in the level of sulphur mineralisation in both soils.

In both soils, when only carbon (Treatment 5) was added, although mineralisation was less than the other treatments described so far micro-organisms were still able to mineralise significant amounts of native sulphur. These results are somewhat different than most closed incubation studies where addition of metabolisable C generally causes net immobilisation of native and added sulphate (e.g. Freney *et al.*, 1971). However, when SO_4^{2-} was applied with carbon (Treatment 6) or with carbon and nitrogen (Treatment 8), both soils showed significant amounts of immobilisation of the added sulphate. This effect was most prominent with treatment 8 (C+,N+,S+) in the Takahe soil where 12.6 μg SO_4-S g^{-1} soil was immobilised during the 14 week period of incubation. In the Teviot soil, some sulphate was released up until the 4th week of incubation but thereafter immobilisation of the added sulphate was observed. This increased level of immobilisation at the later stages of the incubation is the result of increased microbial activity during that period of incubation (see Figures 6.1 and 6.2). These results are consistent with the work of Freney *et al.*, (1971) and McLaren *et al.*, (1985). Both of these workers reported that immobilisation of the added ^{35}S was greatly enhanced in glucose treated soils. Saggar *et al.*, (1981b) also found that addition of a carbon source, in this case cellulose immobilised considerable amounts of added sulphate. The intensity of sulphate immobilisation with treatments including added carbon would largely be determined by the nature of the carbon compound and the microbial population living in that soil. The results show that release of sulphur as sulphate is dependent on the relative of C, nitrate and sulphate in soils. A comparison between treatments 5,6 and 8 shows that in the absence of added S, addition of carbon can encourage mineralisation of native sulphur. In both soils, the degradation of native organic sulphur provided more SO_4^{2-} than that required by micro-organisms for the metabolisation of the added C. However, when sulphate-S was added, mineralisation of native sulphur decreased considerably.

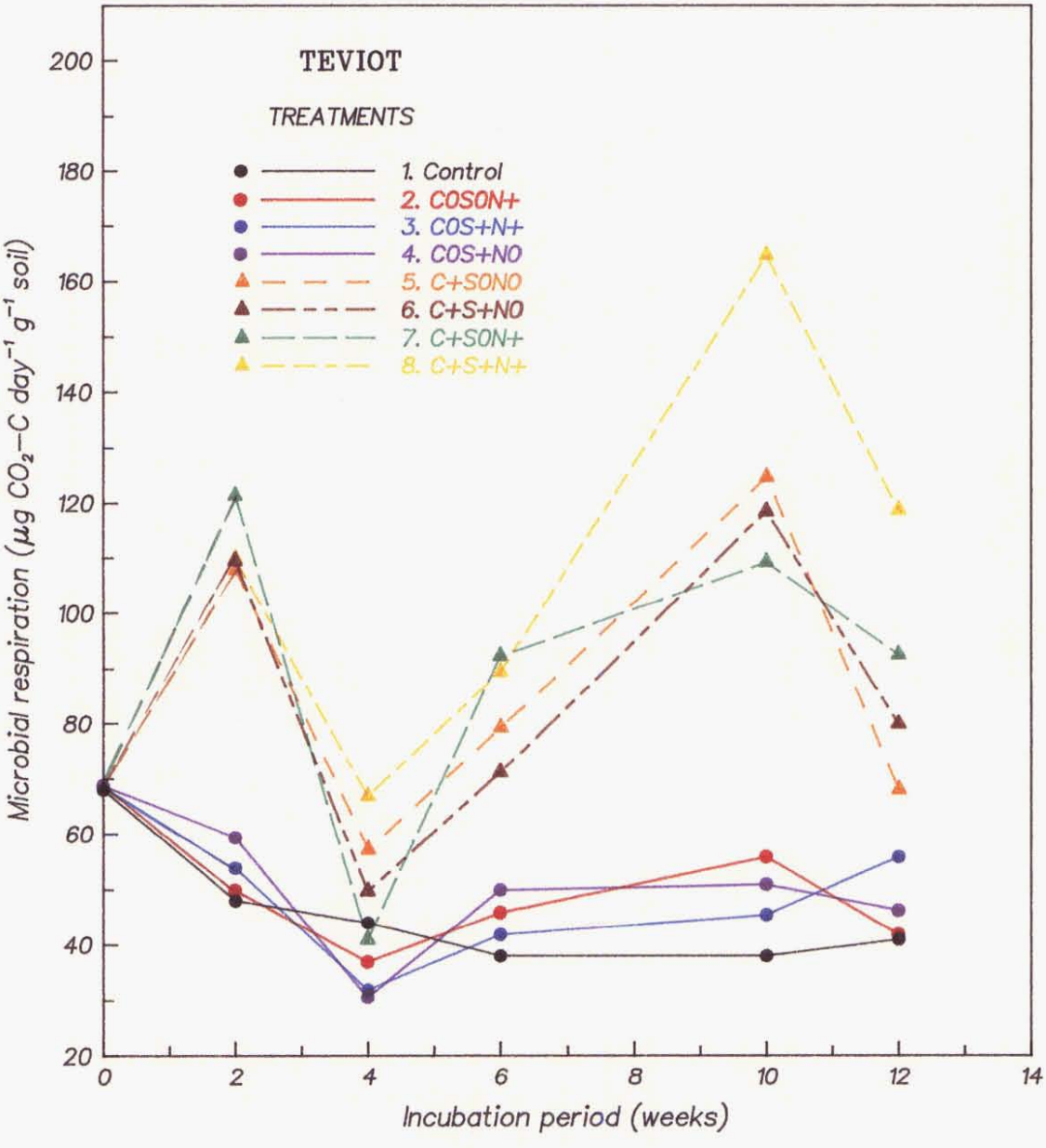


Figure 6.1 The effects of adding carbon, sulphate and nitrate on the rate of CO_2 evolution ($\mu g\ CO_2$–C $day^{-1}\ g^{-1}$ soil) in the Teviot soil during the incubation period.

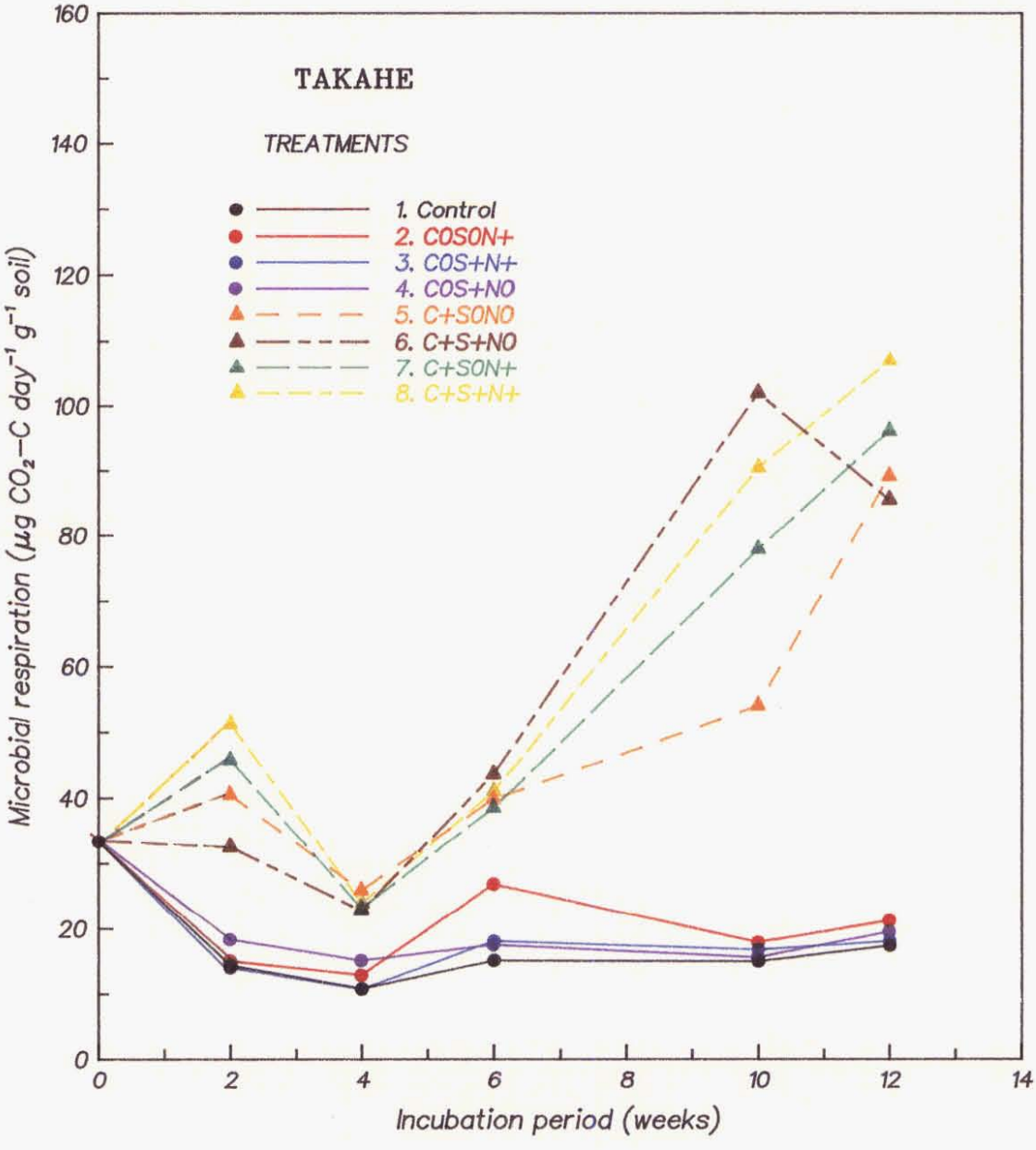


Figure 6.2 The effects of adding carbon, sulphate and nitrate on the rate of CO_2 evolution ($\mu g\ CO_2$–C $day^{-1}\ g^{-1}$ soil) in the Takahe soil during the incubation period.

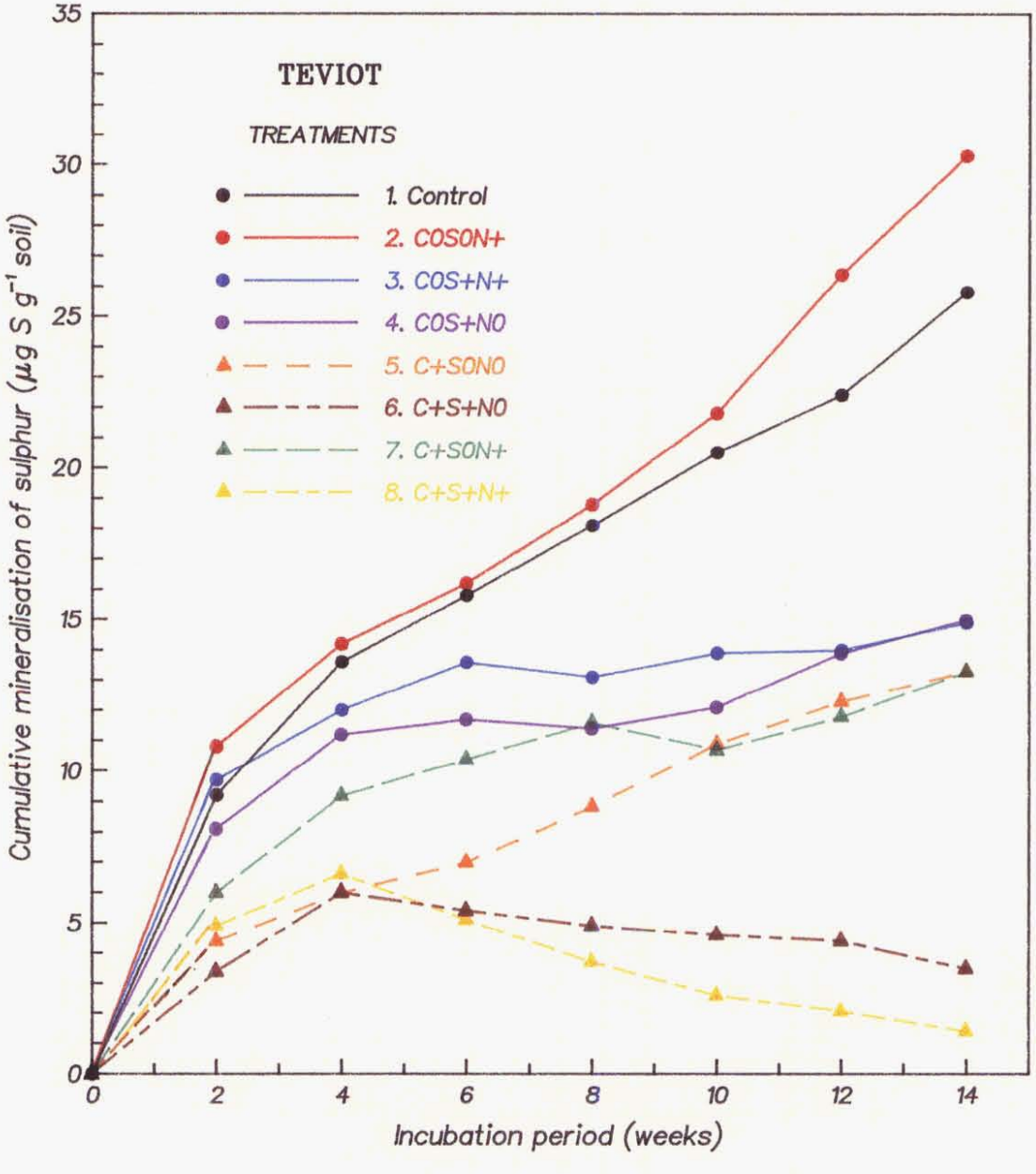


Figure 6.3 The effects of adding carbon, sulphate and nitrate on the mineralisation/immobilisation of sulphur in the Teviot soil.

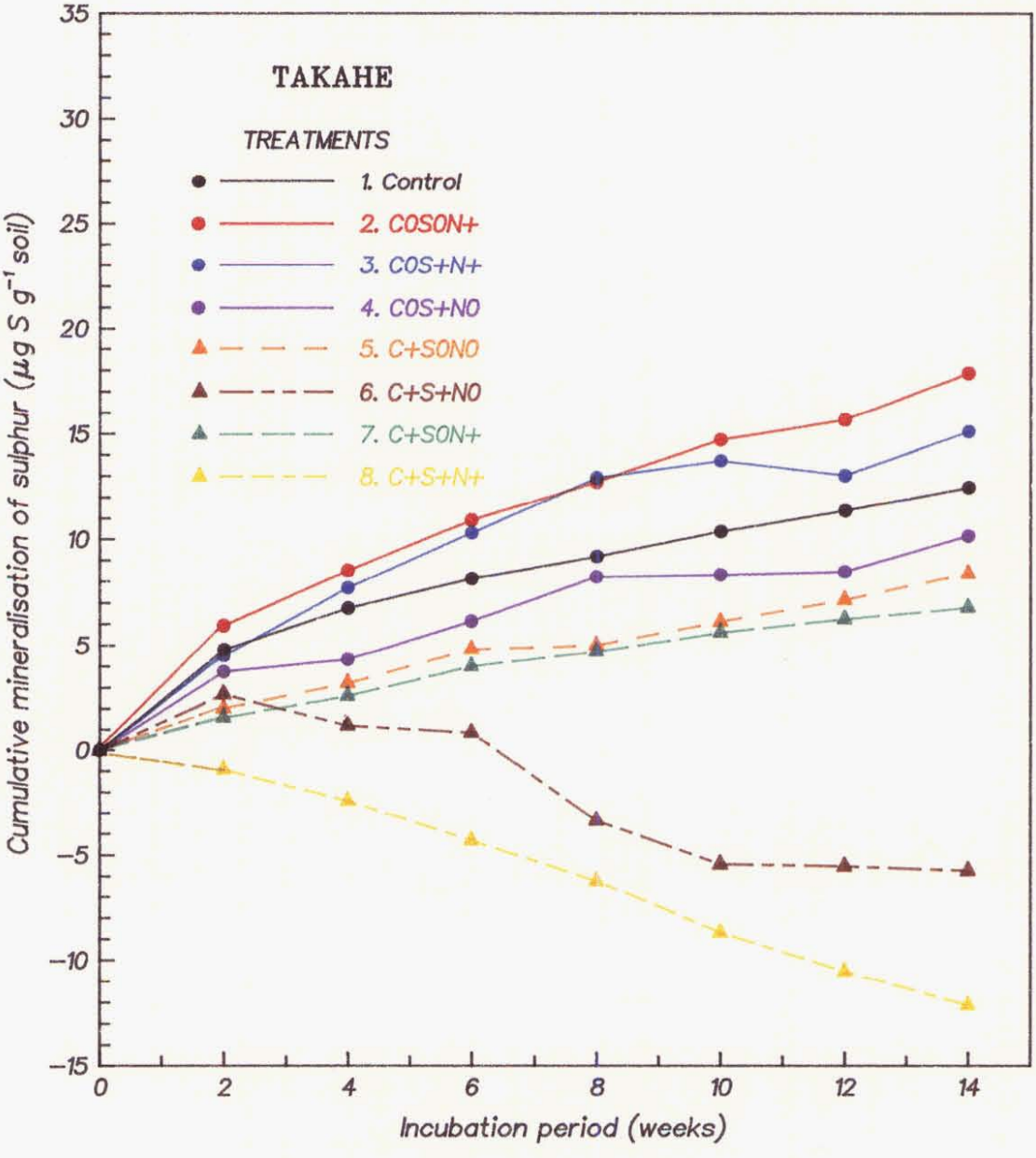


Figure 6.4 The effects of adding carbon, sulphate and nitrate on the mineralisation/immobilisation of sulphur in the Takahe soil.

6.3.3 Interactions between nutrients on the level of sulphur mineralisation and immobilisation

Diagramatic presentations of the significant main effects and interactions of C,N and sulphur on total mineralisation and immobilisation during the 14 week incubation are shown in Figure 6.5. Level of significance reported in these figures ranged between P <0.005 and <0.001. The complete analyses of variance are presented in Table 6.1a and 6.1b. The effects are more pronounced in the Takahe soil than the Teviot soil. Figure 6.5a shows that addition of nitrogen in the Takahe soil decreased the mineralisation of sulphur. Although this effect is not great, it is statistically significant at P <0.005 . It is likely that addition of N encouraged micro-organisms to synthesise more protein, hence utilising more sulphur and reducing the amount of sulphur mineralised as sulphate. It is also possible that availability of nitrate in the soil would have reduced the need for soil microorganisms to obtain nitrogen by degrading soil organic matter. In either case, less mineralisation of sulphur would result. Addition of sulphate in both soils caused a sharp decline in the amounts of mineralised-SO_4^{2-} (Figures 6.5b and 6.5h). Such an effect could be caused by a reduction in biochemical mineralisation which is thought to be responsible for the mineralisation of HI-S, and believed to be controlled by sulphate-S concentration in soil solution (McGill and Cole, 1981). Addition of carbon also decreased the release of sulphate-S in (Figures 6.5c and 6.5i). The reason for such a reduction in the amount of released SO_4^{2-} is due the availability of easily metabolisable C in the soil. This would have enhanced microbial growth with minimum degradation of native soil organic matter, meaning less release of sulphur from native organic matter. At same time, microbial demand for sulphur would have increased, increasing the amounts of sulphur was available for release as sulphate.

Interactions between the nutrients N+S and C+S on sulphur mineralisation were only significant in the Takahe soil. In the presence of added nitrate, the addition of sulphate reduced sulphur mineralisation to a much greater extent than where no nitrate was added (Fig. 6.5.d). Probably, added N+S provided better nutrition for microorganisms, thus more sulphur was incorporated into microbial biomass. As noted above, addition of C alone decreased the mineralisation of sulphur in both soils. Addition of C and sulphur together (C+,S+) showed an even greater decrease in the rate of sulphur

Figure 6.5 Main effects and interactions of added nutrients (C,N and S) on the mineralisation of S in the Takahe (a–g) and Teviot (h–j) soils.

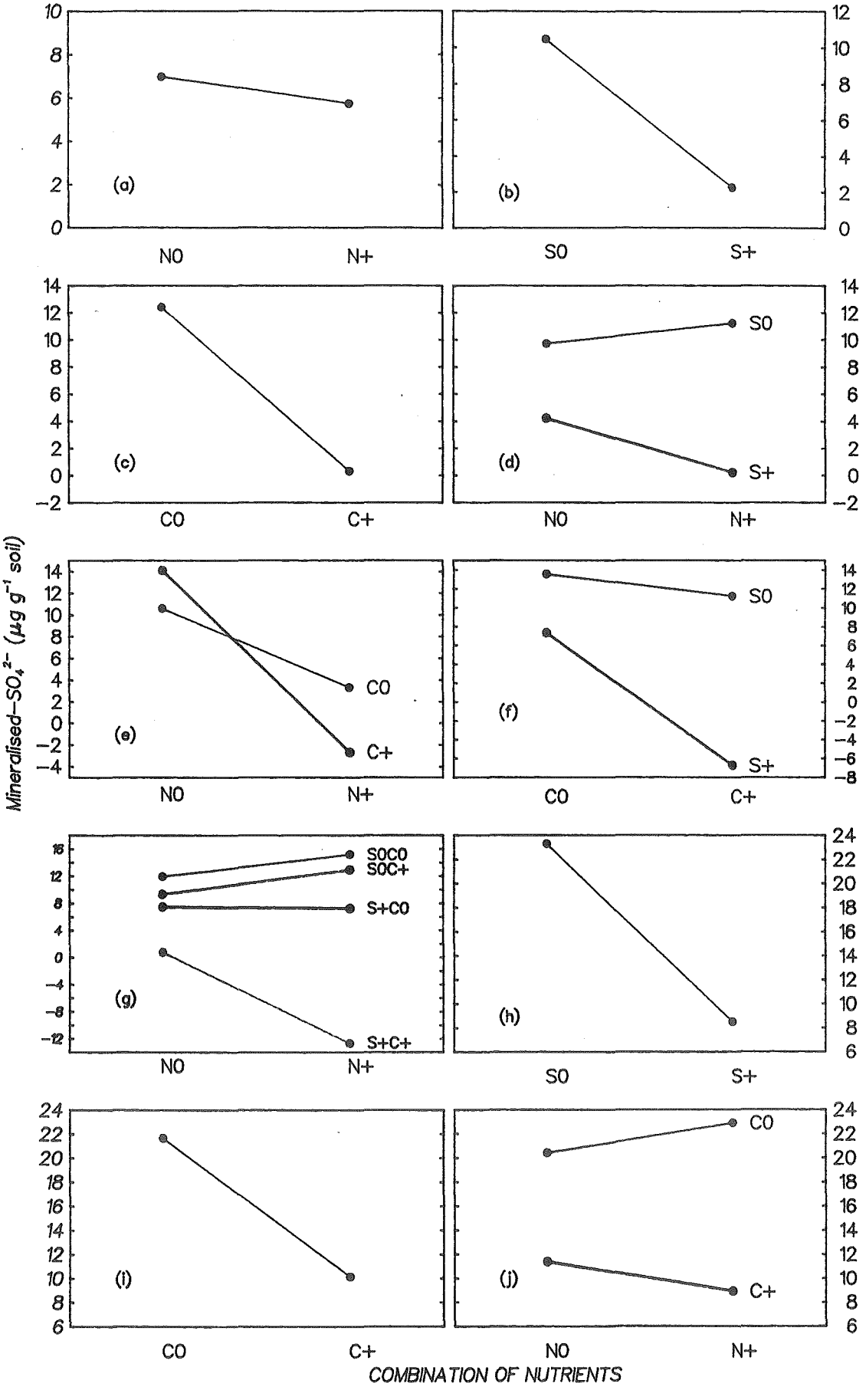


Table 6.1a Main effects and interactions of adding nutrients on sulphur mineralisation and immobilisation in the Takahe soil.

Treatments		Mineralisation/immobilisation (μg S g^{-1} soil)				SED[a]	F PR[b] (Error DF[c] = 21)
Main effects							
Nitrogen		**0**	+				
		6.9	5.7			0.49	0.019
Sulphur		**0**	+				
		10.4	2.2			0.49	<0.001
Carbon		**0**	+				
		12.4	0.3			0.49	<0.001
Two way interactions							
Sulphur		**0**	+				
Nitrogen	**0**	9.7	4.2				
	+	11.2	0.2			0.69	<0.001
Carbon		**0**	+				
Nitrogen	**0**	10.6	3.3				
	+	14.1	-2.6			0.69	<0.001
Carbon		**0**	+				
Sulphur	**0**	13.5	7.3				
	+	11.2	-6.7			0.69	<0.001
Three way interactions							
Sulphur		**0**		+			
Carbon		**0**	+	**0**	+		
Nitrogen	**0**	11.9	7.5	9.3	-0.9		
	+	15.2	7.2	13.1	-12.5	0.98	<0.001

0 without nutrient.
+ with nutrient.
a. standard error of differences between means.
b. the significance ratio associated with F ratio.
c. degrees of freedom.

Table 6.1b Main effects and interactions of adding nutrients on sulphur mineralisation and immobilisation in the Teviot soil.

Treatments		Mineralisation/immobilisation (μg S g^{-1} soil)				SED[a]	F PR[b] (Error DF[c] = 21)
Main effects							
Nitrogen		0	+				
		15.9	15.9			0.78	-.-
Sulphur		0	+				
		23.3	8.5			0.78	<0.001
Carbon		0	+				
		21.6	10.1			0.78	<0.001
Two way interactions							
Sulphur		0	+				
Nitrogen	0	22.5	9.2				
	+	24.9	8.9			1.01	0.069
Carbon		0	+				
Nitrogen	0	20.4	11.4				
	+	22.9	2.1			1.01	0.005
Carbon		0	+				
Sulphur	0	28.3	18.2				
	+	14.9	2.0			1.01	0.088
Three way interactions							
Sulphur		0		+			
Carbon		0	+	0	+		
Nitrogen	0	25.8	19.3	15.0	3.4		
	+	30.9	17.2	14.9	0.6	1.56	0.166

0 without nutrient.
+ with nutrient.
a. standard error of differences between means.
b. the significance ratio associated with F ratio.
c. degrees of freedom.

mineralisation particularly in the Takahe soil where it caused a net immobilisation of added sulphate-S (Fig. 6.5f). Such an immobilisation of sulphur was most likely caused by the growing soil micro-organisms which assimilated the SO_4-S along with easily metabolisable carbon. In both soils N had greater effect on sulphur mineralisation in the presence of added carbon (Figures 6.5e and 6.5j). Addition of these nutrients would have provided sufficient amounts of the two most needed nutrients for microbial growth which should have encouraged the microbial proliferation and as a consequence increased the immobilisation of the soil sulphate. Hence less SO_4^{2-} remained in soil solution. It is noticeable that the interactions of N0C+ for the two soils were different than each other (shown in Figures 6.5e and 6.5j). The reasons for such difference could not be explained from the variations in C:N, N:S or C:S ratios of the two soils.

The combination of all three nutrients produced the greatest decrease in the amounts of mineralisable sulphur in both soils. However, the interaction between C,N and S was only significant in the Takahe soil. When S was added in the presence of N and C, it caused a significant amount of net immobilisation of the added sulphate-S (Fig. 6.5g). This is because supplying these three nutrients would have provided sufficient nutrients for microbial growth, hence reducing the need for the soil micro-organisms to spend energy in degrading organic matter.

The reasons for greater interaction of nutrients in the Takahe soil could be due to the low mineralising ability of this soil. In the case of a high carbon input, this soil would show greater immobilisation of sulphate than the Teviot soil. Nitrogen may also influence sulphur availability in the Takahe soil.

6.3.4 Effect of C,N and S on sulphur transformations

Examination of HI-reducible and C-bonded sulphur in the soils after incubation showed some significant gains and losses in these forms of sulphur. In general, soils with no additional carbon have lost greater amounts of C-bonded sulphur than the soils which had carbon added (Figures 6.6 and 6.7). Theoretically, addition of a readily metabolisable source of carbon might have been expected to completely stop the

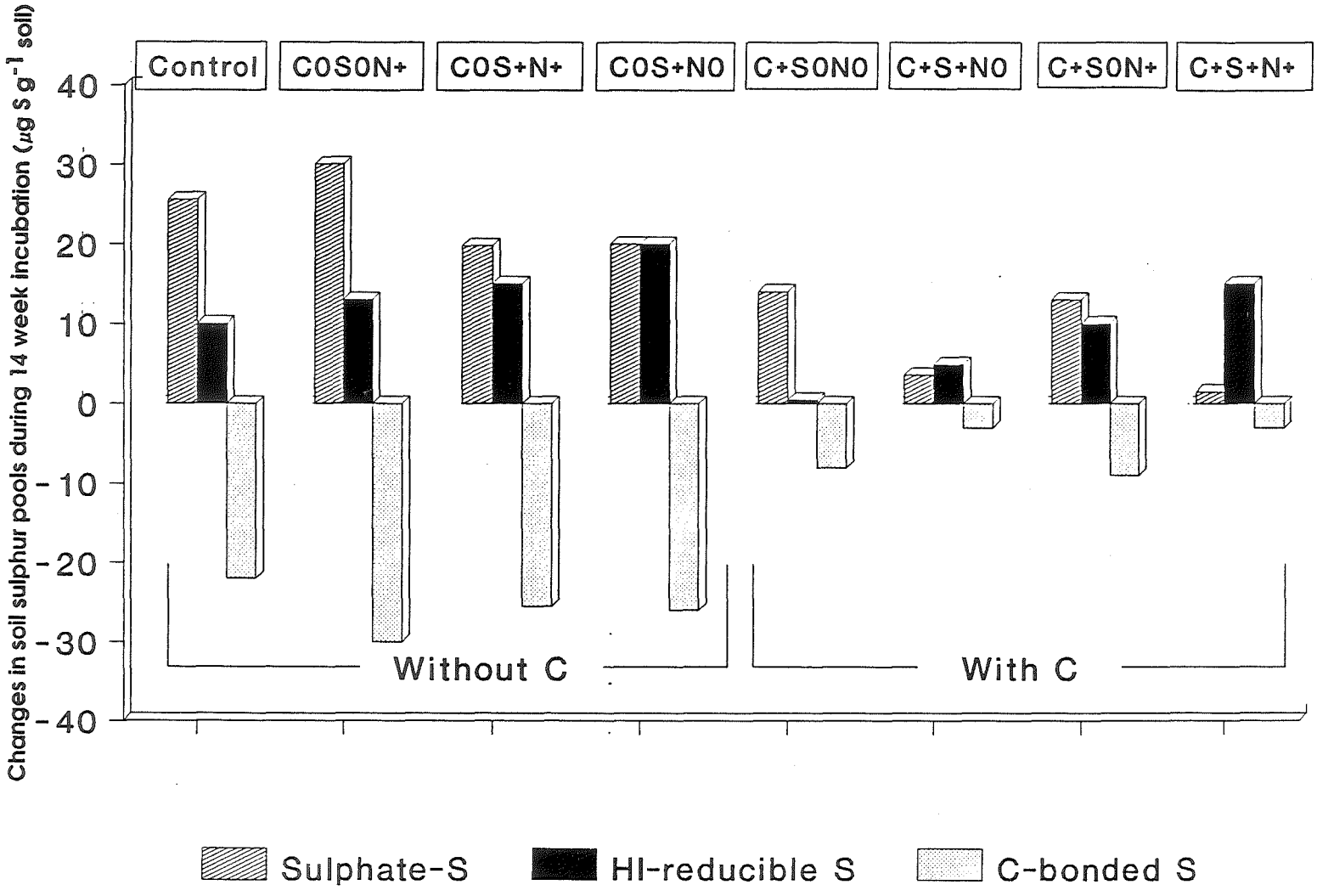


Figure 6.5 Effects of adding nutrients on the mineralisation/immobilisation of sulphur in the Teviot soil.

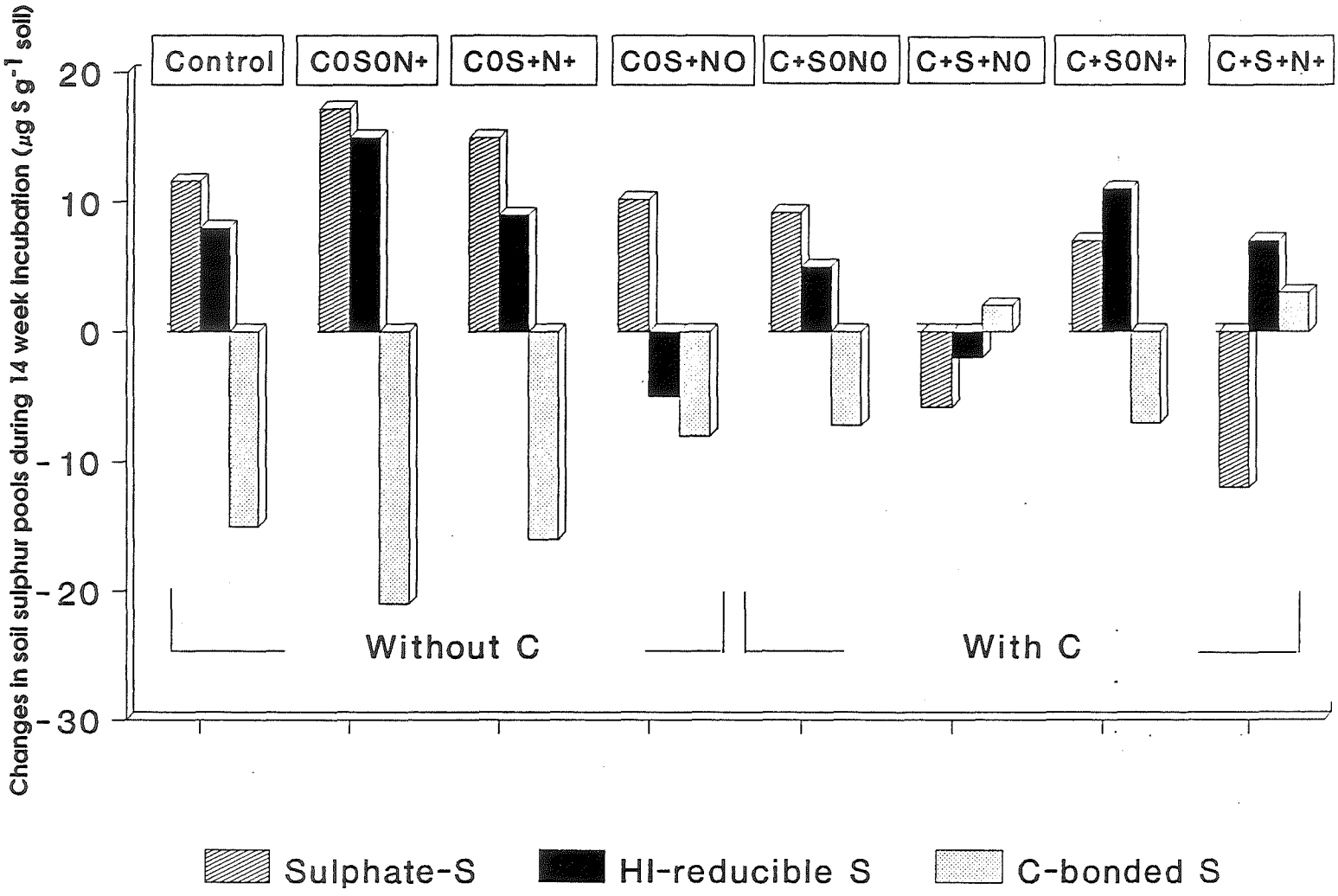


Figure 6.6 Effects of adding nutrients on the mineralisation/immobilisation of sulphur in the Takahe soil.

breakdown of the C-bonded sulphur. However, despite the addition of 500 μg glucose-C g^{-1} soil every two weeks, some C-bonded forms of sulphur was lost through mineralisation during the incubation (specially in the Teviot soil). When sulphate or sulphate and nitrate were added, the losses in C-bonded sulphur were decreased. Thus it appears that compounds containing C-bonded sulphur are broken down not only to obtain C but possibly also to obtain sulphur (or N) to meet microbial requirements. These findings suggest that mineralisation of C-bonded sulphur does not necessarily follow the mechanism suggested by McGill and Cole (1981).

It was expected that treatments providing C but not sulphur would decrease HI-reducible sulphur considerably because a shortage of inorganic sulphate would have induced the biochemical degradation of ester sulphates. McGill and Cole (1981) suggested that in a state of sulphate-S deficiency micro-organisms will preferentially mineralise HI-reducible sulphur through enzymatic action. However, in this present study, for treatments 5 and 7 where sulphur was deficient, there was no reduction in the amounts of HI-S, instead these treatments showed some increase in HI-reducible sulphur. This tends to suggest that biochemical mineralisation as proposed by McGill and Cole (1981) was not operative and regardless of the presence of easily mineralisable carbon in the soil, micro-organisms found it easier to mineralise C-bonded than HI-reducible forms of sulphur. The increase in HI-reducible sulphur in these soils could be due to the transformation of C-bonded sulphur into HI-reducible forms during the incubation. The distribution of organic sulphur in soilstreated with all three nutrients showed an increase in HI-reducible forms of sulphur during incubation (Figures 6.6 and 6.7). It was expected that addition of carbon, sulphate and nitrate would have encouraged the soil micro-organisms to utilise the added sulphur to form sulphur proteins (cysteine and methionine), which should have given a substantial rise in the amounts of C-bonded sulphur during the incubation. Such an increase in C-bonded sulphur was partially observed in the Takahe soil. However, this increase was still relatively small compared to the HI-S increase. These observations suggest that possibly added sulphate-S may have been incorporated into both forms of organic sulphur i.e C-bonded and HI-S simultaneously. Several workers have observed this phenomenon in closed incubation studies (eg. Freney *et al.*, 1975; Maynard *et al.*, 1985; and McLaren *et al.*, 1985) where added SO_4^{2-} has incorporated into both forms of organic sulphur.

6.3.5 Interaction of nutrients with HI-reducible sulphur and C-bonded sulphur in soil

The various effects of added nutrients on net gains or losses of organic forms of sulphur were less statistically significant than the effects on sulphur mineralisation. Part of the reason for this is the error involved in measuring the HI-S and total organic S in soils, thus requiring substantial changes in the amounts present in the fractions to obtain statistical significance. Only statistically significant effects ($P<0.005$ to $P<0.001$) are reported in this section, however, complete factorial analyses of variance for increases in HI-S and decreases in C-S are presented in Tables 6.2a, 6.2b, 6.3a and 6.3b.

6.3.5.1 *Increase in HI-reducible sulphur*

Addition of NO_3^--N to both soils produced a significant increase in HI-reducible sulphur (Figures 6.8a and 6.8c). This tends to suggest that the presence of nitrate in soils affects the microbial metabolic processes whereby soil sulphate is transformed predominantly into HI-S forms of organic sulphur. The main effect of sulphate-S on HI-S was markedly different in the two soils. The Takahe soil showed a much smaller increase in HI-S when sulphate-S was applied compared to when no S was added, while the Teviot soil showed a greater increase in the amount of HI-S with added sulphate than without (Figures 6.8b and 6.8d). The opposite trends in these soils could be due to differences in microbial density and type of microbial population or other soil properties. Addition of carbon to the Teviot soil significantly reduced the increase in HI-reducible sulphur obtained with treatments without added carbon.

6.3.5.2 *Decrease in C-bonded sulphur*

The main effect of NO_3^- on C-bonded sulphur was significant only in the Takahe soil where addition of nitrate to the soil increased the loss of C-bonded sulphur from the soil (Fig. 6.9 a). It is likely that availability of nitrate would have encouraged microbial growth leading to a breakdown of native organic matter to obtain C, with the concurrent release of C-bonded sulphur. In other words, the mineralisation was driven biologically (McGill and Cole, 1981). Addition of sulphate-S decreased the loss of C-S (Fig. 6.9 b). The main effect of adding carbon was highly significant ($P<0.001$) in

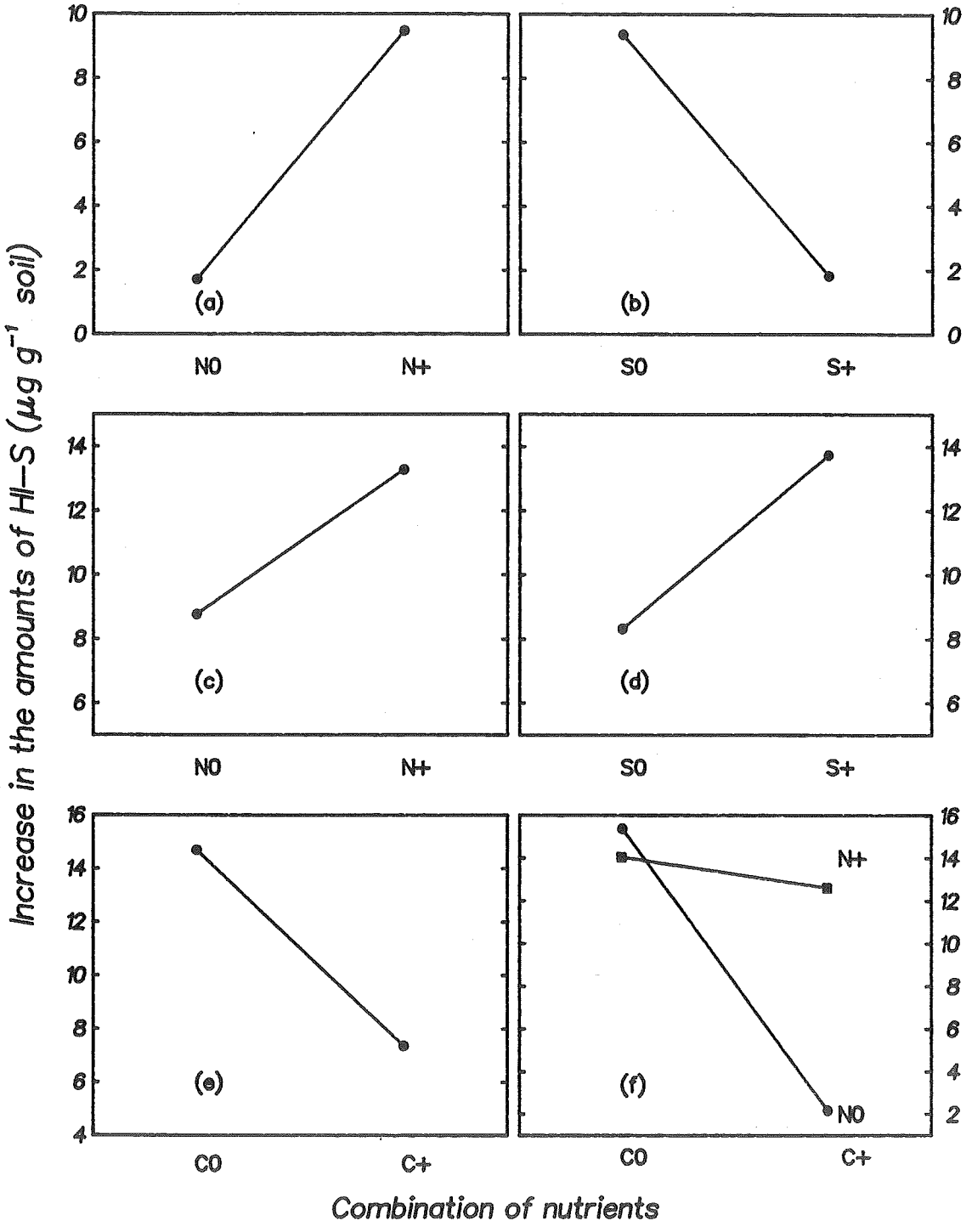


Figure 6.8 Main effects and interactions of added nutrinets (C,N and S) on the amounts of HI–reducible S in the Takehe (a and b) and Teviot soil (c,d,e and f).

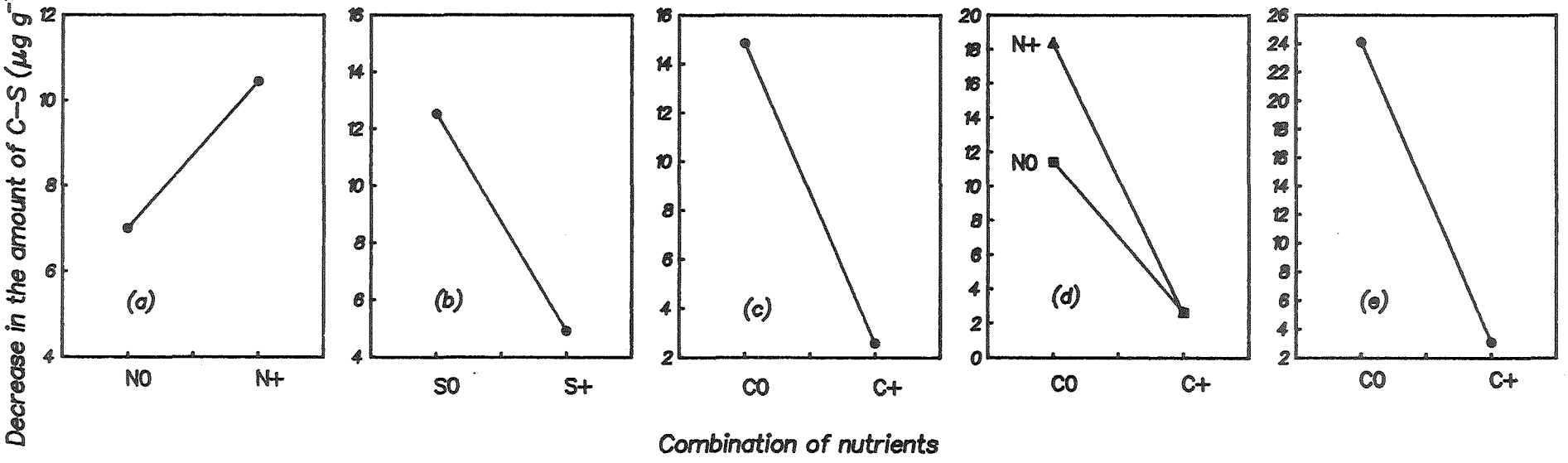


Figure 6.9 Main effects and interactions of added nutrients (C,N and S) on the amounts of C–bonded S in the Takahe (a,b,c and d) and Teviot soil (e).

Table 6.2a Main effects and interactions of adding nutrients on changes in the HI-reducible sulphur in the Takahe soils.

Treatments		Increase in HI-reducible S (μg S g^{-1} soil)				SED[a]	F PR[b] (Error DF[c] = 21)
Main effects							
Nitrogen		0	+				
		1.7	9.4			1.37	<0.001
Sulphur		0	+				
		9.3	1.8			1.37	<0.001
Carbon		0	+				
		6.4	1.8			1.37	0.218
Two way interactions							
Sulphur		0	+				
Nitrogen	0	6.3	-2.9				
	+	12.4	6.5			1.93	0.234
Carbon		0	+				
Nitrogen	0	1.4	2.0				
	+	12.4	6.5			1.93	0.106
Carbon		0	+				
Sulphur	0	11.4	7.3				
	+	1.5	2.1			1.93	0.092
Three way interactions							
Sulphur		0		+			
Carbon		0	+	0	+		
Nitrogen	0	7.8	4.8	-5.0	-0.8		
	+	15.0	9.8	8.0	5.1	2.74	0.371

0 without nutrient
+ with nutrient
a. standard error of differences between means.
b. the significance ratio associated with F ratio.
c. degrees of freedom.

Table 6.2b Main effects and interactions of adding nutrients on changes in the HI-reducible sulphur the Teviot soil.

Treatments		Increase in HI-reducible S (μg S g^{-1} soil)				SED[a]	F PR[b] (Error DF[c] = 21)
Main effects							
Nitrogen		**0**	+				
		8.7	13.3			1.83	0.022
Sulphur		**0**	+				
		8.3	13.7			1.83	0.007
Carbon		**0**	+				
		14.7	7.3			1.83	0.001
Two way interactions							
Sulphur		**0**	+				
Nitrogen	**0**	5.1	12.4				
	+	11.5	15.0			2.58	0.322
Carbon		**0**	+				
Nitrogen	**0**	15.4	2.1				
	+	14.0	12.3			2.58	0.004
Carbon		**0**	+				
Sulphur	**0**	11.5	5.1				
	+	17.9	9.6			2.58	0.603
Three way interactions							
Sulphur		**0**		+			
Carbon		**0**	+	**0**	+		
Nitrogen	**0**	10.0	0.3	20.7	4.0		
	+	13.0	10.0	15.0	15.1	3.6	0.181

0 without nutrient.
+ with nutrient.
a. standard error of differences between means.
b. the significance ratio associated with F ratio.
c. degrees of freedom.

Table 6.3a Main effects and interactions of adding nutrients on changes in the C-bonded S in the Takahe soils.

Treatments		Decrease in C-bonded sulphur ($\mu g\ S\ g^{-1}$ soil)				SED[a]	F PR[b] (Error DF[c] = 21)
Main effects							
Nitrogen		0	+				
		-6.9	-10.4			1.39	0.022
Sulphur		0	+				
		-12.5	-4.9			1.39	<0.001
Carbon		0	+				
		-14.8	-2.6			1.39	<0.001
Two way interactions							
Sulphur		0	+				
Nitrogen	0	-10.7	-3.2				
	+	-14.3	-6.6			1.98	0.968
Carbon		0	+				
Nitrogen	0	-11.4	-2.6				
	+	-18.3	2.5			1.98	0.021
Carbon		0	+				
Sulphur	0	-17.8	-7.2				
	+	-11.9	2.0			1.98	0.253
Three way interactions							
Sulphur		0		+			
Carbon		0	+	0	+		
Nitrogen	0	-14.7	-6.7	-8.0	1.5		
	+	-20.8	-7.6	-15.7	2.5	2.76	0.541

0 without nutrient.
+ with nutrient.
a. standard error of differences between means.
b. the significance ratio associated with F ratio.
c. degrees of freedom.

Table 6.3b Main effects and interactions of adding nutrients on changes in the C-bonded sulphur in the Teviot soil.

Treatments		Decrease in C-bonded S (μg S g^{-1} soil)				SED[a]	F PR[b] (Error DF[c] = 21)
Main effects							
Nitrogen		0	+				
		-12.7	-14.4			3.87	0.676
Sulphur		0	+				
		-13.4	-13.7			3.87	0.940
Carbon		0	+				
		-24.1	-3.1			3.87	<0.001
Two way interactions							
Sulphur		0	+				
Nitrogen	0	-11.1	-14.4				
	+	-15.8	-13.0			5.48	0.433
Carbon		0	+				
Nitrogen	0	-20.1	-5.4				
	+	-28.0	-0.7			5.48	0.118
Carbon		0	+				
Sulphur	0	-22.8	-4.0				
	+	-25.3	-2.1			5.48	0.568
Three way interactions							
Sulphur		0		+			
Carbon		0	+	0	+		
Nitrogen	0	-14.0	-8.1	-26.2	-2.7		
	+	-31.6	0.0	-24.5	-1.5	7.74	0.107

0 without nutrinet.
+ with nutrient.
a. standard error of differences between means.
b. the significance ratio associated with F ratio.
c. degrees of freedom.

reducing the losses of C-bonded forms of sulphur for both soils (Fig. 6.9c and 6.9e). Only one significant interaction between nutrients (C+N) was observed. In the absence of added nitrogen, added carbon had less effect in reducing the loss of C-bonded sulphur than in the presence of added nitrogen (Fig. 6.9 d). The magnitude of the effect of adding carbon is clearly higher than for the effects of adding N and S, indicating that mineralisation of carbon-bonded sulphur is mainly driven by the need of acquiring carbon. However, the results have shown that some of the C-bonded sulphur may be mineralised as a result of requirements for sulphur or N as well.

6.4 CONCLUSIONS

Increases in microbial activity do not necessarily result in a increased release of sulphur for plant consumption. In carbon treated soils where microbial activity was distinctly higher than in non-carbon treated soils, but sulphur mineralisation was very poor and in some instances immobilisation dominated the transformation processes. High microbial activity may increase the speed of sulphur transformation from one form to another but in terms of increasing the amount of available SO_4^{2-} for plant uptake, this may be non-productive. These results are important in relating to field conditions, where freshly added organic matter may increase microbial activity but also promote immobilisation of inorganic sulphate, causing a temporary deficiency of sulphur in the soil. Such incidents will cause more severe effects in low sulphate containing and mineralising soils, such as the Takahe soil.

The results of this present study do not completely agree with the hypothesis of biological and biochemical mineralisation as proposed by McGill and Cole (1981). However, there seems to be some relationship between the presence of easily metabolisable carbon and the mineralisation of C-bonded sulphur in soils. In the presence of metabolisable C, mineralisation of C-bonded sulphur is significantly reduced.

There were indications of substantial transformations of C-bonded sulphur and sulphate-S into HI-reducible sulphur. This was evident particularly for Treatment 8 (C+.N+.S+) which showed either a small increase or a relatively small loss of C-bonded sulphur

during the incubation but at the same time HI-reducible forms of sulphur showed a significant increase.

CHAPTER SEVEN

SULPHUR TRANSFORMATIONS IN SOILS

7.1 INTRODUCTION

Previous parts of this study have mainly concentrated on examining the methods of measuring mineralisable sulphur in soil and the pathways of its release from organic sulphur fractions. This part of the study attempts to examine the transformations of sulphur within the soil system by means of labelling with radioactive sulphur-35. A summary of previous incubation studies using sulphur-35 is presented in Table 7.1. Earlier studies by Freney *et al.*, (1971; 1975); Goh and Tsuji (1979) and more recently by McLaren *et al.*, (1985) have examined sulphur transformations in soils by adding sulphate-S labelled with radioactive ^{35}S. Use of sulphur-35 with carrier sulphate alters the sulphate concentration in soils and hence is likely to affect sulphur transformations. Use of carrier-free ^{35}S which does not interfere with the concentration of indigenous sulphate, is therefore more suited for studying transformations of native soil sulphur. For this reason, some of the recent sulphur transformation studies have been conducted using carrier-free ^{35}S (e.g. Saggar *et al.*, 1981b; Maynard *et al.*, 1983;1985).

There are only two previous studies which have examined the incorporation of added sulphur-35 into organic sulphur fractions within the first few days of incubation (Freney *et al.*, 1971; Saggar et al., 1981b). Other workers have generally made their initial measurements of ^{35}S after at least 7-14 days of incubation. Incorporation of ^{35}S is associated with the rate at which S is cycling in the soil system, a higher rate of incorporation implies higher rate of internal sulphur cycling. Incorporation of ^{35}S within few days could be significant, especially when using air-dried soil. Given the effect of moistening air-dried soil on sulphur mineralisation (Williams, 1967), an initial 7-14 day sampling period is perhaps too late to measure any short-term changes brought about by the flush of microbial activity. It would therefore seem important to measure the

Table 7.1 Experimental details and results of previous incubation studies using sulphur-35 to examine sulphur transformations in the soil.

Reference	type of ^{35}S used	soil (air-dry,moist or preconditioned)	length of incubation (days)	sampling intervals	incubation temperature (°C)	treatments	% of ^{35}S incorporated
Freney *et al.*, (1971)	SO_4-^{35}S	Field moist	168	1,7,14,28,56, 168	30	No glucose Glucose	50 82
Freney *et al.*, (1975)	SO_4-^{35}S	Field moist	64	64	30	Native soil Pasture soil	33.6 (HI-S 73%, C-S 27%) 45.7 (HI-S 57%, C-S 43%)
Goh and Tsuji (1979)	$CaSO_4$-^{35}S	Air-dry	175	14,28,49,70 175	23	Pasture soils	49 (HI-S 60-90%, C-S 10-40%
Saggar *et al.*, (1981b)	Carrier-free	Preconditioned	64	1,2,4,8,16,24 32,64	21	SO_4 Cellulose Cellose + SO_4	Incorporation into fulvic (45-76%) and humic acid (24-55%) fractions.
Maynard *et al.*, (1983)	Carrier-free	Preconditioned	119	7,21,35,49,63, 77,91,105,119	20	-----	30-55
Maynard *et al.*, (1985)	Carrier-free	Preconditioned	88	16,32,48,68,88	20	SO_4 SO_4 + Cellulose Cellulose	30 (HI-S 84-90%, C-S 10-16%) 40 (HI-S 49-66%, C-S 34-51%) 90 (HI-S 49-66%, C-S 34-51%)
McLaren *et al.*, (1985)	SO_4-^{35}S	Air-dry	75	10,25,50,75	20	No glucose Glucose	27 (HI-S 89%, C-S 11%) 48 (HI-S 69%, C-S 31%)

extent of the cycling of applied ^{35}S within a short period (1-2 days) of starting the incubation. Also there is no information available as to what proportion of the sulphur is cycled through the microbial biomass and thus on role of biomass in soil sulphur transformations.

The objectives of this part of the study are to examine the above mentioned areas to improve the understanding of the nature of sulphur transformations in soils. The study has been divided into two parts; the first part examines the transformations of carrier-free sulphur-35 added to soils, and the second part examines the transformation of sulphur-35 added with a sulphate carrier. The later part of the study simulates the transformations of added fertilizer sulphate in soils. The four specific objectives were as follows:

(i) To examine the effect of soil treatment on the nature of carrier-free sulphur-35 transformations in soils. The soil treatments were;

(a) Preconditioned-soil (detail are presented in 7.2.2)
(b) Air-dried soil
(c) Glucose added to air-dried soil

(ii) To examine the role of the microbial biomass in sulphur transformations by measuring the ^{35}S activity in the microbial biomass.

(iii) To compare, the short to medium-term incorporation of ^{35}S applied with carrier K_2SO_4 into preconditioned soil with the incorporation of carrier free sulphur-35.

(iv) To compare the incorporation of ^{35}S between the Teviot (limed) and Meyer soils.

7.2 MATERIALS AND METHODS

7.2.1 Soils

The Teviot (limed) and Meyer soils were used for these studies. The choice of these two soils was made on the basis of their mineralisation characteristics and sulphate-S contents.

The Teviot (limed) soil represents a high S mineralising soil which contains high SO_4^{2-} levels and the Meyer soil is categorized as low S mineralising soil which contains low SO_4^{2-} as well. Parts (i) to (iii) of this study were carried out only on the Teviot soil. Part (iv) of the study was conducted on both soils.

7.2.2 Addition of carrier-free sulphur-35 to the soils

Samples of air-dried soils (40 g) were weighed into 100 ml conical flasks and a solution (6 ml) containing 60 μCi carrier-free ^{35}S as sulphate (Amersham, International Plc, England) was added in two aliquots. The soil was thoroughly mixed after each addition to ensure that the distribution of applied ^{35}S was as homogeneous as possible. The moisture content of the soil was then increased to 75% of the field capacity by adding distilled water and samples were then again thoroughly mixed. The flasks were loosely plugged with non-absorbing cotton wool to reduce rapid loss of moisture and at the same time maintain aerobic conditions.

A second set of samples were prepared as above but with the addition of 10 ml of a 10% glucose solution to provide (1% organic carbon) a source of readily metabolisable carbon for soil microbial growth.

A third set of samples were prepared using soil that had been preconditioned by maintaining at 75% field capacity for two weeks before addition of the ^{35}S. The soil was allowed to dry to 50% field capacity immediately prior to weighing into the flasks to avoid over moistening when adding the ^{35}S solution. All samples were incubated at 20 °C in darkness (closed incubation). Moisture level were maintained at 75% field capacity by weight throughout the incubation period.

Three flasks were sampled from each soil on the basis of : (1) short-term (1,2,3 and 5 days), (2) medium-term (10,16,24 and 32 days) and (3) long-term (60,90 and 120 days) time period. Soil samples were analysed for extractable sulphate-sulphur, biomass-sulphur, HI-reducible sulphur and total organic sulphur as described in chapter 3. The amount of radioactivity was also determined in each of the sulphur fractions (see section 7.2.5).

7.2.3 Addition of ^{35}S-labelled K_2SO_4 to soils

The Teviot (limed) and Meyer soils were preconditioned for two weeks as described above and then were weighed (30 g air-dried) into 100 ml conical flasks. Radioactive solution (4 ml, giving 5.866 μCi ^{35}S and 8.10 μg SO_4-^{32}S g^{-1} soil.) was added to each flask in a similar way to that described in section 7.2.4. Soils were incubated at 20 °C in darkness. Three flasks were sampled from both soils at 1,2,3,5,8,16,24 and 32 days.

7.2.4 Radioactivity assay of ^{35}S

The amount of sulphur-35 present in soils as sulphate-S, biomass-sulphur and total sulphur was determined by counting the radioactivity present in the extracts used to determine ^{32}S. Aliquots (1 ml) of the filtered extracts were mixed with 10 ml of scintillation cocktail. The cocktail was a 2:1 mixture of toluene and a surfactant, triton X-100 (scintillation grade). A small amount (6.0 g l^{-1}) of 2,5-Diphenyloxazole was also added to increase the scintillation energy of the cocktail. Addition of triton X-100 to the cocktail maintained a clear and stable emulsion of toluene with either KH_2PO_4 or NaOH based solution. The counting efficiency of the ^{35}S in solution was in the range of 94 to 97% . This was a vast improvement over the Blair and Croft (1969) 1,2-dioxane based cocktail where addition of KH_2PO_4 extracted solution or NaOH entrapped H_2S solution resulted in a cloudy suspension, which caused high quenching of the β particles, resulting in a counting efficiency of only 50-60%. The ^{35}S activity was measured using a Phillips PW-4700 β-liquid scintillation counter. Sulphur-35 present as HI-reducible sulphur was determined by measuring the activity in the NaOH solution used to entrap H_2S gas. The ^{35}S present in each fraction was expressed as a percentage of the total ^{35}S initially added to soils. Isotopic decay was taken into account by comparing the activity of samples with standards counted at the same time.

7.3 RESULTS AND DISCUSSION

7.3.1 Results from carrier-free ^{35}S experiments

7.3.1.1 *Net mineralisation/immobilisation of ^{32}S*

The net mineralisation/immobilisation of sulphur was calculated by subtracting the initial concentration of KH_2PO_4 extractable sulphate-S from the concentration of sulphate-S present in the soils at the end of each sampling interval. Initial concentrations of sulphate-S in the soil are shown in Table 7.2. It is noticeable that the amounts of sulphate-S are higher in the preconditioned soil than the other two treatments. This increase is due to mineralisation which occurred during the two week period of preconditioning. The higher figure of 18.9 μg S g^{-1} soil was used for calculating the net mineralisation in the preconditioned soil.

The type of soil treatment had a significant effect on the amount of sulphur mineralised or immobilised. The preconditioned soil showed a small net mineralisation of SO_4^{2-} during the course of the incubation (Table 7.3). The air-dried sample showed a greater amount of mineralisation, and the addition of glucose caused immobilisation of soil sulphate-S in the short to medium-term, followed by net mineralisation in the long-term (in this case 60 and 90 days). These effects of adding a carbon source on sulphur mineralisation are consistent with those observed by previous workers (Saggar *et al.*, 1981b; Swift, 1983).

The amounts of sulphur mineralisation/immobilisation reported in Table 7.3 show an overall picture of the processes taking place during the short, medium and long-term. A more detailed picture, based on all the individual sampling periods is plotted in Fig. 7.1. Mineralisation of sulphur in the preconditioned soil fluctuated a little during the first 5 days of incubation and reached a plateau at about the 16th day. Thereafter, there was very little change in the amount of net mineralised-SO_4^{2-} in this treatment. In the air-dried soil, mineralisation increased until the 60th day of incubation followed by a significant drop in mineralisation between 60 and 120 days. Glucose treated soil showed a rapid immobilisation of soil sulphate-S, with approximately 25% of the soil sulphate-S (3.8 μg S g^{-1} soil) being immobilised within 24 hours of incubation. A maximum of 90% of the soil sulphate-S was immobilised by the 5th day of the incubation. The amount of net

Table 7.2 Concentration of KH_2PO_4 extractable sulphate-S in soils prior to incubation.

Treatment	Sulphate-S (μg S g^{-1} soil)
Preconditioned	18.9
Air-dried	15.2
Glucose-treated	15.2

Table 7.3 Net mineralisation/immobilisation of sulphur as sulphate in preconditioned, air-dried and glucose-treated soils.

Treatment	Sulphate-S mineralised/immobilised* (μg S g^{-1} soil)		
	short-term[1]	medium-term[2]	long-term[3]
Preconditioned	1.8 ± 0.5	3.2 ± 0.2	3.4 ± 0.3
air-dried	3.3 ± 0.7	7.5 ± 0.7	9.5 ± 0.6
glucose-treated	-8.7 ± 1.8	-2.5 ± 2.3	7.2 ± 0.7

* negative values indicate immobilisation.

1. mean ± s.e of mineralisation/immobilisation at 1,2,3 and 5 days.
2. mean ± s.e of mineralisation/immobilisation at 10,16,24 and 32 days.
3. mean ± s.e of mineralisation/immobilisation at 60,90 and 120 days (glucose-treated values were calculated from 60 and 90 days only).

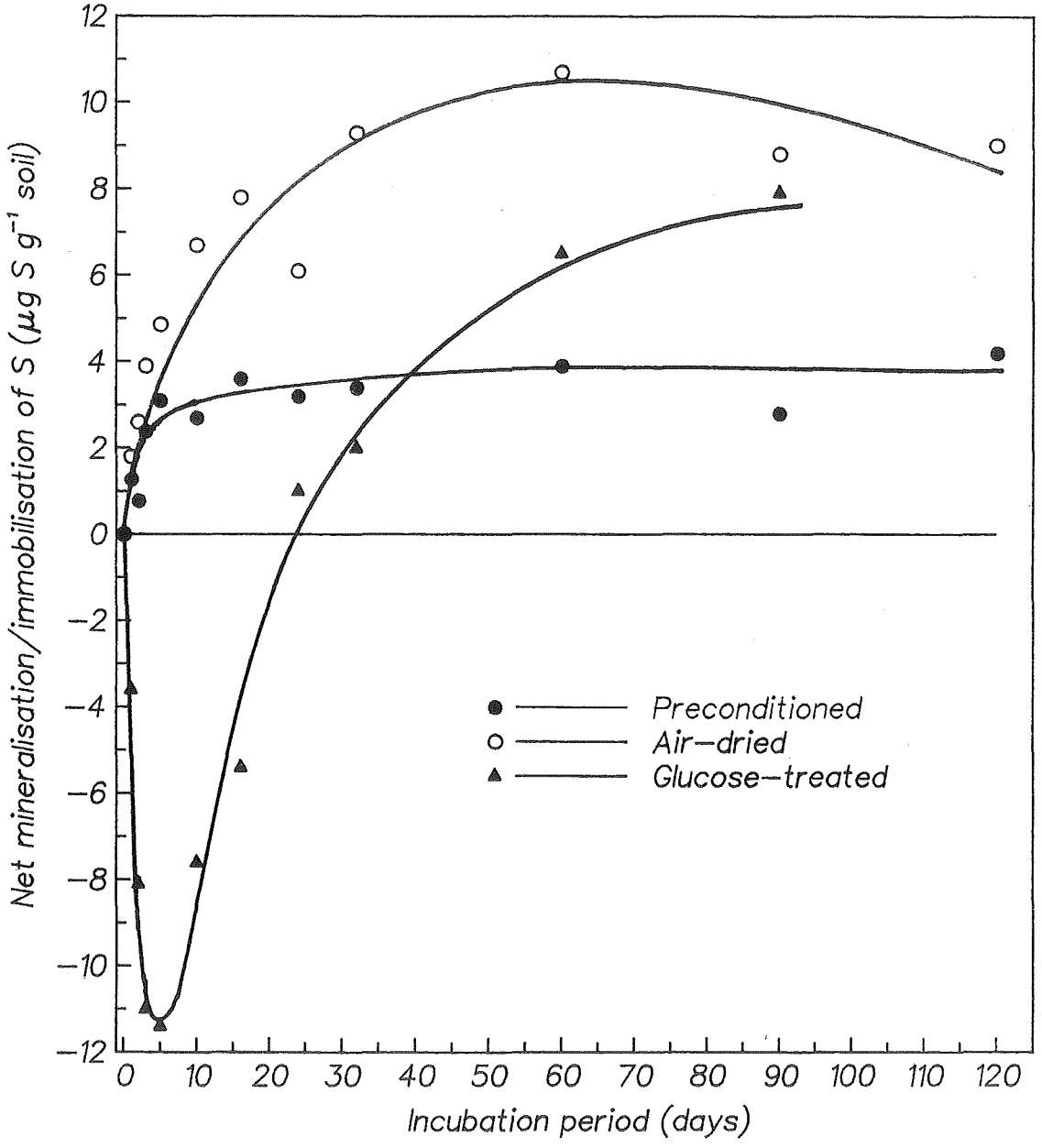


Fig. 7.1 Effect of soil treatments on the pattern of sulphur mineralisation and immobilisation in soils.

Table 7.4 Biomass-sulphur (K_S = 0.40) in preconditioned, air-dried and glucose-treated soils.

Treatment	Biomass-sulphur (μg S g^{-1} soil)		
	short-term[1]	medium-term[2]	long-term[3]
Preconditioned	6.5 ± 0.5	8.9 ± 1.3	7.3 ± 1.8
air-dried	5.0 ± 1.2	8.6 ± 1.4	5.6 ± 0.5
glucose-treated	10.6 ± 2.2	8.2 ± 0.8	6.3 ± 0.4

1. mean ± s.e of biomass-S at 1,2,3 and 5 days.
2. mean ± s.e of biomass-S at 10,16,24 and 32 days.
3. mean ± s.e of biomass-S at 60,90 and 120 days (glucose-treated values were calculated from 60 and 90 day only)..

immobilised-SO_4^{2-} dropped sharply between days 10 and 24, indicating the end of the log phase of soil microbial growth. Net mineralisation in the glucose-treated soil could only be measured after the 24th day of incubation. Thereafter, considerable amounts of sulphur were mineralised.

The size of the organic sulphur fractions i.e. HI-reducible sulphur and C-bonded sulphur remained relatively stable throughout the incubation period (Appendix 3). There were significant differences however in biomass-sulphur between the treatments, especially in the short-term (Table 7.4). The differences in biomass-sulphur between the preconditioned and air-dried soils in the short-term were not significant. However, the glucose-treated soil had approximately twice as much biomass-sulphur compared to the other two treatments. This clearly shows that the immobilisation observed in the glucose-treated soil in the short-term was related to the increase in biomass-sulphur, with microbes utilising soil sulphate-S to assimilate the added glucose-carbon. Such increases in biomass-sulphur levels brought about by the addition of glucose appear to be relatively short-lived because the biomass-sulphur content in these soil samples decreased in the medium to long-term. This decrease is again directly related to the decrease in the amounts of immobilised-SO_4^{2-} reported in Table 7.3. In contrast, biomass-sulphur in the other treatments showed a slight increase in the medium-term. In the long-term, the amount of biomass-sulphur decreased in all soils. However, there was no significant difference in the biomass-sulphur between treatments in the medium to long-term (10-120 days).

7.3.1.2 *Incorporation of the added ^{35}S*

There were very high recoveries of the added ^{35}S throughout the incubation period. Recoveries of ^{35}S were not affected by the soil treatments. On average between 94 and 103% of the added ^{35}S was recovered during the incubation. These figures are consistent with recoveries of nearly 100% reported by McLaren *et al.*, (1985) when carrying out similar studies. The results reported here in the Figures and Tables are the means of six determinations. The standard errors involved in determining the values between replicates were relatively small (generally less than 2%).

7.3.1.2.1 Preconditioned soil

The amount of added carrier-free sulphur-35 present as extractable sulphate-S decreased with time and at the same time there was a corresponding increase in the ^{35}S incorporation into organic sulphur fractions (Fig. 7.2). A high proportion of the total incorporation took place within the first 16 days of the incubation during which time approximately 32% of the added ^{35}S was incorporated (representing 76% of the total incorporation). Thereafter, incorporation was at a slower rate, reaching 42% after 32 days. There was very little change in the amount of incorporated ^{35}S between 32-120 days, indicating a state of approximate equilibrium between the incorporation and the release of ^{35}S from organic sulphur.

The specific activity of the extractable sulphate-S decreased steadily until the 16th day, but thereafter remained relatively constant (Fig. 7.5), suggesting that incorporation of ^{35}S into organic sulphur and the production by mineralisation of sulphate-^{35}S from recently incorporated organic sulphur was taking place at a similar rate. Maynard *et al.*, (1983) and McLaren *et al.*, (1985), both found similar patterns in the specific activity of extractable sulphate-S. These workers have suggested that a constant specific activity of sulphate-S during this period could be due to the release of sulphate from an organic fraction which had a similar specific activity to the extractable sulphate-S form.

In the first two days, incorporation of ^{35}S was predominantly into HI-reducible forms of sulphur, accounting for between 80 and 100% of the sulphur incorporated into organic fractions (Fig. 7.2). The proportion of ^{35}S incorporated into this form of organic sulphur decreased after this, and throughout the rest of the incubation averaged 65% of the total incorporation. With increased time of incubation, the specific activity of HI-reducible sulphur also increased (Fig. 7.6). On average, ^{35}S present in C-bonded forms of S accounted for 35% of the total incorporation.

Although traces of ^{35}S were detected in the biomass sulphur in the early stages of the incubation, there was no significant labelling of the microbial biomass until the 24th day of the incubation (Table 7.5). From that time onwards, although there was considerable

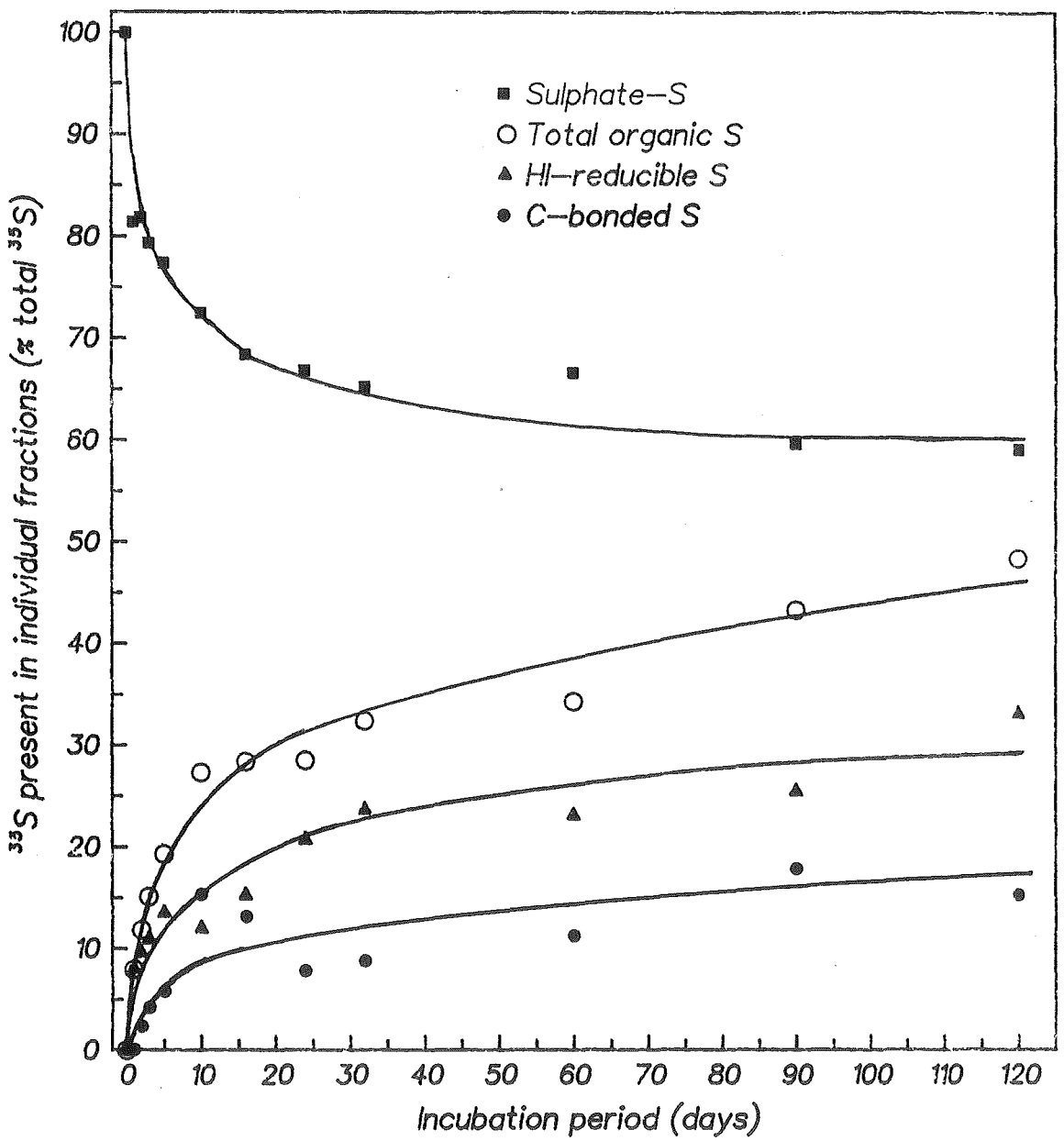


Fig. 7.2 Incorporation of sulphur–35 into different sulphur fractions of the preconditioned soil.

fluctuation between the sampling periods, a significant proportion of the incorporated ^{35}S (between 6-18%) appeared in the microbial biomass.

7.3.1.2.2 Air-dried soil

Fig 7.3 shows that by the end of the incubation (120 days), there was a similar level of ^{35}S incorporation in the air-dried soil, as was found for the preconditioned soil. However, there were some significant differences in the pattern of incorporation between the two treatments. During the first five days, incorporation of ^{35}S occurred at a faster rate in the air-dried compared to the preconditioned soil. This could be due to a sudden flush of microbial activity caused by wetting the air-dried soil which enhanced the incorporation in that early period of incubation. Sulphur-35 incorporation gradually increased to approximately 42% after the 32nd day of incubation. Thereafter, a slight decrease in ^{35}S incorporation was noted together with an increase in ^{35}S present as extractable sulphate-S. The amount of ^{35}S incorporation observed in this study is reasonably consistent with the work of Freney *et al.*, (1971) who reported about 50% incorporation of added sulphur-35 in some Australian soils. However, similar studies conducted on Scottish soils showed only 27% incorporation (McLaren *et al.*, 1985). This illustrates that there can be a considerable difference in the amounts of incorporated ^{35}S between soils.

The specific activity of sulphate-S in the short-term showed a greater decline in the air-dried than the preconditioned soil (Fig. 7.5). This could have been due to relatively higher amounts of incorporation of ^{35}S into organic fractions, and also at the same time more ^{32}S mineralisation. The specific activity in the later stages of the incubation was similar to that found in the preconditioned soil and likewise showed minimal changes after the 16th day.

In the first three days, a smaller proportion of the ^{35}S incorporated was present in HI-reducible forms compared to the preconditioned soil (Fig. 7.3) Approximately 60% of the total incorporated ^{35}S being found in HI-reducible forms. This proportion fell to approximately 50% until the last 60 days of the incubation, during which time, although the total level of ^{35}S incorporated remained relatively constant, there was a gradual increase in ^{35}S labelled as HI-reducible S and a corresponding decrease in the ^{35}S

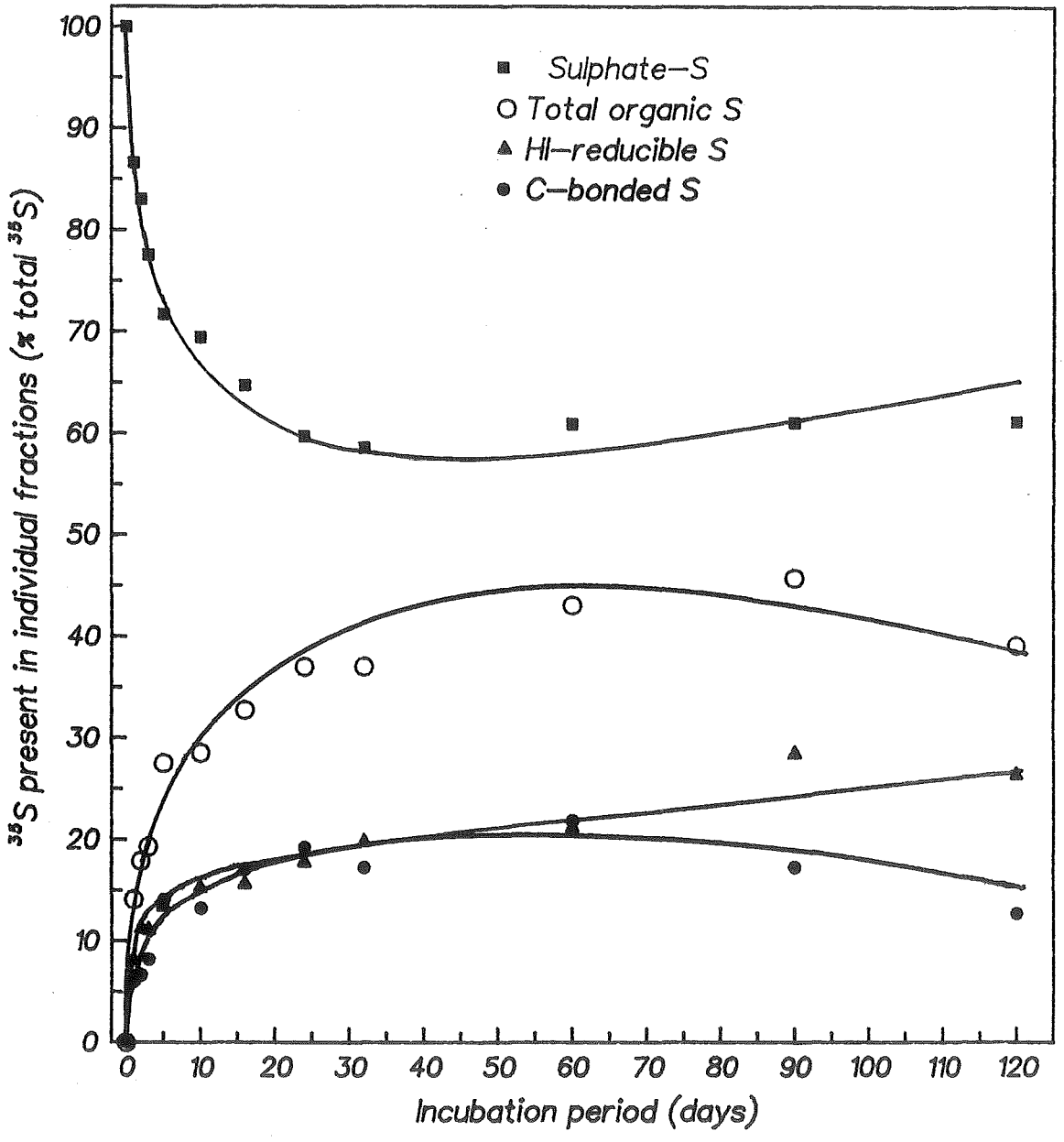


Fig. 7.3 Incorporation of sulphur–35 into different sulphur fractions of the air–dried soil.

present in C-bonded form of sulphur (Fig. 7.3). This is illustrated even more clearly in Fig. 7.6 which shows similar specific activities in these two organic fractions until the 60th day. After this period, labelling of the HI-reducible fraction increased significantly with a corresponding decrease in the labelling of C-bonded forms of sulphur. This suggest that the increase in the HI-S was due to the transformation of some of the C-bonded sulphur into HI-reducible forms of sulphur. After 120 days, the proportional labelling of HI-S and C-bonded S were very similar to that found in the preconditioned soil. The proportional labelling of these two organic sulphur forms are significantly different from that reported by McLaren *et al.*, (1985), who found that the incorporation was mainly into HI-reducible forms (89% into HI-reducible forms and 11% into C-bonded sulphur).

Labelling of the biomass with ^{35}S occurred much more quickly with the air-dried soil compared to the preconditioned soil. Significant amounts of ^{35}S were observed in the biomass after 3 days of incubation (Table 7.5). Once again, the amounts of ^{35}S measured in the biomass-S fluctuated between samplings. However, from day three onwards a substantial proportion of the incorporated ^{35}S (6-24%) appeared in the microbial biomass. The specific activity of biomass-S fluctuated greatly throughout the incubation (Fig 7.7). Biomass from air-dried soil exhibited greater amounts of labelled ^{35}S than the preconditioned soils. However, at the end of the incubation, biomass from both treatments were labelled at the same rate.

7.3.1.2.3 Glucose-treated soil

The incorporation of ^{35}S into the glucose-treated soil was much more rapid, and the amount of incorporation was far greater compared to the other two treatments (Fig. 7.4). By the third day, approximately 67% of the added ^{35}S was incorporated into organic sulphur forms, and by the 24th day a maximum incorporation of over 80% was observed. The specific activity of the extractable sulphate-S decreased rapidly until the 24th day (Fig. 7.5). From the 32nd day and onwards, the specific activity of extractable sulphate-S again increased slightly but significantly. Unlike the other two treatments i.e. air-dried and preconditioned soils, the total incorporation of the ^{35}S decreased after this time and was at a level of 75% when the incubation was discontinued after 90 days. The amount of incorporation found in the glucose-treated soil was of a similar magnitude to that

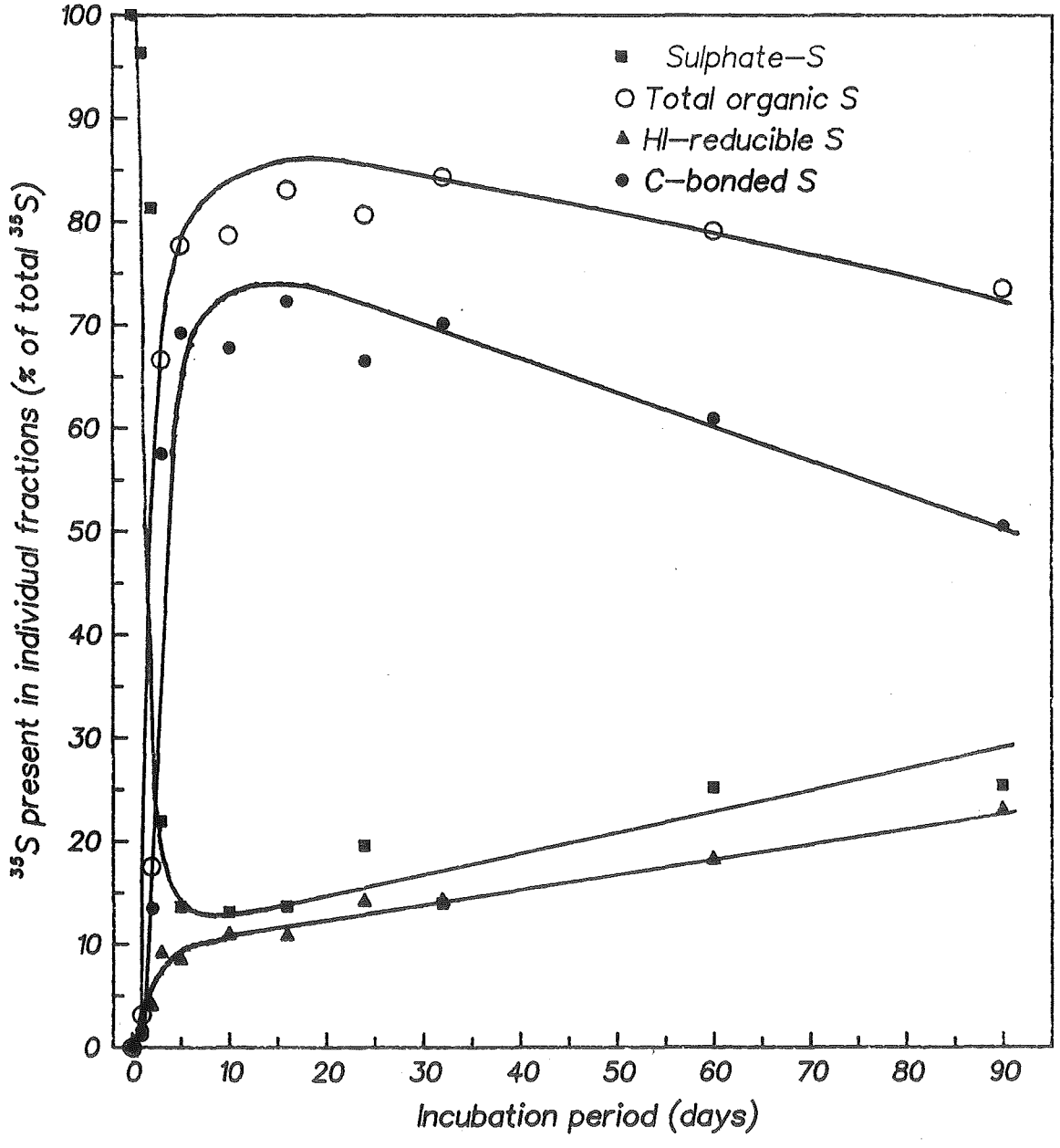


Figure 7.4 Incorporation of sulphur–35 into different sulphur fractions of the glucose–treated soil.

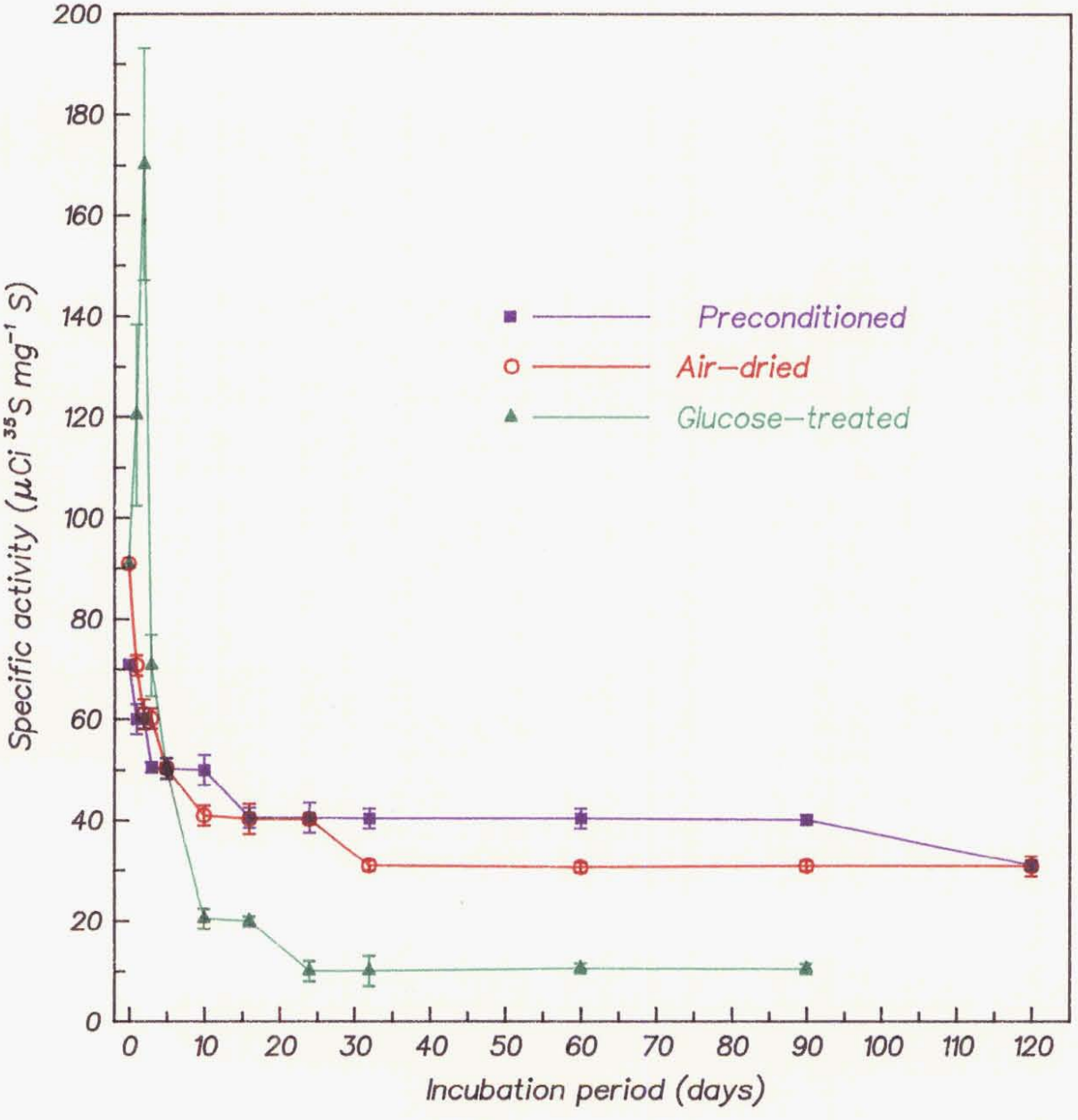


Figure 7.5 Specific activity of KH_2PO_4 extractable sulphate–S pool in the preconditioned, air–dried and glucuse–treated soils.

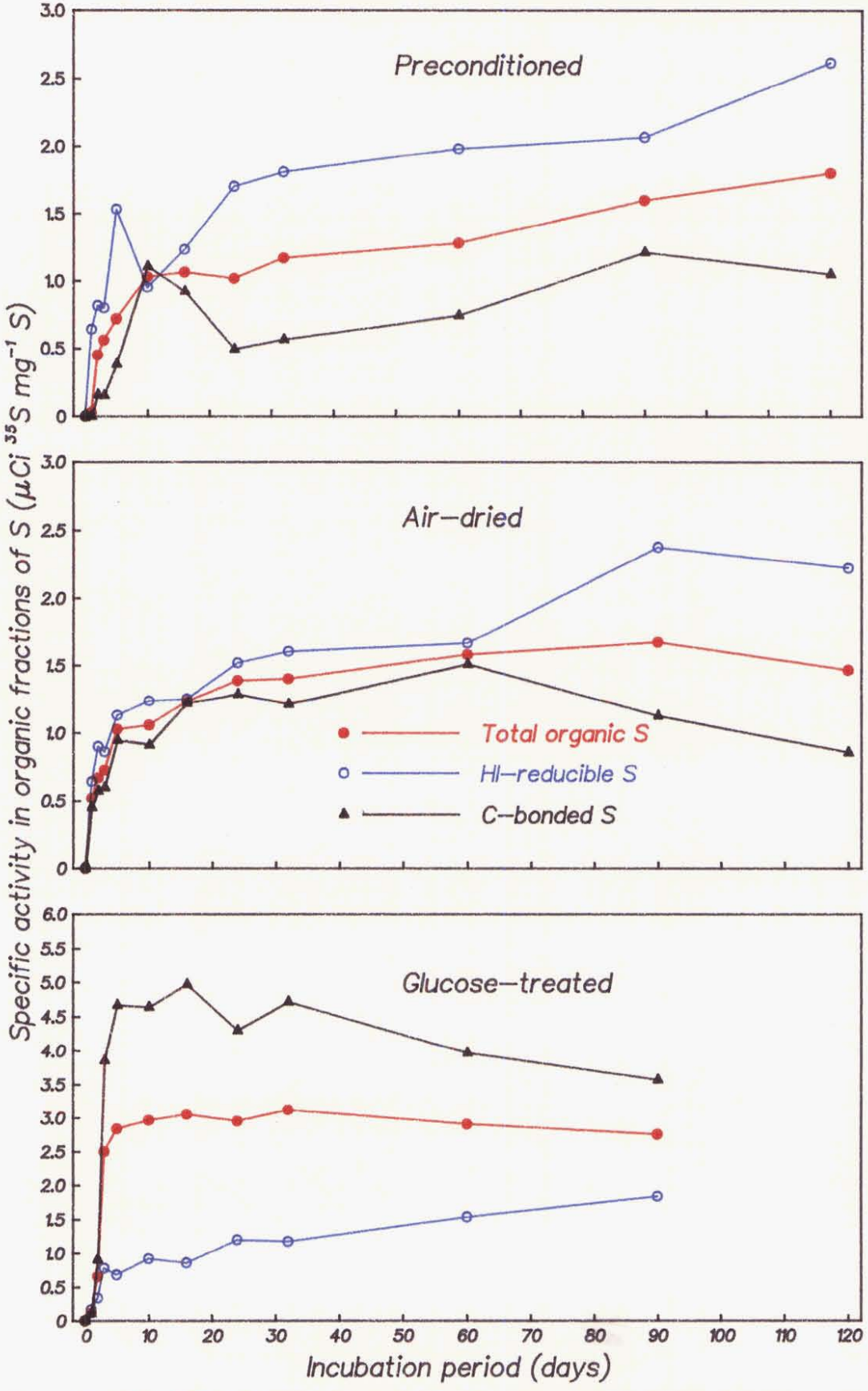


Figure. 7.6 Specific activity of total organic sulphur, HI–reducible S and C–bonded S in the preconditioned, air–dried and glucose–treated soils.

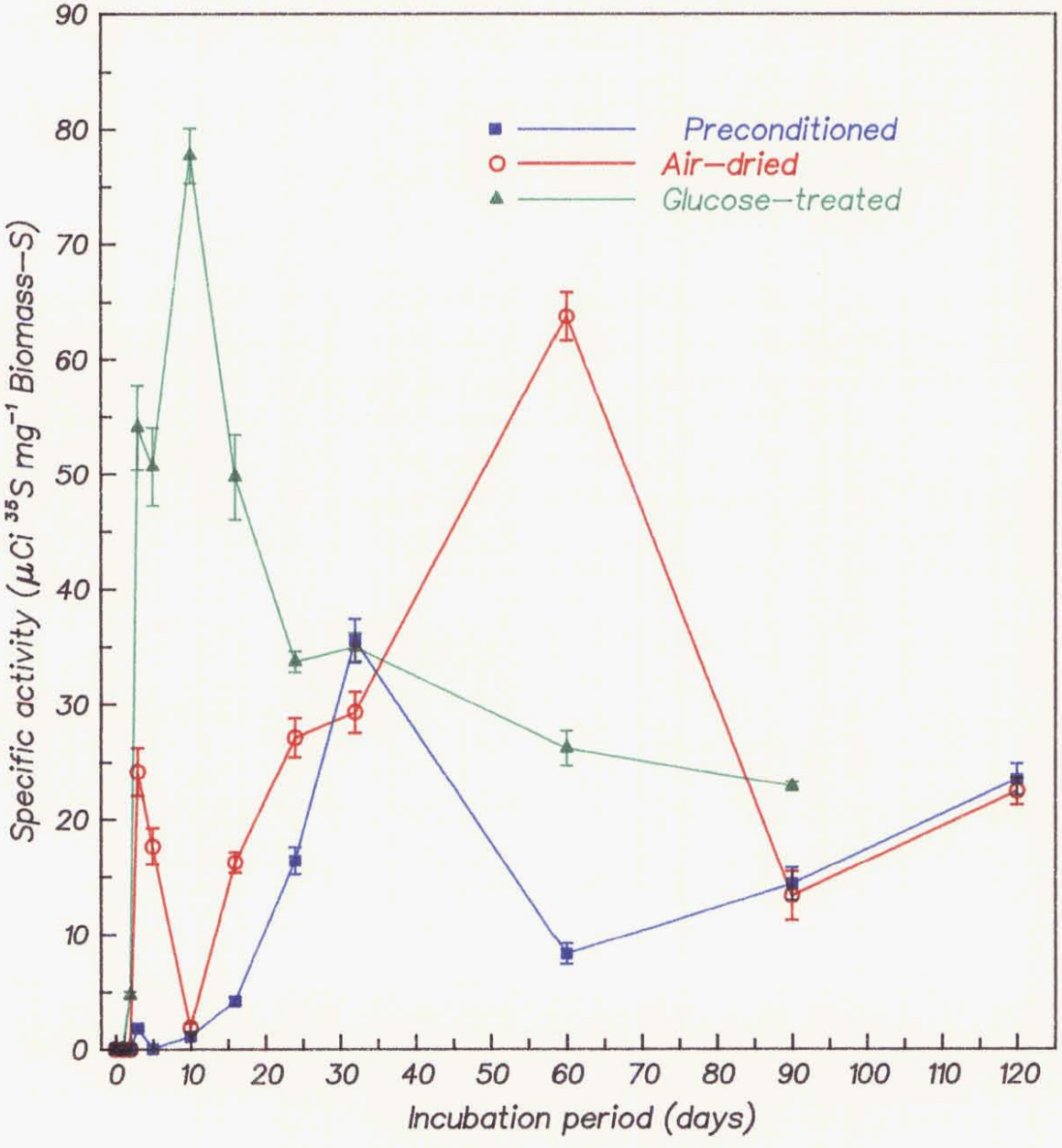


Figure 7.7 Specific activity in biomass–sulphate–S pool of the preconditioned, air–dried and glucose–treated soils.

Table 7.5 Percentage of applied ^{35}S present as biomass-Sulphur.

Incubation Period (days)	Treatment		
	preconditioned	air-dried	glucose-treated
	(% of applied ^{35}S)[1]		
1	(0.0)[2]	(0.0)	(0.0)
2	(0.0)	(5.0)	(2.4)
3	(1.6)	8.0	59.9
5	(0.0)	8.1	50.4
10	(1.0)	7.6	46.5
16	(1.9)	7.9	33.5
24	9.5	15.6	14.0
32	18.0	12.0	14.9
60	6.0	24.0	9.4
90	10.0	6.0	11.0
120	6.1	10.4	n.d.

1. $K_S = 0.40$

2. values presented inside the parentheses are not significantly different from zero.

n.d = not determined

reported by Freney *et al.*, (1971), who also found that addition of glucose resulted in approximately 80% incorporation. However, in contrast to their studies, where most of the incorporation occurred in HI-reducible forms of sulphur, this present study showed 77-89% incorporation into C-bonded forms. Results from this present study tend to be more in agreement with those reported by McLaren *et al.*, (1985) who also found that addition of glucose increased the ^{35}S incorporation into C-bonded forms of sulphur. In this study, these effects are very pronounced. The reason for such an increase is based on the availability of metabolisable carbon which increases the synthesis of microbial cells in which most of the sulphur-35 is incorporated into sulphur containing amino acids. As the time of incubation increased, the proportion of C-bonded forms of sulphur-35 decreased from 84 to 73%. During the same period, the incorporation of ^{35}S in HI-reducible forms actually increased, as did the ^{35}S present as sulphate-S indicating a redistribution of ^{35}S from C-bonded ^{35}S into these forms of sulphur.

The amounts of ^{35}S determined in the biomass during the first 16 days of the incubation were significantly higher for the glucose-treated soil than for the other two treatments (Table 7.5). At day three, approximately 60% of the added ^{35}S (90% of the incorporated ^{35}S at this time of the incubation) appeared to be present in the biomass. However, the amount of ^{35}S in the biomass decreased substantially after this time. In the long-term (60-90 days), ^{35}S present in the biomass had fallen to a similar level to the other treatments. During the early period of incubation, the biomass immobilised large amounts of soil sulphate-S (Table 7.4) and also utilised the added ^{35}S. During this period, the specific activity of the biomass-S increased to very high level (up to 78 μCi mg^{-1} biomass-S)(Fig. 7.7). As the time of incubation increased, the large amounts of ^{35}S were released from the biomass-S, reducing the specific activity considerably. However, even at the later stages, the biomass in the glucose-treated soil retained relatively more labelled ^{35}S than either of the other two treatments.

7.3.1.3 Discussion (Carrier-free ^{35}S)

Results obtained from the above study show a significant effect of soil treatment on net sulphur mineralisation/immobilisation, biomass-sulphur and also on the amounts and nature of ^{35}S transformations between inorganic and organic forms. Preconditioning

before ^{35}S addition, which brought the soil system to a relatively steady state both chemically and biologically, showed a lower net mineralisation and a steadier rate of sulphur-35 transformation compared with the other two treatments. When microbial activity was enhanced at the time of ^{35}S addition, by adding moisture to air-dried soil, either with or without the addition of glucose, higher rates of ^{35}S transformation were observed. The glucose treatment in particular would have stimulated microbial growth and increased the demand for sulphur for the synthesis of amino acids. This caused large amounts of sulphate-S to be immobilised by the micro-organisms as shown by the biomass sulphur values (Table 7.4). Approximately 57% of the extractable sulphate-S was immobilised in the short-term by this treatment. Most of the immobilised sulphate-S during this period was utilised by micro-organisms. A difference between the glucose-treated and air-dried soil biomass-S in this period shows that about 65% of the immobilised sulphate-S in the glucose treated soil was retained in the microbial bodies. This was further confirmed by the ^{35}S results (Table 7.5) which show a high percentage, between 50-60%, of the added ^{35}S was incorporated in microbial tissues.

The concurrent nature of sulphur mineralisation and immobilisation observed by other workers such as Maynard *et al.*, (1983) and Swift, (1983) was also apparent in this study. With the air-dried and preconditoned soils, a continuous net mineralisation was observed throughout the incubation period, and at the same time the amount of incorporation of the added ^{35}S into organic fractions increased with time. This illustrates the co-occurrence of the mineralisation and immobilisation processes, where ^{32}S was mineralised at the same time as a substantial amounts of ^{35}S were immobilised. Quantification of these two processes together in the soil system is very difficult, especially when transformations have not reached equilibrium. However, if equilibrium has been reached then a quantification of the over-all cycling of sulphur is possible, as has been suggested by McLaren *et al.*, (1985) who calculated the amount of sulphur actively being cycled during the incubation. This is only possible when the specific activity of the inorganic sulphate-S and the sulphur in organic fractions remain at a constant level. During the course of this study, especially in the long-term, specific activity has been relatively constant in the total organic sulphur pool but it has been changing in the HI-reducible and C-bonded forms throughout the incubation. Such a change was also noted in the extractable sulphate-S pool to some extent.

Generally, the specific activity of HI-reducible sulphur increased with an increase in the incubation period. This increase was at the expense of C-bonded forms of sulphur which showed a decrease at the later stages of the incubation period. This effect was particularly pronounced in the air-dried and glucose-treated soils. Since the amount of C-bonded sulphur in the organic fractions did not change significantly during the period when specific activity decreased (Appendix 1), it would appear that recently incorporated sulphur-35 in the C-bonded sulphur had been mineralised. This was obvious in the air-dried and glucose-treated soils where the amounts of sulphur-35 retained in the carbon-bonded fraction decreased significantly (Fig 7.3 and 7.4). Such decreases in the C-bonded forms of sulphur after the maximum incorporation of ^{35}S in glucose treated soil, were accompanied by an increase in the labelling of HI-reducible forms of sulphur but also showed a significant increase in extractable sulphate-S. This shows that sulphur recently incorporated into C-bonded forms is particularly susceptible to remineralisation to sulphate or possibly conversion to HI-reducible forms. It is most likely the nature of organic compounds with which carbon-bonded sulphur is associated rather than the C-bonded sulphur itself which determines the ease of mineralisation. Therefore, the amount of remineralisable C-bonded sulphur is probably being limited by the nature of complexing e.g., whether C-S is present in free amino acid form or held in peptide chains or is further complexed with high molecular weight organic acids. The mineralising behaviour of the recently incorporated C-bonded sulphur is very similar to that observed for the sulphur-containing amino acid methionine, when incubated with forest soil (e.g., Fitzgerald and Andrew, 1984; Fitzgerald *et al.*, 1984). The methionine, in which sulphur is retained as carbon-bonded sulphur was rapidly converted to sulphate-S and HI-reducible forms of organic sulphur. However, the rate at which added methionine has been mineralised in these studies is considerably faster than what we observed in this study. This is because the added methionine is comparatively easier to utilise than the C-bonded S found/formed in soils and therefore micro-organisms would find this easier to degrade. Hence, the difference between the rate of mineralisation of recently incorporated C-bonded sulphur and added methionine is due to the difference in the complexity.

Differences in the short-term incorporation of ^{35}S between treatments also raise some interesting points. When the soil was preconditioned, to avoid a sudden burst of microbial

activity at the time of ^{35}S addition, most of the short-term incorporation was into the HI-reducible fraction (Fig. 7.2). For the other two treatments, the glucose-treated sample in particular, a much larger proportion of the initial incorporation was into carbon-bonded forms of organic sulphur. The short-term incorporation in the preconditioned soil may have been predominantly by biochemical processes, where extracellular enzymes transformed inorganic ^{35}S into organic fractions without the direct involvement of the soil micro-organisms (McGill and Cole, 1981). Extracellular formation of sulphated polysaccharide has been found in certain bacterial strains (Taylor and Novelli, 1969). These workers were able to isolate a soil bacterum which when grown in $^{35}SO_4$ synthesized a polysaccharide ester sulphate, which can be classified as HI-reducible S. This could be one of the reasons in this case why despite the appreciable incorporation of the added sulphur-35 into organic fractions, there was significant radioactivity found in the biomass during the incubation. However, determination of low activity the in biomass may also have been due to the method employed to measure microbial biomass-S. The method could under-estimate biomass-s for two main reasons. Firstly, all micro-organisms may not have behave in the same way towards the fumigation technique used, and secondly, the proportion of the biomass sulphur not released by fumigation could have contained a disproportionate amount of incorporated ^{35}S. In contrast to the preconditioned soil, in the glucose-treated soil in particular, the large increase in microbial biomass would have ensured a high level of incorporation of ^{35}S into microbial tissues, predominantly as carbon-bonded sulphur in amino acids and proteins. It would explain why specific activity in biomass during this period increased to very high levels (Fig. 7.7). This also caused high labelling of carbon-bonded sulphur (Fig. 7.6).

Specific activity in the biomass fluctuated considerably throughout the incubation period. These fluctuations are significantly different between the sampling intervals (Fig. 7.7) and appear to be following a time series pattern, where incorporation of ^{35}S into biomass-S reaches a maximum and then decreased sharply. At least two such cycles were observed in the air-dried and preconditioned soils. In the glucose-treated soils this event was observed only once during the incubation period. Such a rapid change in the specific activity indicates the speed at which S is transformed through the biomass. It also reflects the transitory nature of the S held in this pool. Clear evidence of this can be cited from the glucose-treated soil where sharp decrease in the specific activity of the biomass

is related to a decrease in the C-bonded S specific activity. This would suggest that C-bonded sulphur in the biomass has been remineralised during the long-term. This prersent study tends to agree to the suggestion made by Biederbeck (1978), who stated that sulphur held in the microbial tissues is of a labile nature.

7.3.2 Results from labelled sulphate-^{35}S

7.3.2.1 *Net mineralisation/immobilisation*

Addition of sulphate-S has shown relatively small amounts of S mineralisation in both soils. There was no net change in the amount of sulphate-S in the Teviot (limed) soil during the short-term (Table 7.6). In the medium-term, relatively low levels of sulphur mineralisation were observed, giving a net mineralisation of 2.2 μg SO_4-S g^{-1} soil. These values are directly comparable to the preconditioned soils used in the carrier-free sulphur-35 experiments (Table 7.3), show considerably lower amounts of net mineralisation of sulphur. The Meyer soil which is classified as a sulphur deficient soil, showed a net immobilisation of sulphate in the short-term, but towards the end of the incubation it had shown a net mineralisation. Due to preconditioning of the soil, there was an appreciable amount of sulphur retained as microbial biomass-sulphur. The Teviot (limed) soil contained significantly higher amounts of biomass-sulphur in the short-term than the Meyer soil (Table 7.7). In the medium-term, the differences in biomass-sulphur between the two soils were non-significant.

7.3.2.2 *Incorporation of labelled sulphate-^{35}S*

There were significant differences in the incorporation of ^{35}S when added with or without carrier sulphate. Results from this study for the Teviot soil are directly comparable with that of the preconditioned soil used in the Part I of this study. Addition of ^{35}S with sulphate-S markedly reduced the incorporation of the added ^{35}S. With carrier sulphate in the short and medium-term 10 and 23% respectively of the added ^{35}S was incorporated into organic fractions (Fig. 7.8), compared to 23 and 35% incorporation with carrier-free ^{35}S at a similar periods of time. In the first 3 days, relatively higher amounts of ^{35}S were incorporated in the Meyer soil compared to the Teviot (limed) soil but in the medium-term both soils incorporated similar amounts of added ^{35}S. Specific activity in the extractable

Table7.6 Net mineralisation/immobilisation of sulphur in sulphate-^{35}S treated soils during the 32 day incubation period.

Soils	Sulphate-S mineralised/immobilised* (μg S g^{-1} soil)	
	short-term[1]	medium-term[2]
Teviot (limed)	0.3 ± 0.4	2.20 ± 0.6
Meyer	-2.0 ± 0.3	1.70 ± 0.2

* negative values indicate immobilisation.

1 mean ± s.e. of mineralisation/immobilisation occurred at 1,2,3,5 days.

2 mean ± s.e. of mineralisation/immobilisation occurred at 8,16,24 and 32 days.

Table7.7 Biomass-sulphur (K_S =0.40) in the Teviot and Meyer soil.

Soils	Biomass-sulphur (μg S g^{-1} soil)	
	short-term[1]	medium-term[2]
Teviot (limed)	8.6 ± 3.1	5.1 ± 0.9
Meyer	4.8 ± 0.3	4.0 ± 1.1

1. mean ± s.e. of biomass-S at 1,2,3 and 5 days.
2. mean ± s.e. of biomass-S at 8,16,24 and 32 days.

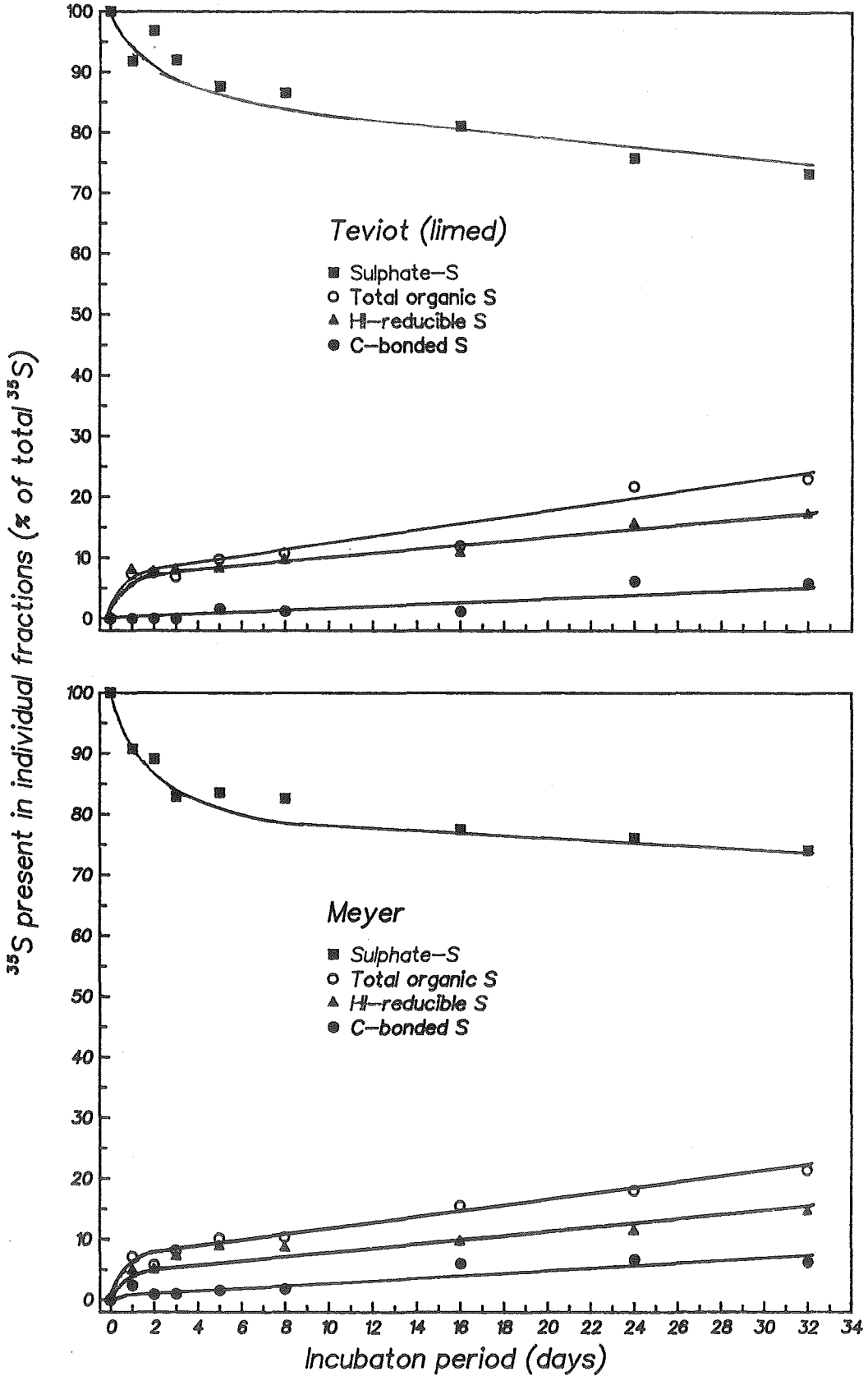


Figure 7.8 Incorporation of sulphate–^{35}S into different fractions of sulphur in the Teviot (limed) and Meyer soils.

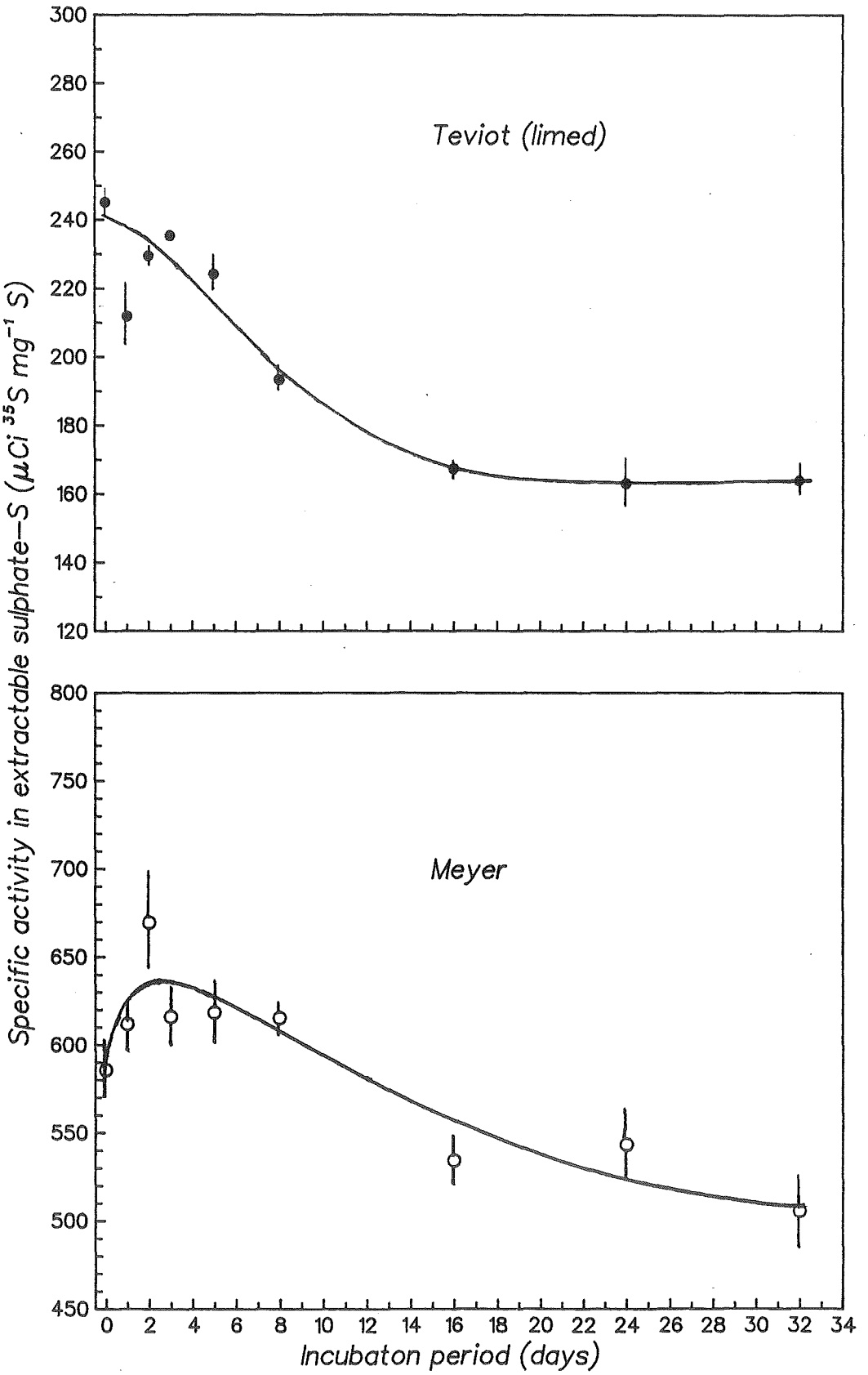


Figure 7.9 Specific activity in the KH_2PO_4 extractable sulphate–S in the Teviot (limed) and Meyer soils.

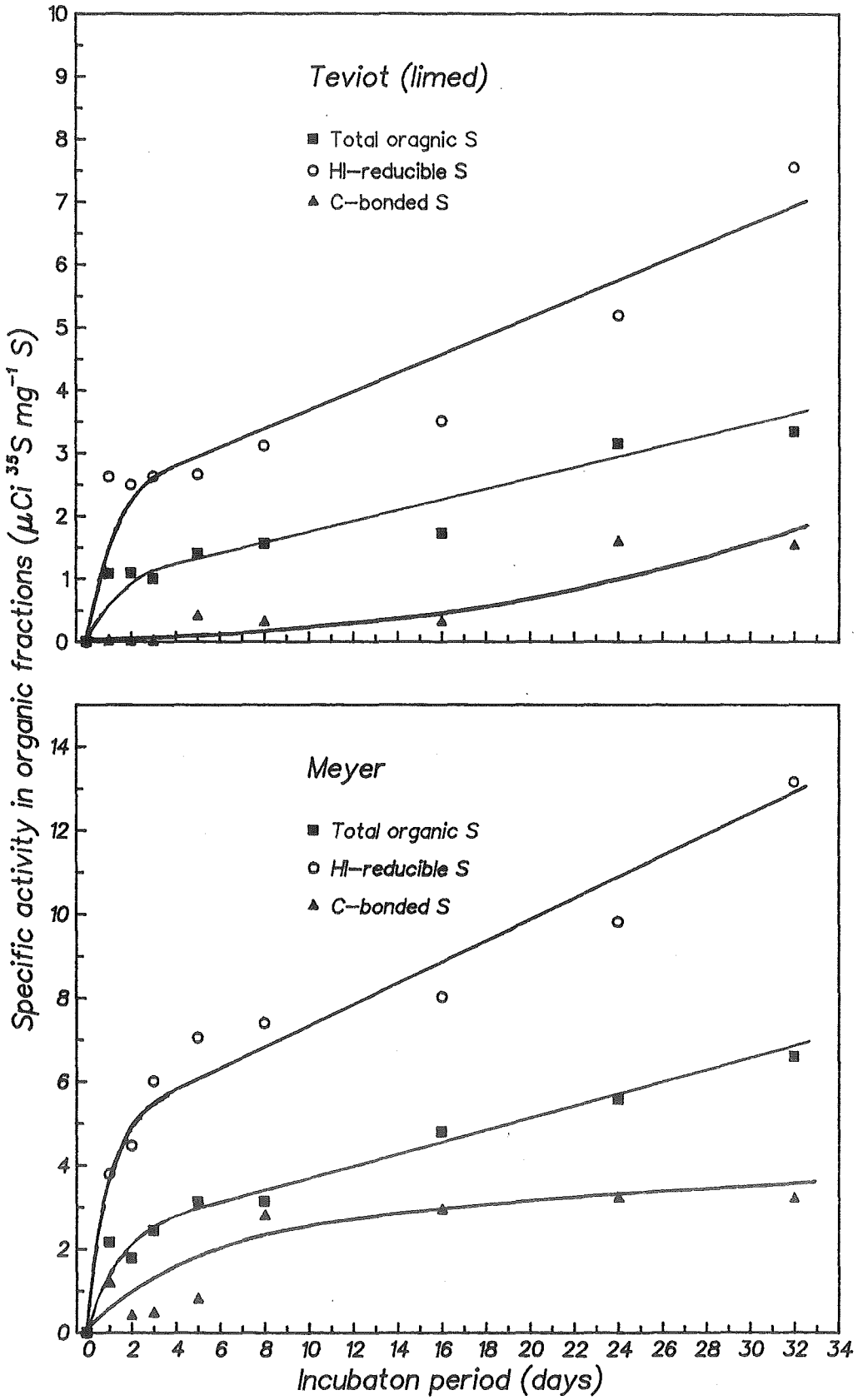


Figure 7.10 Specific activity in organic fractions of sulphur during the incubation in the Teviot (limed) and Meyer soils.

sulphate-S of the Teviot (limed) soil generally decreased with an increase in incubation period (Fig. 7.9). The decrease in specific activity between 24 and 32 days was non-significant, showing an approximate equilibrium between incorporation and mineralisation of ^{35}S from the freshly incorporated organic fraction. The Meyer soil showed an apparent increase in the specific activity during the first five days of incubation, however this was largely due to errors involved in determining the specific activity. There was a significant decrease in the specific activity after the 5th day. Unlike the Teviot (limed) soil, specific activity in this soil was still decreasing when the incubation stopped, indicating that sulphur transformations had not reached an equilibrium.

Measurements of ^{35}S in the biomass pool gave inconsistent results, thus a comparison of involvement of the biomass in the incorporation of sulphate-^{35}S with the carrier-free ^{35}S cannot be made. After 32 days of incubation, the incoporation of added sulphur-35 was predominantly into HI-reducible forms of S. The Teviot soil contained 74% of the total incorporated ^{35}S into HI-reducible forms of S while in the Meyer soil a lesser amount (69%) was incorporated into the HI-S pool. However, most of the early incorporation in both soils was mainly into HI-reducible fractions, especially in the Teviot (limed) soil where, during the first 3 days, all the ^{35}S incorporated was recovered in HI-reducible fractions (Fig. 7.10). As the time of incorporation increased the proportion of HI-reducible labelling decreased and consequently ^{35}S incorporation into carbon-bonded forms increased slowly. Lower labelling of C-bonded forms of S would tend to suggest that incorporation in these soils was mainly carried out by extracellular mechanisms as discussed earlier. It is possible that addition of sulphate-^{35}S could have encouraged incorporation into HI-reducible forms. This would also explain the reason for poor and inconsistent recoveries of ^{35}S in the biomass-sulphur, since there would be a very small fraction of the added sulphur present within microbial tissues. However, increased specific activity in the C-bonded forms during the later stages of incubation would suggest that micro-organisms started utilising the added ^{35}S and consequently cycled it into S amino acids, thus increasing the amounts of ^{35}S incorporated into C-bonded forms. A comparison of the Teviot preconditioned soil with the carrier-free ^{35}S indicates that the C-bonded form was labelled more quickly (Fig 7.6) than where sulphate was added as carrier (Fig. 7.10) showing that addition of sulphate delayed the labelling of the C-bonded fraction.

Table 7.8 A comparision of carrier-free and carrier labelled sulphur-35 incorporation in the Teviot soil (preconditioned).

Incubation period (days)	% of the originally added ^{35}S incorporated					
	carrier-free ^{35}S			sulphate-^{35}S		
	T.O.S.*	HI-S	C-S	T.O.S.*	HI-S	C-S
1	7.9	8.0	0.0	6.9	7.0	0.0
2	11.8	9.5	2.3	7.0	7.0	0.0
3	15.1	10.9	4.2	6.9	7.0	0.0
5	19.3	13.5	5.8	9.8	8.4	1.4
8	n.d	n.d	n.d	10.8	9.6	1.2
10	27.4	15.0	12.4	n.d	n.d	n.d
16	28.4	22.2	5.8	12.0	10.8	1.2
24	28.5	21.6	6.9	21.8	15.6	6.2
32	32.4	23.4	8.8	23.2	17.2	6.0

* total organic sulphur.

7.3.2.3 *Discussion (labelled sulphate-^{35}S)*

The results in Table 7.6 and the incorporation of ^{35}S shown in Fig. 7.8 suggests that in both soils immobilisation was the dominant process in the short-term. The Teviot (limed) soil showing no ^{32}S mineralisation during this period and incorporated about 10% of the added ^{35}S. These effects were more pronounced in the Meyer soil where approximately 16% of the added ^{35}S was incorporated along with significant immobilisation of $^{32}SO_4$. It is evident that the addition of sulphate-S has slowed the cycling of the added sulphate-35.

Cycling of the sulphate-^{35}S simulates the fate of sulphate fertilizer in these soils. By the end of the 32 days, both of these soils had incorporated between 22-24% of the total ^{35}S into organic fractions. Short-term incorporations were higher in the Meyer soils, indicating that a sulphur deficient soil can immobilise greater quantities of added fertilizer than soils which already contain sufficient sulphate-S. It was incouraging to note that niether of these soils showed large amounts of immobilisation of the added ^{35}S, more than 70% of the applied sulphur was still present as extractable sulphate. This indicates that a large proportion of the added sulphur fertilizer would be available for plant growth in these soils. However, this may change if an extra source of carbon is provided e.g. by fresh plant residues. In that case, large amounts of added sulphate-S can become immobilised quite quickly, as has already been found in studies with carrier-free ^{35}S. The rate of such an immobilisation and remineralisation will entirely depend on the type and number of micro-organisms in soils. The predominant incorporation into HI-reducible forms of sulphur found in both soils of added sulphate-^{35}S can be explained from the studies conducted on fungi, which increased HI-reducible sulphur content when grown in sulphate rich culture (Fitzgerald , 1976; Saggar *et al.*, 1985). When fungi were grown in low sulphate-containing solution they contained only 12% organic sulphur in the HI-reducible form but in a high sulphate solution this proportion increased to 37% of the organic sulphur. Therefore, ^{35}S distribution in soil organic matter is perhaps related to the concentration of sulphate-S in soil solution and it depends also on the type of microbial population involved in the cycling of sulphur.

7.4 CONCLUSIONS

Incubation studies showed the effect of soil treatment on sulphur cycling in soil which implies that cycling of sulphate-S is dependent on soil conditions before and at the time of observation. Differences in the level of ^{35}S incorporation were thought to be caused mainly by the effects of soil treatment on microbial activity. Preconditioned soil showed a slow but steady incorporation of the added carrier-free ^{35}S. Air-dried soil showed a faster incorporation in the beginning but after reaching a maximum level of incorporation some of the recently incorporated sulphur was remineralised. The remineralisation occurred mainly from C-bonded forms of sulphur, which were mineralised to sulphate-S and also transformed into HI-reducible forms. Glucose-treated soil showed the fastest and highest level of incorporation of the added carrier-free ^{35}S. After reaching a maximum of 84% incorporation at 32 days, it also showed considerable amounts of remineralisation and transformation from the C-bonded forms. These studies further confirm that recently incorporated fraction of carbon-bonded sulphur represents a mineralisable pool of sulphur. It is likely that this fraction is largely composed of microbial tissues.

It can be concluded from these studies that there are two means by which inorganic sulphate can be incorporated into organic fractions. Firstly, by direct utilisation of sulphate-S by soil micro-organisms, where most of the incorporation is predominantly into C-bonded forms (e.g. glucose-treated soil), and secondly, incorporation by extracellular enzymes where incorporation is mainly into HI-reducible forms (e.g. preconditioned soils). Addition of sulphate-^{35}S which altered the sulphate levels in soils showed considerably slower and lower levels of incorporation compared to carrier-free ^{35}S. The two soils used, representing sulphur sufficient and sulphur deficient soils, were not significantly different in their incorporation characteristics, both resulting in between 22-24% incorporation after 32 days incubation. The results obtained here also show that the interpretation of the sulphur-35 incorporation data must be made with caution as the soil pretreatment and use of carrier sulphur greatly influence the overall cycling of sulphur.

CHAPTER EIGHT

MINERALISATION OF SULPHUR RECENTLY INCORPORATED INTO SOIL ORGANIC MATTER

8.1 INTRODUCTION

This part of the study is a continuation of the ^{35}S incorporation experiments reported in Chapter 7 in which the incorporation of radio-active sulphur into soil organic sulphur fractions was discussed. After a period of incubation in the closed system and removal of inorganic sulphate-S, these soils were re-incubated using the open incubation system, which allowed a study of the mineralisation of the recently incorporated sulphur. The different soil treatments applied in the incorporation studies (chapter seven) resulted in varying amounts of ^{35}S incorporation into HI-reducible and C-bonded forms of sulphur. Samples from these treatments therefore provided an opportunity to measure the mineralisation of organic sulphur held in those two forms.

8.2 OBJECTIVES

The main thrust of this study was to examine the mineralisation potential of sulphur recently incorporated into soil organic matter and to identify the form or forms of organic sulphur which are most readily mineralised to sulphate-S. The study also aimed to examine the effect of length of the original incorporation period on the subsequent release of sulphate-^{35}S from organic sulphur fractions.

8.3 MATERIALS AND METHODS

The soils used in this study and the analytical methods required for determining the various forms of ^{32}S and ^{35}S were identical to those described in section 7.3.

8.3.1 Re-incubation

Three flasks from each of the soil treatments (preconditioned, air-dried and glucose-treated) were taken after 32, 60, 90 and 120 days of closed incubation. These soils were then packed into leaching columns and re-incubated in an open system (details of this method been described in section 4.2.4). Sulphate-^{32}S and sulphate-^{35}S were removed from soils by a slow leaching with 100 ml KH_2PO_4 solution (containing 500 μg P ml^{-1}). A further leaching with 100 ml distilled water was carried out to remove any phosphate extracted sulphate which may have been left in the soil columns. The soil columns were then incubated at 20 °C in darkness. Mineralised-SO_4 was leached from the columns using 100 ml 0.01 M $CaCl_2$ solution every two weeks for a period of ten weeks. Both ^{32}S and ^{35}S were measured in the leachates. At the end of the re-incubation period, after the final leaching, soils from the columns were analysed for adsorbed sulphur, biomass-sulphur, HI-reducible sulphur, total sulphur and carbon-bonded sulphur.

8.4 RESULTS AND DISCUSSION

8.4.1 Mineralisation of ^{32}S and ^{35}S organic sulphur

Mineralisation results are separated into three sub-headings based on the pre-treatment of soils during the closed incubation. These pre-treatments had distinct effects on the nature of ^{35}S incorporation into organic fractions (see chapter seven), therefore it is appropriate to distinguish them from each other and describe their mineralisation characteristics separately. The organic sulphur which was labelled with ^{35}S during the closed incubation is regarded as recently formed organic sulphur and has been described as recently incorporated sulphur.

8.4.1.1 *Preconditioned soil*

The period of closed incubation (32,60,90 and 120 days) of the preconditioned soil had no significant effect on the amount of SO_4-S mineralised during the 10 weeks of re-incubation. Between 5 and 6% of the total organic sulphur was mineralised from the soil during this period (Fig. 8.1). Mineralisation of sulphur-35, when expressed as a % of the

total sulphur-35 incorporated into the soil, was much higher than the percentage of total sulphur-32 mineralised by the soil (see Figure 8.1). This trend was apparent throughout the re-incubation period, although the difference was greatest during the the first two weeks of incubation. Where proportion of the ^{35}S was mineralised approximately 8-10 times faster than the proportion of sulphur-32. The mineralisation of a greater percentage of recently incorporated ^{35}S in this soil indicates that recently formed organic sulphur is more easily mineralised compared to the bulk of the organic sulphur present in the soil. More than 50% of the total ^{32}S and ^{35}S released during the ten week incubation was mineralised within the first 2 weeks of incubation. With each successive removal of mineralised-SO_4^{2-}, the percentage of ^{35}S and ^{32}S released during the next two week incubation period decreased. The ratio between ^{32}S and ^{35}S, as measured by the specific activity of the mineralised sulphate-S fluctuated considerably throughout the re-incubation period.

The length of the original closed incubation period had a significant effect on the release of ^{35}S during re-mineralisation, particularly during the first 2-4 weeks of re-incubation (see Fig. 8.1). The differences over the first 2-4 weeks are largely responsible for the cumulative differences observed over the whole 10 week period (Table 8.1). Between 25 and 50% of the labelled ^{35}S was mineralised from the soil during the re-incubation. The longer the period of ^{35}S incorporation in the closed system, the smaller was the proportion of ^{35}S released from the organic fractions during re-mineralisation.

8.4.1.2 *Air-dried soil*

The mineralisation characteristics of sulphur for the air-dried soil (Fig. 8.2) were similar to those observed for the preconditioned soil. Approximately 3.6-5.5% of the total organic sulphur was mineralised during the re-incubation. The pattern of ^{35}S release from organic fractions in this treatment was very similar to that of the preconditioned soil where most of the release occurred within 2-4 weeks of re-incubation and thereafter decreased considerably, with the increase in the incubation period. In comparison to the preconditioned soil, for samples incubated originally for 32 days relatively higher amounts of ^{35}S were mineralised from the air-dried soil (38% compared to 28% of the total incorporated ^{35}S). As with the preconditioned soil, the cumulative mineralisation of

^{35}S decreased with an increase in the incorporation period i.e from 32 day to 120 day (Table 8.1). A maximum of 54% of the labelled sulphur-35 was mineralised from the soil incubated for 32 days whereas 29% mineralised from soil incubated for 120 days. As for preconditioned soil, the specific activity of the mineralised-SO_4^{2-} fluctuated considerably throughout the incubation period.

8.4.1.3 *Glucose-treated soil*

Sulphate-S mineralised during the re-incubation of glucose-treated soil was slightly lower than for the preconditioned and air-dried soils. Only 3.6 to 4.2% of the total organic sulphur was mineralised. The glucose-treated soil contained large amounts of ^{35}S incorporated into the C-bonded sulphur fraction (see Fig 7.4) and showed a significantly lower release of the ^{35}S compared with the other two treatments. Only 7-8% of the incorporated sulphur was released during the first 2 weeks of re-incubation. The pattern of release of this sulphur was similar to that found with the other two treatment, that is, the release of sulphur decreased with an increase in the re-incubation period. On average, 15-19% of the labelled sulphur was mineralised (Table 8.1). This would suggest that the nature of the labelled organic sulphur in these soils incubated for 32,60 and 90 days was not affected by the duration of incubation period.

The mineralisation characteristics of the labelled sulphur-35 from all treatments described above clearly demonstrates that recently incorporated sulphur is more easily mineralised than the non-labelled native sulphur. These results are consistent with the work of Freney *et al.*, (1975), who also incorporated ^{35}S into organic fractions for 8 weeks and then examined the uptake of the labelled and non-labelled (native) sulphur by sorghum (*Sorghum vulgaris*). They found that recently incorporated organic sulphur was more available to sorghum plants than the native sulphur. The higher mineralisation of recently incorporated sulphur is more likely due to the relative ease of the breakdown by soil micro-organisms.

In relation to the recently incorporated organic sulphur, native soil organic sulphur is more resistant to mineralisation. It appears that the longer the sulphur-35 remains in the soil organic sulphur pool, the more it resembles to native sulphur (in terms of

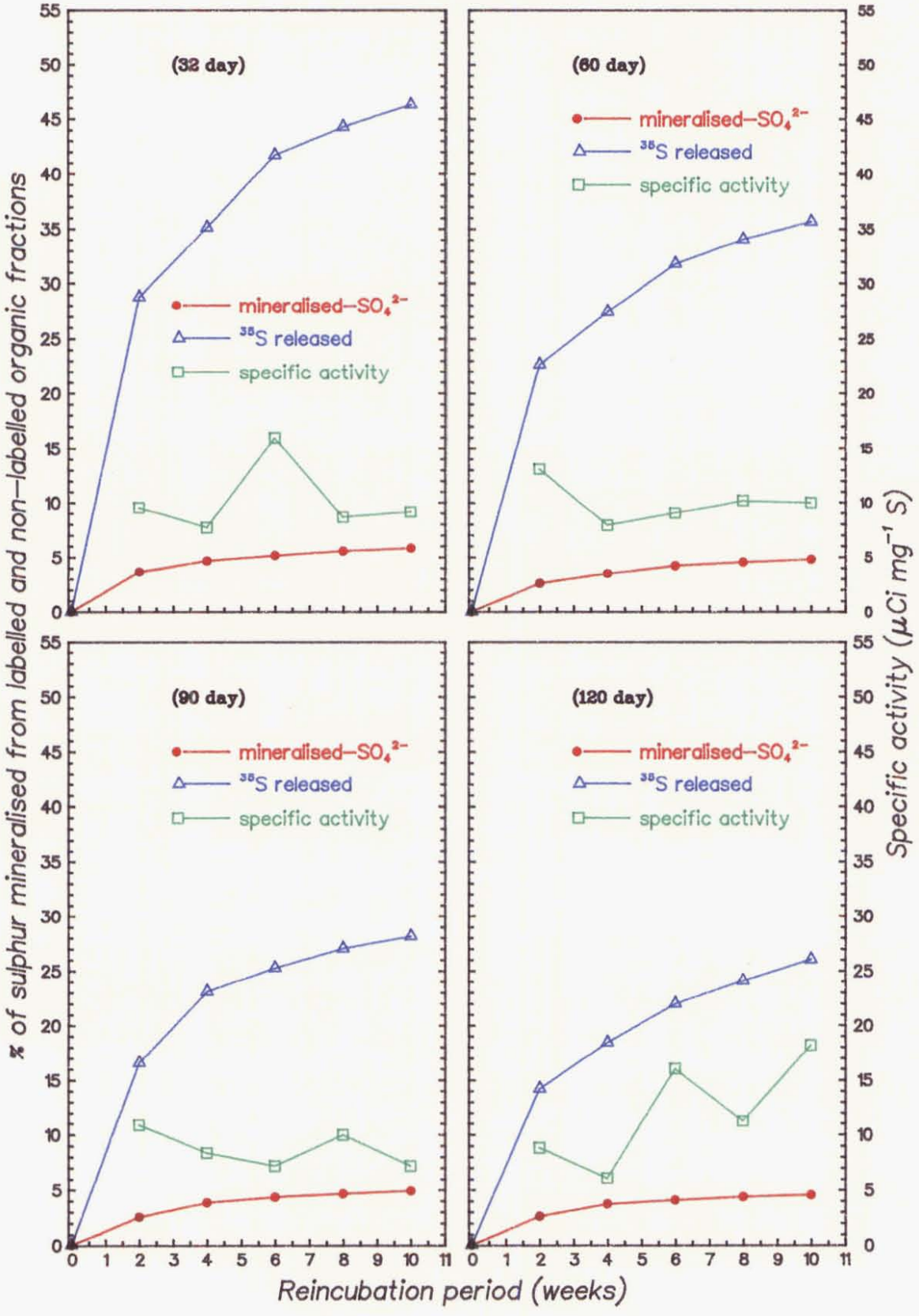


Figure 8.1 Mineralisation of the recently incorporated sulphur–35 and native sulphur in the preconditioned soil.

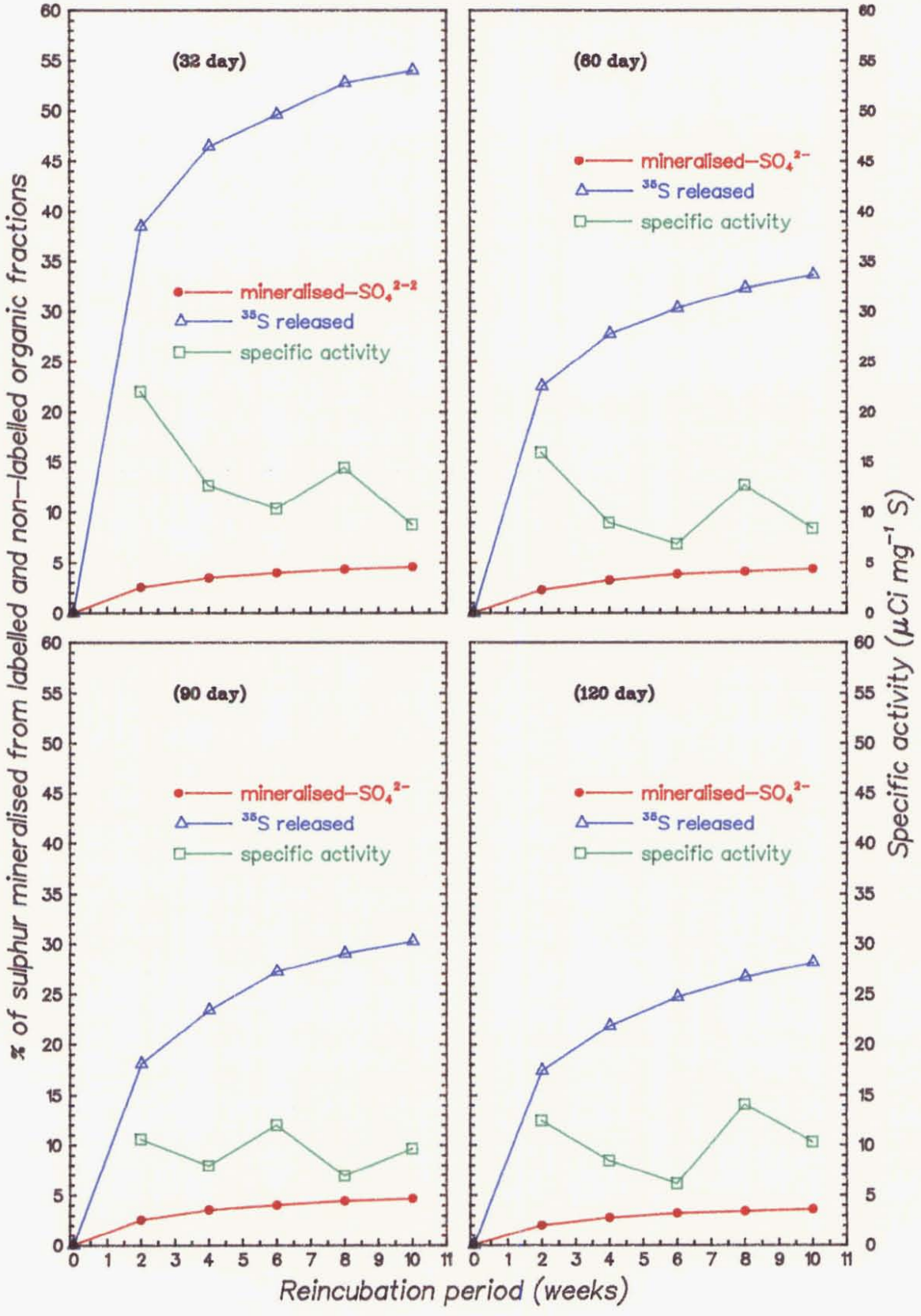


Figure 8.2 Mineralisation of the recently incorporated sulphur–35 and native sulphur in the air–dried soils.

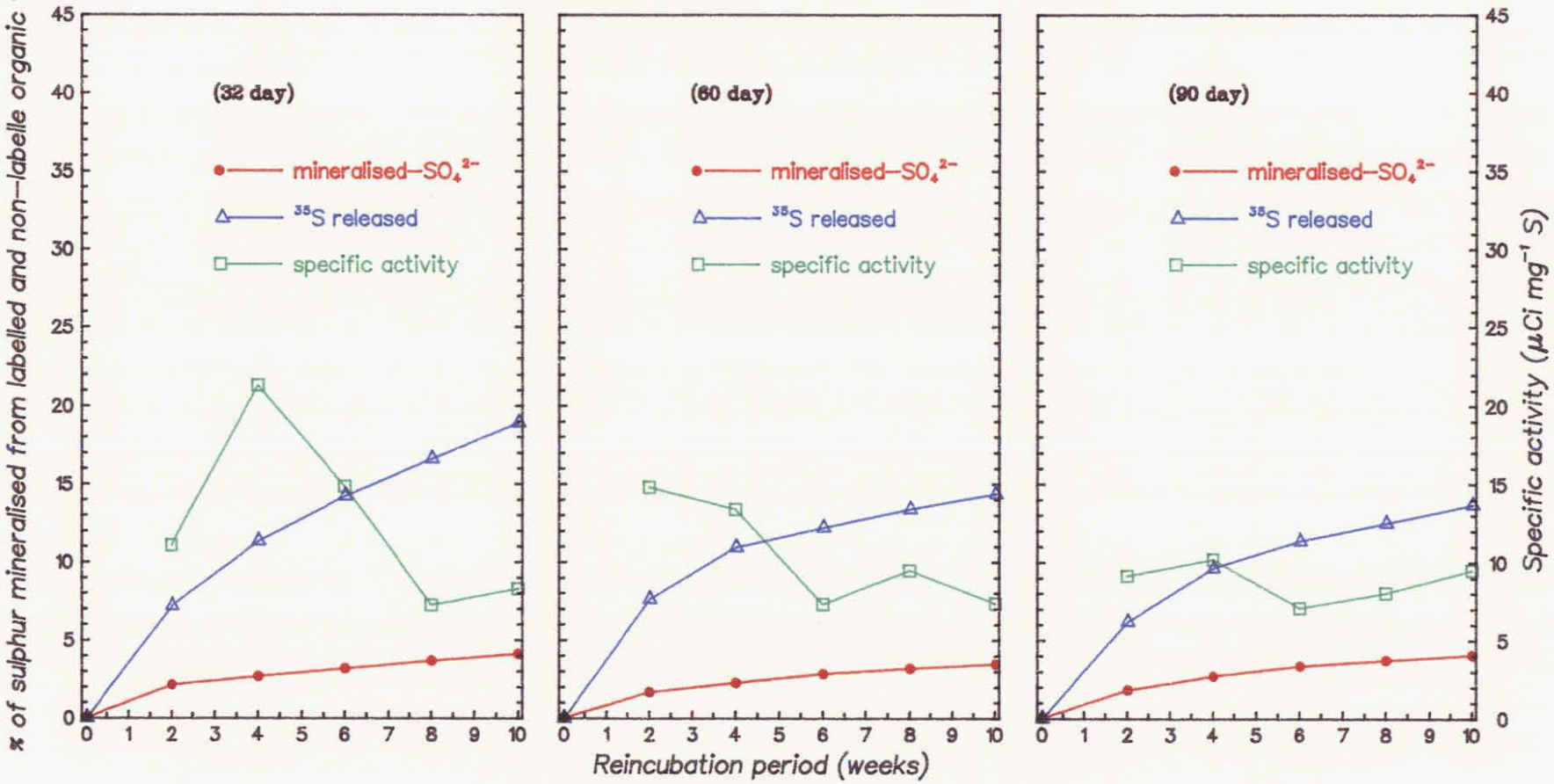


Figure 8.3 Mineralisation of the recently incorporated sulphur-35 and native sulphur in the glucose-treated soil.

mineralisation). It has been noted in these experiments that native sulphur was mineralised at slower rates than the recently incorporated sulphur.

It is possible that sulphur cycling between inorganic sulphate and organic forms of sulphur takes place faster than that can be monitored with ^{35}S. the longer a soil is incubated the greater the number of times individual ^{35}S atoms are likely to be incorporated into organic compounds and remineralised to sulphate-S. It is hypothesised that with each successive cycling of ^{35}S a small proportion of the ^{35}S is incorporated into relatively non-mineralisable forms of sulphur. Therefore, as an incubation proceeds the amount of ^{35}S in such forms will gradually increase. This would explain why in the studies described above the extent of mineralisation of incorporated sulphur-35 decreased with an increased in the length of the original closed incubation. These effects are highlighted with the preconditioned and the air-dry soils (Table 8.1). However, in the case of the glucose-treated soil, length of incubation period had very little effect on the subsequent release of ^{35}S. Addition of glucose would have increased microbial activity and thus the added ^{35}S would have been cycled at a greater rate than in either the preconditioned or air-dried samples, and the of less mineralisable organic sulphur would have build-up at a faster rate. Therefore, the proportion of sulphur-35 mineralised from these samples was relatively less compared with the preconditioned and air-dried soils.

8.4.2 Sources of mineralisable organic sulphur from the labelled and non-labelled pool

Since only 4-5% of the total organic sulphur was mineralised during the 10 weeks of reincubation, relatively very little net change was detected in the amounts of total organic sulphur (non-labelled, native) (Appendix 4). These results are consistent with the studies reported in Chapter 5. However, the ^{35}S data (Fig. 8.4) shows that sulphur was mineralised not just from C-bonded sulphur forms of sulphur but from HI-reducible sulphur as well. In some cases HI-reducible ^{35}S has been major contributor to the mineralised-$^{35}SO_4$ e.g. the preconditioned soil which was originally incubated for 60 day and 90 days and similar results were also found for the air-dried soil originally incubated for 120 days (Fig. 8.4). This indicates that there is much greater movement to and from the HI-reducible sulphur pool than non-labelled experiments would suggest.

Mineralisation is not only dependent on the chemical forms of organic sulphur but also to a large extent on the processes operating to decompose the organic sulphur fractions. It is likely that in the open system, sulphur would be mineralised from HI-reducible fractions by means of enzymatic reactions (biochemical) and also microbes would decompose C-bonded sulphur to acquire the carbon for metabolic purposes as has been suggested by McGill and Cole (1981). The extent of the biochemical process will depend on whether the decomposition of organic matter releases sufficient sulphur for microbial use or not. In case of deficient release, enzymatic mineralisation will also be continued. Therefore, in this case, both fractions of organic sulphur will be mineralised.

A comparison of specific activity in sulphate pool prior to reincubation (shown in Fig. 7.5) and the specific activity in the mineralised sulphate-^{35}S pool showed considerable difference between the two values. This indicates that mineralisation of sulphur during the reincubation period is taking place from a non-homogeneous labelled material.

The mineralisation of labelled organic sulphur can be classified as **specific** or **non-specific** mineralisation. An example of **specific mineralisation** is shown in Figure 8.5 where the samples showed an identical mineralisation pattern to that observed with non-labelled organic fractions. These samples were treated with glucose and therefore incorporated most of the sulphur-35 into C-bonded fractions. When re-incubated they showed a significant decrease in this fraction and at the same time sulphur-35 in the HI-reducible either increased slightly or stayed at the preincubation levels. Therefore, most of the mineralisation of the labelled organic fractions in these soils has occurred from a specific form of sulphur i.e., C-bonded S, thus this type of mineralisation is referred to **specific mineralisation.** Examples of **non-specific mineralisation** are shown in Fig. 8.4 where mineralisation has occurred from both fractions (HI-reducible and C-bonded). Also, there is no set pattern as to when and how much of the each fraction was mineralised. In some cases mineralisation of labelled sulphur was predominantly from the HI-reducible fraction (32 days incorporated, preconditioned soil) while in other case mineralisation was mainly from the C-bonded fractions (60 days incorporated, air-dried soil). Since mineralisation was not from any specific fraction, it is called **non-specific mineralisation.**

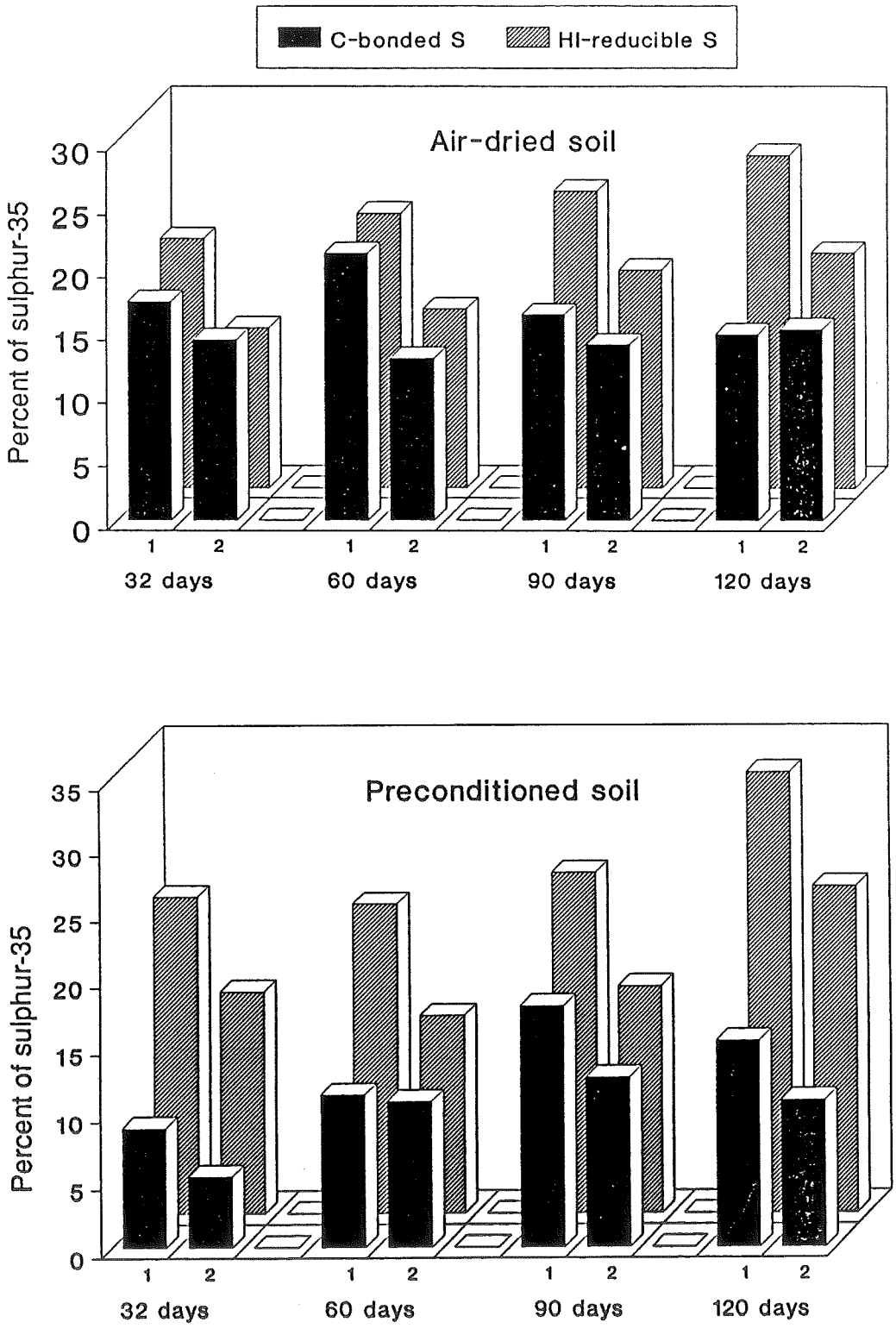


Figure 8.4 Mineralisation of incorporated sulphur-35 from soils; (1) shows the amounts of sulphur-35 incorporated into HI-S and C-bonded sulphur after 32,60,90 and 120 days of closed incubation, (2) shows the amounts of sulphur-35 retained after 10 weeks of incubation.

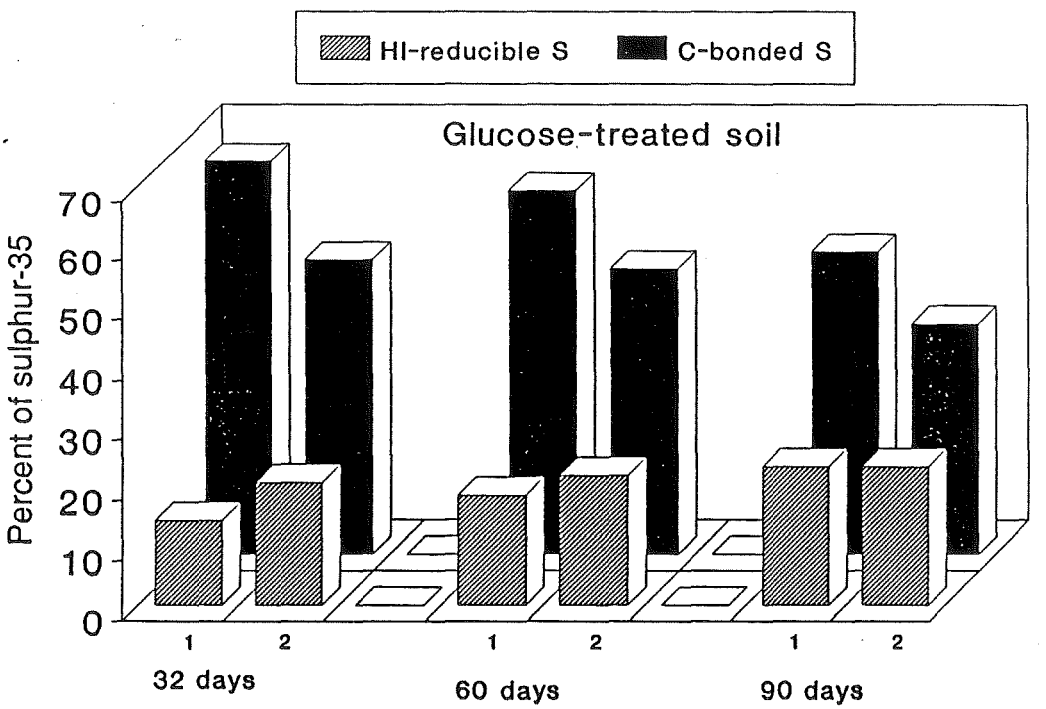


Figure 8.5 Mineralisation of incorporated sulphur-35 from soils; (1) shows the amounts of sulphur-35 incorporated into HI-S and C-bonded sulphur after 32,60, and 90 days of closed incubation, (2) shows the amounts of sulphur-35 retained after 10 weeks of incubation.

Table 8.1 Total percentage of ^{35}S mineralised during the 10 weeks of re-incubation period at 20°C.

Soil treatments	Period of incorporation in a closed incubation (days)			
	32	60	90	120
	(% of total organic sulphur mineralised)			
Preconditioned	49.17	39.88	31.36	25.43
Air-dried	54.64	35.64	27.80	28.88
Glucose-treated	18.94	16.12	15.51	n.d.

n.d = not determined

8.5 CONCLUSIONS

Results from this study show that sulphur incorporated recently into organic fractions is likely to be mineralised faster than that which has resided in the organic matter for longer time in native sulphur. The amount of minerlisation from these pools is likely to depend on the period of incorporation. Both forms of organic sulphur (HI-S and C-S) can be mineralised depending on the sulphate status in soil solution and the micro-organisms involved in the decomposition of the organic sulphur. The practical implications of these findings would relate to the mineralisation of the immobilised sulphur under field conditions where some of the immobilised sulphur can become available for plant uptake. None of the soils showed a complete mineralisation of the recently incorporated sulphur during the 10 week of reincubation. Mineralisation in soils from the labelled pool varied between 15-55%. This gives an idea of the magnitude of the likely mineralisation from the recently incorporated sulphur.

CHAPTER NINE

REMOVAL OF HI-REDUCIBLE SULPHUR FROM SOIL ORGANIC MATTER AND SUBSEQUENT MINERALISATION OF THE REMAINING (C-BONDED) SULPHUR

9.1 INTRODUCTION

It is believed that organic sulphur held in C-bonded forms is either mineralised directly to sulphate-S or transformed via an intermediary step into HI-reducible sulphur before final mineralisation as sulphate-S (see section 5.3.2). However, there is no conclusive evidence for the intermediary step, the hypothesis for its existence appear to be based on results obtained from organic sulphur fractionation schemes (Freney *et al.*, 1971; McLaren and Swift, 1977; Bettany *et al.*, 1979). The fraction of soil organic sulphur present as fulvic acid is often rich in HI-reducible forms of sulphur and therefore thought to represent an easily mineralisable sulphur fraction. However, in a pot experiment, Freney *et al.*, (1975) found that most of the sulphur mineralised from organic sulphur came from C-bonded forms. A similar observation was made by McLaren and Swift (1977) studying long-term changes under field conditions. They found that the amount of C-bonded sulphur decreased more than the HI-reducible sulphur over 20 years of continuous cultivation. More recent studies by Fitzgerald and co-workers (e.g. Fitzgerald *et al.*, 1984; Fitzgerald and Andrew, 1984) have shown that large proportions of C-bonded compounds (e.g. methionine) added to soil are readily mineralised to sulphate-S, which would suggest that sulphur held in C-S forms represents readily mineralisable sulphur in soil. The rate of mineralisation of methionine reported by Fitzgerald and co-workers was many times higher than that observed by Freney et al., (1975) and McLaren and Swift (1977) for mineralisation of recently incorporated/native C-bonded sulphur in soils. Perhaps, a direct comparison of the rate of mineralisation of pure amino acids with that for native C-bonded sulphur is not appropriate. The later form of C-bonded sulphur is likely to be much more complex than simple amino acids.

The confusion and the contradiction in sulphur studies reported so far is largely brought about by a lack of understanding of the pathways of sulphur transformation in soils. David *et al.*, (1982), on the basis of studies to that date, suggested a conceptual model involving the transfer of sulphur between C-bonded and HI-reducible forms of sulphur. Experiments on sulphur transformations carried out by Strickland and Fitzgerald (1983) show that sulphate-S is incorporated by soil micro-organisms into covalent compounds (ester sulphates), which can then be re-mineralised enzymatically to sulphate-S. However, studies conducted using $^{35}SO_4$ show that sulphur is incorporated not only into HI-reducible forms but considerable amounts are also immobilised as C-bonded sulphur (Freney *et al.*, 1971; McLaren *et al.*, 1985). In both of these latter studies, there was a simultaneous incorporation of ^{35}S into HI-reducible sulphur and C-bonded sulphur forms.

Unless the processes in sulphur transformation are fully understood, it is difficult to assess the effect of soil organic sulphur in supplying sulphate-S for plant growth. If the two organic forms of sulphur i.e. HI-reducible or C-bonded sulphur could be separated from each other, a study of their individual transformation and mineralisation characteristics could be made. This approach could provide a better opportunity to identify sulphur transformation processes more precisely. The study reported in this chapter attempts to remove the HI-reducible forms of sulphur from the soil and then study the mineralisation characteristics of the remaining C-bonded sulphur. This type of approach does not appear to have been attempted previously in studies of soil sulphur transformations.

9.2 PRELIMINARY STUDIES

A series of preliminary studies were carried out to develop a method which could remove HI-reducible sulphur without affecting the C-bonded forms of sulphur in soil. This involved acid hydrolysis of the soil which decreased the soil pH to an extremely low value and also altered the nutritional status of the soil. Thus a rehabilitation or regeneration of the soil was required after the HI-reducible sulphur removal before mineralisation/transformation studies could proceed. The soil used for these

experiments was the Teviot (limed) soil which originally contained 174 μg sulphur g^{-1} as HI-reducible sulphur.

9.2.1 Removal of HI-reducible sulphur

The technique involved in the removal of HI-reducible sulphur from the soil organic fraction should ideally extract maximum amounts of HI-reducible forms of sulphur while having a minimal effect on the biological and physical status of the soil. Any disruption to the chemical composition of the remaining soil organic matter (especially C-bonded S) should also be avoided. However, given the nature of HI-reducible sulphur in soils (Freney *et al.*, 1969), it is unlikely that any extraction procedure will meet exactly the above criteria. The HI-reducible forms of sulphur consist mainly of covalent ester-sulphates. By extracting soil with 0.04 N methanolic HCl for 3 days and then hydrolysing the extracts with 6 N HCl, Freney (1961) managed to recover between 45 to 81% of the HI-reducible sulphur from five Australian soils. Although the extractant used in Freney's study was of relatively low strength (0.04 N HCl) which may have kept the organic matter relatively intact, the duration of the extraction (3 days) would have severally disrupted the soil aggregates. Also, the effectiveness of the solution for extracting HI-reducible sulphur was poor, 19-55% of the HI-reducible sulphur remained in the soil (within the organic fraction). Therefore, to extract greater amounts of HI-reducible sulphur from the soil in a shorter period of time, a stronger hydrolytic treatment was selected for this study.

9.2.1.1 *Extraction of HI-reducible sulphur by shaking with 2N HCl*

A series of extraction experiments were carried out using 2 N HCl solution for different lengths of time. Soil (10 g) was weighed into 100 ml polyproplyene tubes and 40 ml of HCl solution was added into each tube. The mixture of soil and HCl was shaken on an end-over-end shaker for 1.5, 3 and 6 hours respectively. The sample were then centrifuged at 10,000 rpm for 10 minutes. The supernatant solution was filtered through Whatman filter paper No. 42 and analysed for sulphur using the reduction method (see 3.4.1).

Table 9.1 Extraction of HI-reducible sulphur with 2 N HCl, either by shaking on an end-over-end shaker or by heating to 120 °C for different lengths of time.

Treatment	Time (min)	Amounts of S removed (μg S g^{-1}soil)*	% of HI-S removed
Shaking			
	90.0	16.0±0.40	9-10
	180.0	54.0±1.80	30-32
	360.0	58.0±1.60	32-34
Boiling			
	10.0	146.0±4.2	82-84
	20.0	168.0±5.0	94-99

* mean ± s.e of four replicates

A maximum of 31-34% of the HI-reducible sulphur was extracted with 6 hours of shaking (Table 9.1). There was little increase in the amounts of extracted HI-reducible sulphur between 3 and 6 hours shaking, hence extraction for longer period of time was not considered. The lower extractability of HI-reducible sulphur using these treatments would tend to indicate that the hydrolytic energy provided by the 2N HCl is insufficient to cause major hydrolysis of the HI-reducible forms of sulphur.

9.2.1.2 *Extraction of HI-reducible sulphur by boiling with 2N HCl*

A second set of experiments was carried out in which samples of soil (10 g air-dry) were weighed into 150 ml conical flasks and 40 ml of 2N HCl added. The contents of the flask were boiled for 10 and 20 minutes. During the heating, flasks were gently swirled 4 to 5 times to prevent foaming/bumping. The flasks were then allowed to cool for 30 minutes before the supernatant solution was transferred into a 40 ml polypropylene tube and centrifuged at 10,000 rpm for 10 minutes. The samples were then filtered and the filtrate was analysed for sulphur by the reduction method (see section 3.4.1). The soil remaining in the conical flask was washed twice with 20 ml of KH_2PO_4 solution (containing 500 μg P ml^{-1}) followed by another washing with 20 ml of distilled water. These washings ensured that any hydrolysed soluble sulphate was removed from the soil. Soils were then air-dried in the laboratory at 20 °C. The sulphur remaining as organic sulphur was analysed for total organic sulphur, HI-REDUCIBLE sulphur and C-bonded sulphur using the methods described in section 3.5.

$$R.OSO_3^- + HOH \xrightarrow[\text{Heat}]{H^+} ROH + SO_4^{2-} + H^+ \qquad .....(9.1)$$

(hydrolysis of the sulphate ester when reacted with HCl)

Boiling the soils with HCl extracted significantly higher amounts of HI-reducible sulphur than by shaking alone at room temperature. Heating appeared to increase the hydrolysis of ester sulphate compounds. A 10 minute boiling removed between 82-84% of the total HI-reducible sulphur from the soil (Table 9.1), and when extended to 20 minutes removed

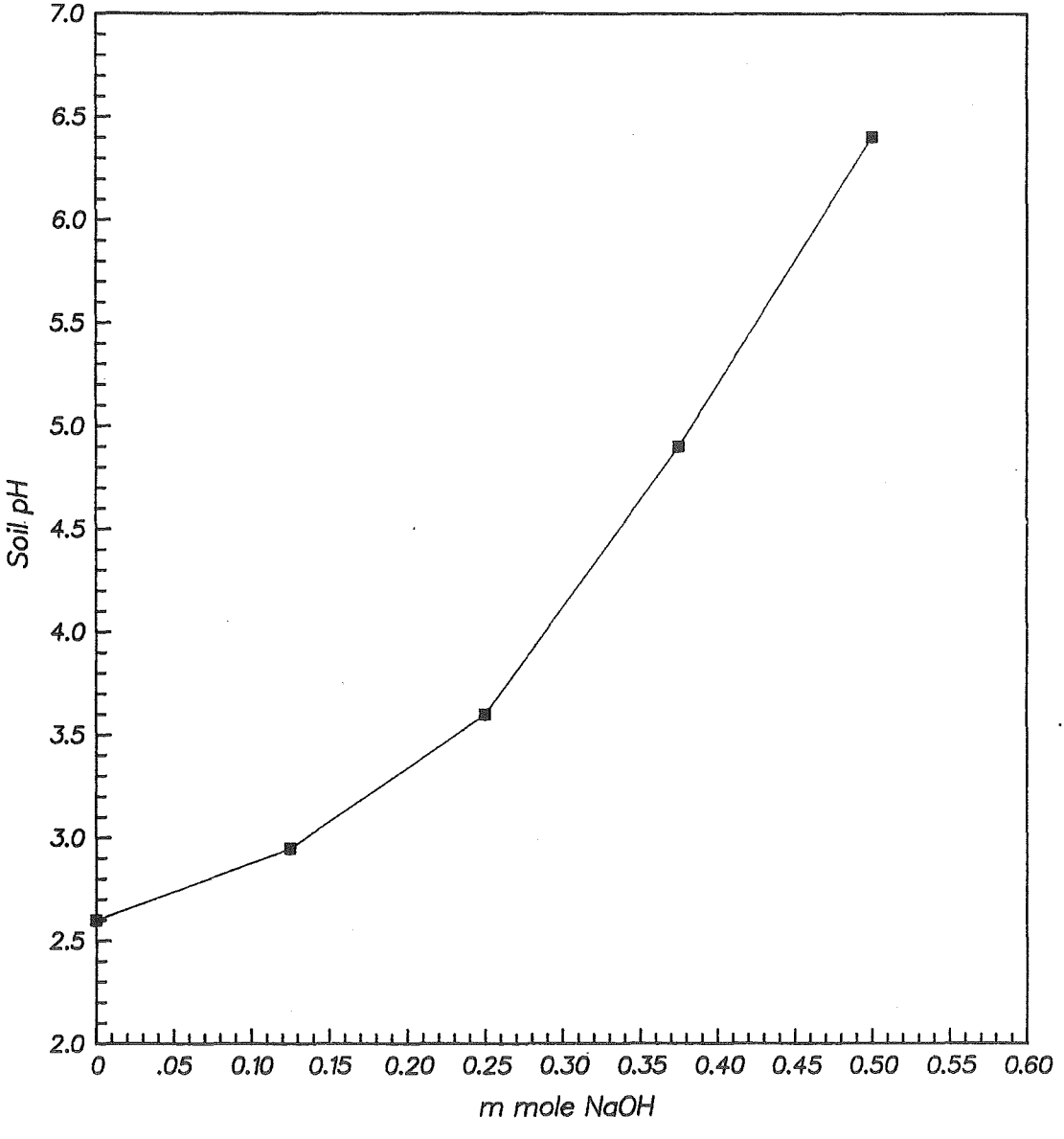


Figure 9.1 Buffer curve of the treated soil.

94-99%. This indicates that the 20 minutes boiling with 2N HCl hydrolysed most of the HI-reducible S. However, because the amount of sulphur determined in these extracts may not be all from the HI-reducible S, the residual soil had to be analysed for organic sulphur fractions to check whether the amount measured in the extract was truely derived from HI-reducible forms of S. Results presented in Table 9.2 confirm that most of the sulphur hydrolysed during the boiling process had come from the HI-reducible fraction. Analysis of the treated soils showed that during boiling, small proportions (3-5%) of the C-bonded sulphur were also removed. Since about 95% of the C-bonded sulphur was retained in the soil and most of the HI-reducible sulphur was removed from the 20 minutes boiling, this separation technique was chosen for further studies.

9.2.2 Rehabilitation of soil

Prior to studying the mineralisation of the residual (C-bonded S) sulphur, it was necessary to rehabilitate the soils by re-introducing soil micro-organisms, adding nutrients and increasing soil pH. The treatment applied to remove the HI-reducible sulphur from the soil would have had adverse affects on the microbial population. Therefore, it was essential to ensure that prior to incubation, the soil was inoculated with the micro-organisms present in the soil prior to the hydrolysis treatment. The HI-reducible sulphur separation treatment also lowered the soil pH to an extremely acidic level (pH ≤ 2.4). If soils were reincubated at that pH, it would have severely affected the re-establishment of a microbial population. Hence, the soil pH was raised to the original level. The acid treatment and extensive washing of the soil with KH_2PO_4 carried out essentially to remove the hydrolysed sulphur would also have removed various soluble nutrient cations and anions and some carbohydrates and possibly proteins. Therefore, essential cations and anions were added to the soil prior to incubation. The following reagents and materials were used in the rehabilitation of the treated soil;

(a) ***Buffering capacity of the treated soil*** Five samples (5 g) were weighed in 50 ml beakers and varying amounts of 0.5 M NaOH solution were added to the soil. The soil solution ratio was adjusted to 1:2.5 by adding distilled water. This mixture was stirred well to form a slurry and left to stand for 2 hours before the pH of each sample was measured with a glass electrode pH

meter. Each successive addition of NaOH increased the pH of the soil (Fig. 9.1). It was calculated from this buffer curve that approximately 0.75 g of $Ca(OH)_2$ was required for every 25 g of the treated soil to increase the soil pH to its original level (4.9).

(b) ***Inoculum*** Samples of soil (untreated) which were preconditioned at 75% field capacity moisture and 20 °C for two weeks were weighed (5.0 g) into 40 ml polyproplyene centrifuge tubes. These samples were extracted with 20 ml distilled water on an end-over-end shaker for 2 hours and were then allowed to settle for 1-2 hours. One ml of supernatant solution together with 0.1 g preconditioned soil were then used as an inoculum for each 25 g of the treated (hydrolysed) soil. The samples were well mixed after addition of the inoculum.

(c) ***Nutrient solution*** A nutrient solution was prepared containing 80 ppm nitrogen as NO_3^-, 10 μg of Ca^{2+} and Mg^{2+} and 2 μg Cu^{2+},Mn^{2+},Zn^{3+},Mo^{6+} and B^{3+}per ml solution. Phosphate and potassium were not included in the nutrient solution because during the last washing, soils were treated with KH_2PO_4 (containing 500 μg P ml^{-1}) which would have left sufficient amounts of these two nutrients in the soil. 2 ml of the nutrient solution was added to each 25 g of treated soil.

(d) ***Glucose solution*** A 31.25% D-glucose solution was prepared in distilled water and 4 ml of this solution was added to each flask (containing 25 g air-dry soil). This addition of glucose provided 2% organic C (easily metabolisable source of C) on air-dry weight basis.

Samples of the treated soil were weighed (25 g) into 120 ml glass bottles. The soil was then moistened to 20% (w/w). Soil pH was increased from 2.4 to 4.9 by adding $Ca(OH)_2$ to the soil. Soil samples were thoroughly mixed twice with an interval of 15 minutes in between. Soil microbial inoculum (1 ml) and 0.1 g of preconditioned soil as culture were also added to each bottle. Nutrient solution (2ml) as described above was added and soils were re-mixed.

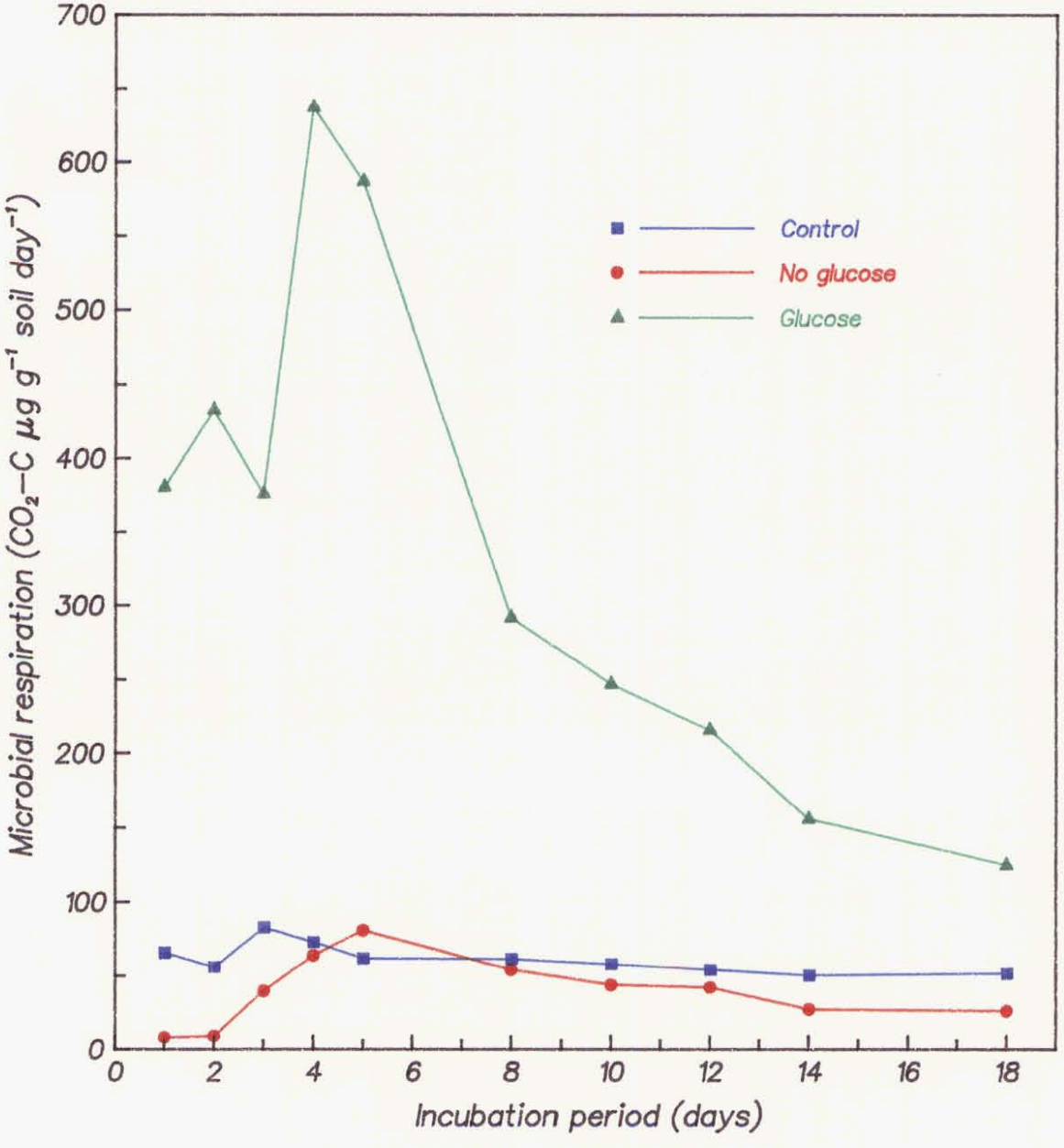


Figure 9.2 Microbial activity in the acid treated soils after the rehabilitation (preliminary experiments).

To examine whether a source of carbon was also required to stimulate microbial activity, a comparison was made between samples containing 2% C added as glucose and samples with no addition of carbon. To measure the microbial activity in the samples, CO_2 evolution was measured over a period of 14 days. CO_2 evolution from these samples was also compared with the evolution from the original preconditioned, non-treated soil. Results from the three types of samples are shown in Fig. 9.2. Soil without any additional carbon showed a lower microbial activity than the control (original preconditioned soil) during the first three days of incubation. Between 4 days and 12 days of incubation, the respiration rates in the treated soil was similar to the control but thereafter microbial activity in the treated sample decreased significantly below that of control. These results suggest that microbial activity in the treated (hydrolysed) soil is limited by the availability of a suitable carbon substrate. Addition of glucose to the treated soil produced significantly higher microbial activity throughout the incubation period. However, a sharp decline in the microbial activity was observed after 4 days suggesting that the added carbon was quickly assimilated or metabolised.

9.3 MINERALISATION OF C-BONDED FORMS OF SULPHUR

It has been shown in the preliminary experiments that HI-reducible sulphur can be removed successfully from soil organic sulphur with minimal loss of carbon-bonded forms of S. Such a removal of HI-reducible sulphur allows a study of the mineralisation and transformation of the remaining organic sulphur i.e. C-bonded sulphur. However, small changes in the size of the C-bonded sulphur pool (less than 2 μg sulphur g^{-1} soil) would be difficult to detect using current analytical techniques. This problem could be overcome by labelling of the C-bonded sulphur with sulphur-35, thus enabling the transformations of sulphur to be measured accurately by tracing the appearance of ^{35}S in the HI-reducible fraction or in the KH_2PO_4 extractable sulphate-S pool. It is known from the previous studies (see section 7.3.1.2.3) that (in the short-term) soils treated with glucose-C, incorporated added ^{35}S mainly into the C-bonded forms of S. Following this procedure would therefore enable C-bonded form sulphur to be labelled with ^{35}S.

9.3.1 Materials and methods

9.3.1.1 *Incorporation of ^{35}S into C-bonded forms of sulphur*

Three kg of air-dried Teviot (limed) soil was taken for ^{35}S incorporation into C-bonded forms of S. It is difficult to apply carrier-free radio-active sulphur homogeneously to such a large sample. Therefore, it was essential to subdivide the soil into smaller portions so that the applied sulphur-35 could be thoroughly mixed in. Six sub-samples (500 g each) were weighed into crystallising dishes (230 mm diameter). Carrier-free sulphur-35 solution, prepared in distilled water (100 ml, containing 10 μCi ml^{-1}) was added in four portions to each sub-sample (2 μCi sulphur-35 g^{-1} soil). Soils were mixed thoroughly after each addition of radioisotope solution. In order to facilitate incorporation of the added ^{35}S into C-bonded forms, 125 ml of 10% glucose solution was also added to each sub-sample (1% glucose-C on a soil air-dry weight basis). After a final mixing each sub-sample was then transferred to a 5 l conical flask. The soil moisture content were adjusted and maintained at 75% of the field capacity and the flasks were incubated at 20 °C for 24 days. On the 16th day of the incubation, 20 ml of the glucose solution was added to each flask to further ensure maximum incorporation of the added sulphur-35 is into the C-bonded fraction. At the end of the incubation period, soils were sub-sampled (two samples, each sample 5 g) to determine the percent incorporation of the added sulphur-35 and also the amounts of sulphur present in the different soil sulphur pools (see table 9.3).

9.3.1.2 *Separation of HI-reducible forms of labelled and non-labelled sulphur*

The soil in each conical flask (490 g) was treated with 1.96 l of 2N HCl solution. The flasks were then heated to boiled for 20 minutes. The heated mixture was then allowed to stand for 2 hours. Small volumes of supernatant solution (20 ml in duplicate) were then placed in 40 ml polypropylene tubes and centrifuged at 10000 rpm for 10 minutes and filtered as described in the preliminary studies. These solutions were analysed for sulphur to check the amount of sulphur removed from the soil. The amounts of sulphur in the separated extracts were corrected for sulphate-S which was present as KH_2PO_4 sulphate in soil. The remaining contents of the flask were then filtered using buchner funnels. To ensure that the hydrolysed sulphur and excess HCl was removed from the soils, the treated soils were

washed initially with 1 l of KH_2PO_4 solution (containing 500 μg P ml^{-1}) followed by subsequent washing with 1 l distilled water and 1 liter of KH_2PO_4. The treated soil was then air-dried at 20 °C in the laboratory. The soil was analysed for KH_2PO_4 extractable sulphate-S, HI-reducible sulphur and total organic sulphur (see section 9.3.4).

9.3.1.3 *Rehabilitation*

All the treated soil (six sub-samples) was bulked together and thoroughly mixed before weighing subsamples (25.0 g) into 150 ml conical flasks. The soil in each flask was rehabilitated by adding soil inoculum, nutrient solution, $Ca(OH)_2$ and distilled water (for details see section 9.2.2).

9.3.2 Soil treatments

The amount of mineralisation from the C-bonded sulphur fraction can be controlled by providing an extra source of carbon (see section 6.3.5). Addition of sulphur along with carbon should reduce the mineralisation of carbon-bonded sulphur and addition of C,S, and N should certainly decrease the mineralisation of C-bonded sulphur even further. Therefore, the following four treatments were applied to the rehabilitated soil in order to examine the biological mineralisation of C-bonded sulphur in relation to the dichotomous model of sulphur cycling as proposed by McGill and Cole (1981).

1. Control	rehabilitated soil.
2. Glucose	1% glucose-C was added to the rehabilitated soil.
3. Glucose, sulphate	1% glucose-C and 5 μg sulphate-S g^{-1} soil were added to rehabilitated soil.
4. Glucose, sulphate, nitrate	1% glucose-C, 5 μg sulphate-S and 20 μg nitrate-N g^{-1} soil were added to the rehabilitated soil.

Soils were incubated in conical flask (closed system) at 30 °C and 75% moisture. Three flasks were sampled from each treatment after 4,7,14,28 and 40 days of incubation.

Microbial activities in each treatment were determined by measuring the rate of CO_2 evolution as described in section 3.7.

9.3.3 Results

9.3.3.1 *Removal of HI-reducible sulphur*

Most of the HI-reducible sulphur was removed from the soil by 20 minutes boiling with 2N HCl (Table 9.3). Less than 1 μg sulphur g^{-1} soil could be detected as HI-reducible sulphur in the treated soil. More than 99% of the native HI-reducible ^{32}S and 96% of the labelled HI-reducible ^{35}S was removed by the separation method adopted here. However this procedure also hydrolysed 10% of the native C-bonded forms of sulphur and approximately 40% of the incorporated C-bonded ^{35}S. Thus the sulphur released from this readily hydrolysed C-bonded sulphur fraction was more highly labelled than that remaining in the soil. The amount of C-bonded sulphur hydrolysed during the HI-reducible sulphur separation was slightly higher than that observed during the preliminary studies (Table 9.2).

9.3.3.2 *Microbial activity during the incubation*

Microbial activity in the soil increased to a maximum at about 14 days in the glucose treated samples and thereafter decreased with the increase in the incubation period (Fig. 9.3). The control treatment showed a significantly lower level of microbial activity compared to the soils treated with glucose (treatment 2,3 and 4). The differences in the early stages of the incubation were particularly large, where the glucose treated samples showed 8 to 10 fold more CO_2 evolution than the control. During the later stages of the incubation, the differences in CO_2 evolution between the control and the glucose-treated samples narrowed down considerably. This would have been caused by the depletion of easily metabolisable carbon during the later stages of the incubation.

9.3.3.3 *Mineralisation and transformation of the C-bonded sulphur*

There was very little effect of adding glucose, nitrate or sulphate on either the net amount

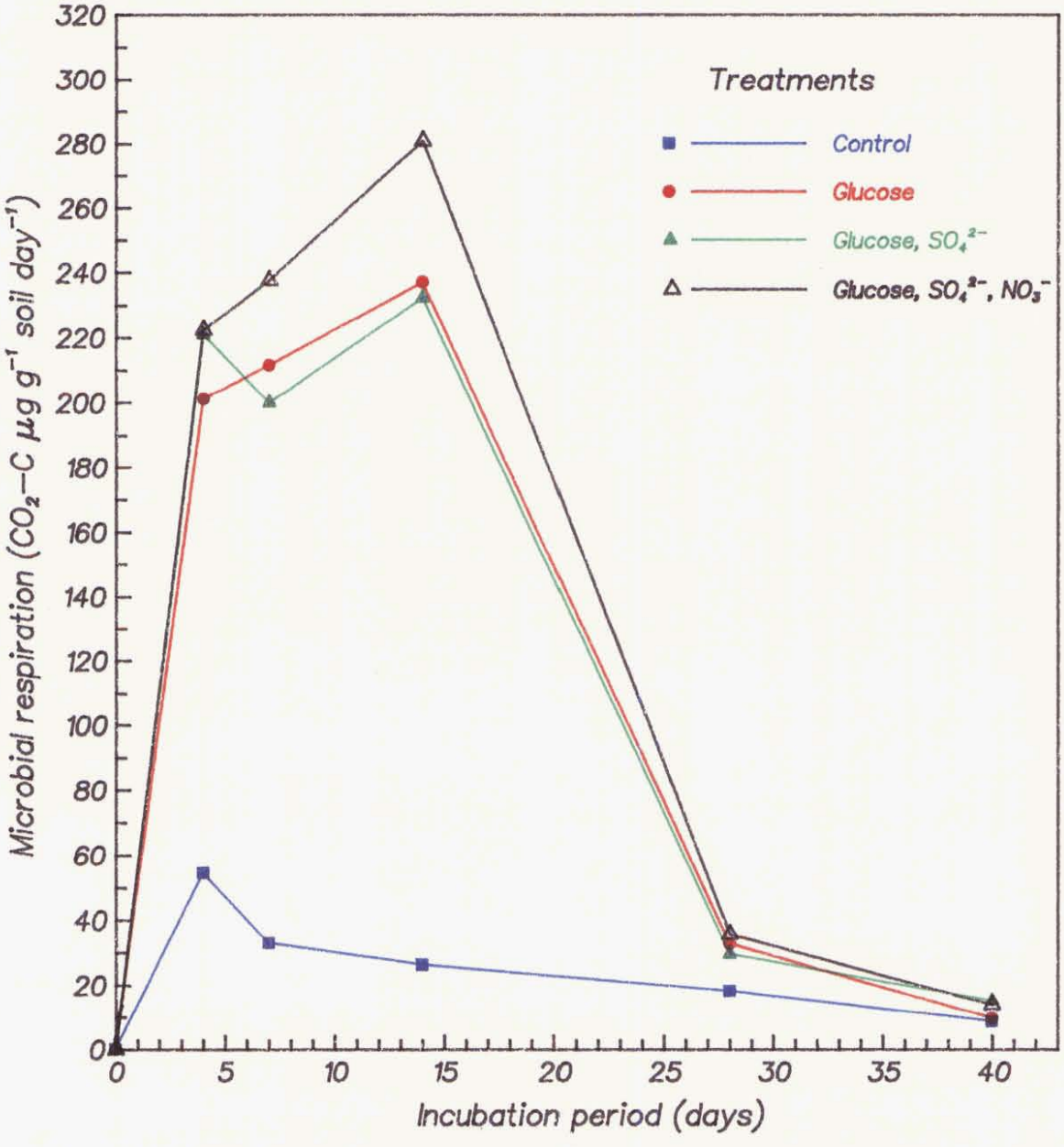


Fig. 9.3 Effect of soil treatments on the microbial respiration in the rehabilitated soil.

Table 9.2 Amounts of organic sulphur remaining in the soil after boiling with HCl for 10 or 20 minutes.

Treatments	Total organic S	HI-reducible S	C-bonded S
	(μg sulphur g^{-1} soil)		
Control	406.0(100)[1]	174.0(100)	232.0(100)
10 minutes	265.0(65)	35.0(20)	225.0(97)
20 minutes	224.0(55)	2.6(1.5)	219.4(95)

1. Values in parentheses are the percentages of the original organic sulphur remaining in individual fractions.

Table 9.3 Amount of sulphur-35 and sulphur-32 in the soil before and after the removal of HI-reducible sulphur. Values reported in this table are the means of the six sub-samples. There was less than 3% s.e. from the mean values.

Treatment	Total organic S	HI-reducible S	C-bonded S	SO_4-S
Before HI-reducible S removal				
Sulphur-35*	88.0	16.0	74.0	12.0
Sulphur-32 (μg S g^{-1} soil)	406.0	174.0	232.0	15.0
After HI-reducible S removal				
Sulphur-35*	44.4(50.5)[1]	0.6(3.5)	43.5(58.7)	0.2(1.9)
Sulphur-32 (μg S g^{-1} soil)	210.0(51.7)	<1.0(0.5)	≤210.0(90.5)	<0.5(2.5)

*. percent of the originally added activity (2 μCi g^{-1} soil).

1. values presented inside the parentheses represent the percentage of ^{32}S and ^{35}S remaining before and after the HI-reducible S separation in individual sulphur fractions.

of sulphur mineralised from the C-bonded sulphur as sulphate-^{35}S or its pattern of mineralisation during the incubation. On average, 3-4% of the total sulphur-35 retained in the C-bonded sulphur fraction was mineralised after 40 days (Fig. 9.4). Most of the mineralisation occurred in the early stages of incubation, where between 60% and 75% of the total mineralised sulphate-^{35}S was released within 4 days. It appears that addition of sulphate and nitrate to glucose (Treatment 3 and 4) increased the mineralisation of labelled organic sulphur. These effects were obvious in the early period (between 4 and 7 days of incubation) where significantly higher amounts of $^{35}SO_4^{2-}$ was released compared to the control and glucose treated soils.

Addition of glucose, nitrate-N and sulphate-S had a significant effect on the amount of net mineralised sulphur, shown as sulphate-S in Fig 9.4. The control treatment showed a rapid release of sulphate in the initial stages of the incubation followed by a slight re-immobilisation of some of the mineralised sulphate, and finally a significant increase in net mineralised-SO_4 between 28 and 40 days. Soil treated with glucose alone (Treatment 2) showed a similar pattern of sulphate release to the control soil. However, the amount of sulphate released during the first 14 days was comparatively lower than the control soil. This was probably due to the higher microbial activity during that period (Fig. 9.3) resulting the immobilisation of the mineralised-SO_4 within the microbial biomass. When microbial activity declined after the 14th day of incubation, some of the immobilised sulphur was released from the microbial tissues as sulphate-S, giving an increase in the amount of net mineralised-SO_4^{2-}. However, Treatments 3 and 4 showed a reverse trend of sulphur mineralisation/immobilisation than that observed with the glucose-treated soil. Addition of sulphate and nitrate with glucose increased the mineralisation of C-bonded sulphur in the early stages but later on the effect of these nutrients appeared to have somewhat disappeared (Fig. 9.4). This was evident also from the microbial activity which was reduced considerably between 14 to 40 days (as it was with the Treatment 2). This might have been expected to result in a release of sulphate-S from the declining biomass as observed with glucose treated soils, but rather than release of sulphate-S in there was gradual immobilisation of sulphate-S.

The amount of C-bonded sulphur transformed into HI-reducible forms of sulphur during the 40 days incubation period is shown in Fig 9.5. The transformation shown in this figure has

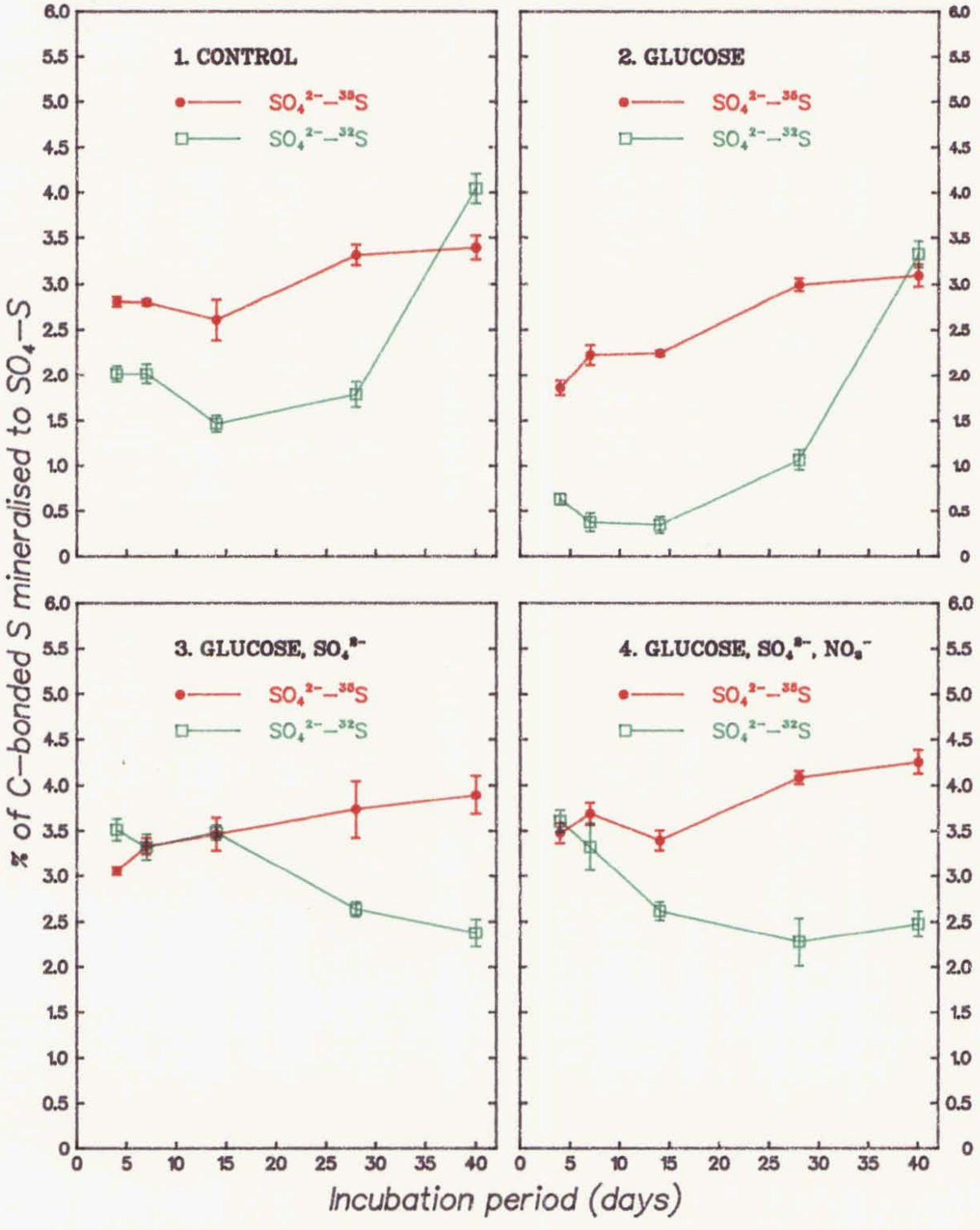


Figure 9.4 Amount of C–bonded sulphur mineralised to sulphate–S following the treatments 1,2,3 and 4. Plotted lines are means of six replicates and the bars represent standard error between the replicates.

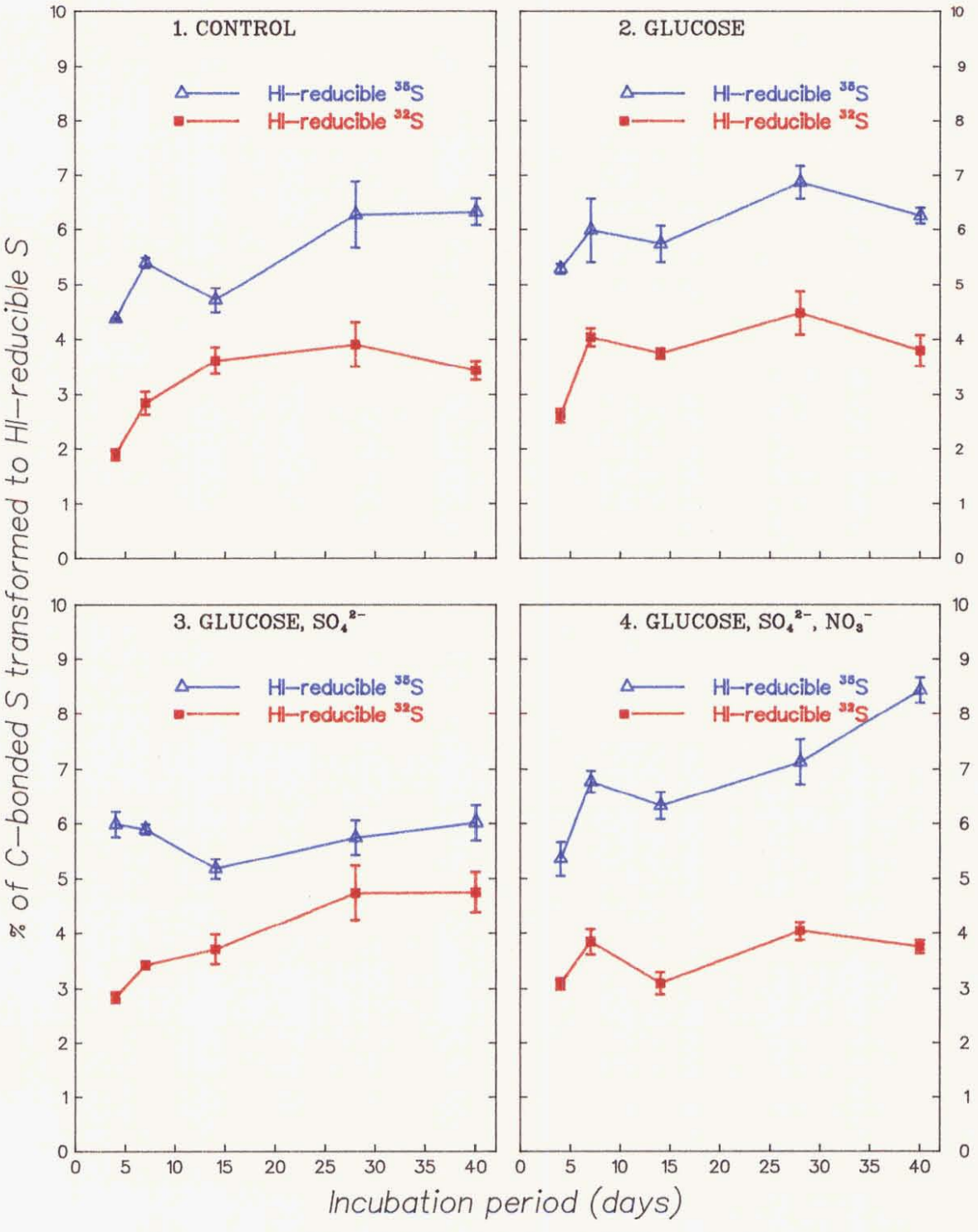


Figure 9.5 Amount of C-bonded sulphur transformed into HI-reducible forms of sulphur following the treatments 1,2,3 and 4. Plotted values lines are means of six replicates and the bars represents standard error between the mean.

been corrected for the amount of HI-reducible ^{32}S and ^{35}S which were either added with 0.1 g soils as inoculum or remained after the removal of the bulk of the HI-reducible sulphur from the soil. Most of the transformation of C-bonded sulphur to HI-reducible sulphur occurred in the first four days of the incubation. There was no significant difference in the amount of ^{35}S transformed to HI-reducible sulphur between treatments 1,2 and 3, where approximately 6 to 7% of the C-bonded sulphur was transformed. However, it appears that addition of glucose, sulphate and nitrate to the soil (Treatment 4) has encouraged the transformation process, resulting in significantly higher amounts of C-bonded sulphur transformation than in the other treatments (8.5% of the total C-bonded ^{35}S was transformed to HI-reducible sulphur after 40 days of incubation, Fig. 9.5).

9.3.4 Discussion

The procedure used to remove HI-reducible sulphur showed nearly complete removal (99%) of HI-reducible sulphur from the soil, which is a significant improvement over the method used by Freney (1961) which removed only 45 to 81% of the total HI-reducible sulphur. However, the treatment employed in this study to remove HI-reducible sulphur is non-selective in its hydrolytic action. It also hydrolyses part of the C-bonded sulphur from the soil organic matter. The fraction of the C-bonded sulphur lost during the separation of HI-reducible sulphur contains a high proportion of recently incorporated sulphur. About 42% of the labelled C-bonded sulphur which was incorporated during the original 24 day incubation was lost during the HI-reducible sulphur removal process. Loss of a relatively high proportion of labelled C-bonded sulphur which has been recently incorporated into organic forms of sulphur suggests that the fraction of C-bonded sulphur lost during the hydrolysis may have been a relatively highly active pool of C-bonded sulphur. Since it has been found that recently incorporated sulphur is more easily mineralised than sulphur which has been part of the organic fraction for a longer period of time (see section 8.4), this loss could have had a major effect on the subsequent mineralisation of sulphur from the treated soil. The ^{35}S remaining as C-bonded sulphur may not have been as labile as that lost in the hydrolysis. This may be one of the reasons why very low amounts of C-bonded sulphur were mineralised during these experiments. However, the remaining C-bonded ^{35}S was apparently still more labile than the native (non-labelled) C-bonded sulphur. The proportion of ^{35}S mineralised to sulphate-S was either equal to or greater

than the proportion of total sulphur mineralised (Fig. 9.4). More evidence of the labile nature of the labelled fraction is shown in the transformation of C-bonded sulphur to HI-reducible sulphur. A higher proportion of C-^{35}S was transformed to HI-reducible ^{35}S than for non-labelled sulphur (^{32}S) (Fig. 9.5). On average, 8 to 10% of the C-bonded ^{35}S was transformed during 40 days of incubation, 3-4% was mineralised to sulphate and 5-6% was transformed to HI-reducible sulphur. Formation of HI-reducible sulphur during these studies confirms at least one step in the sulphur cycle that is C-bonded forms of sulphur are transformed to HI-reducible forms of sulphur in soil. The actual pathway of C-bonded sulphur transformation to HI-reducible sulphur however, can not be confirmed from these studies, because the appearance of HI-reducible sulphur and sulphate-S occurred simultaneously.

9.4 CONCLUSIONS

Results have shown that the HI-reducible sulphur form of sulphur can be removed completely from the organic sulphur fraction with a minimum loss of C-bonded sulphur.

The remaining organic sulphur (i.e. C-bonded S) studied in isolation has been shown to be mineralsed to sulphate-S and transformed to HI-reducible forms of sulphur. On the basis of the results obtained in this study it can be concluded that C-bonded sulphur in soils is a primary source of mineralisable sulphur. Approximately 10% of the C-bonded sulphur was mineralised/transformed to SO_4^{2-} and HI-reducible sulphur during the 40 day incubation. However, the amount of mineralisation of C-bonded sulphur which occurred in this experiment cannot be compared directly to the likely amount of C-bonded sulphur mineralised in soils. The reasons for this reservation are that the use of acid hydrolysis for separating HI-reducible sulphur may have affected the nature of C-bonded sulphur in soil and altered microbial activity. Studies reported in this chapter can be used as mean of examining the nature of soil-oriented C-bonded sulphur. However, further work is needed to improve the method of HI-separation so that the losses of C-bonded sulphur can be minimised.

CHAPTER TEN

SEASONAL FLUCTUATIONS OF SULPHATE-S AND BIOMASS-S IN SOILS

10.1 INTRODUCTION

Analysis of soil sulphur is used to estimate the likely amounts of sulphur available for plant uptake. Since plants utilise the SO_4^{2-} form of S, soils are commonly analysed for extractable sulphate to predict deficiency or sufficiency. In some cases soil tests for sulphate-S have shown good relationships with plant yield (Spencer and Freney, 1980; Saunders and Cooper, 1984; Westerman, 1974). However, soil testing for sulphate-S in temperate areas has had variable success because the size of the soil sulphate pool varies over short periods of time (Blair, 1979). The main problems are caused by short-term fluctuations in moisture and temperature which greatly affect microbial activity (Williams, 1967). Therefore soil tests which measure only SO_4-S at the time of sampling, and assume that it is a constant value, have given less than satisfactory results. In New Zealand, seasonal fluctuations in moisture and temperature are rapid and hence fertilizer recommendations based on soil tests alone have often shown poor plant yield (Cornforth *et al.*, 1983).

The study presented in this chapter was designed to examine the seasonal fluctuations in soil sulphate-S in pasture, fallow and ploughed land. The study also measured seasonal fluctuations in biomass-S levels, which is believed to represent a labile pool of soil sulphur. Examination of these two sulphur pools should provide a better understanding of sulphur fluctuations in the soil system. Attempts are made to explain the reasons for observed SO_4-S and biomass-S fluctuations in the soil.

10.2 MATERIAL AND METHODS

A field trial was conducted on Wakanui silt loam soil, classified as a recent yellow/grey earth/gley integrade. This soil has <60% P retention and receives between 500 and 750 mm of rain annually, therefore can be classified as a slow to moderate sulphate leaching soil, having a sulphate leaching index of 3 on a 0-6 scale (Sinclair and Saunders, 1984). The area chosen for this experiment had been under pasture (white clover and ryegrass) for at least five years, and had been top dressed annually with superphosphate @ 25 kg P ha^{-1} for the last two years (1984-85). An area 17 m x 17m was selected was selected for the trial. The spatial variability of KH_2PO_4 extractable SO_4-S in the soil was determined prior to the application of treatments. A total of 12 samples were collected from the area of the trial surface layer (0-7.5 cm) and analysed for sulphate-S. Results ranged from 5.9 to 6.8 μg sulphate-S g^{-1} soil with a mean value of 6.4±0.09 μg SO_4-S g^{-1} soil. These values of sulphate-S indicate less than 5% deviation from the mean value across the proposed experimental area. Other soil properties are listed in Table 3.3 and 3.4 respectively.

10.2.1 Experimental design

The trial was laid down in a completly randomized design. A layout of the trial is shown in Fig. 10.1. Treatments were randomly allocated to 3 X 3 m^2 plots. A group of three plots (4,6 and 8) were maintained as pasture plots, plots 3,5 and 9 were ploughed and the remaining three plots (1,2 and 7) were maintained as fallow land.

Fallow and ploughed plots 1,2,3,5,7 and 9 were sprayed with 1% Roundup (active ingredient glyphosate) and 1% Velpar (active ingredient 3-Cyclohexyl-6(dimethylamino)-1-methyl-1,3,5-triazine-2,4(1H,3H)-dione) in mid November, 1986. These herbicides were effective in killing most of the plants within 6-10 days. Dead plants were pulled out from the plots. This was necessary to avoid the decomposition of plant material in soil. Five weeks later, plots 3,5 and 9 were cultivated using a rotary hoe, and they were cultivated again in September, 1987. To maintain the fallow plots 1,2, and 7 free from vegetation, another spraying of 1% Velpar herbicide was carried out early in September, 1987.

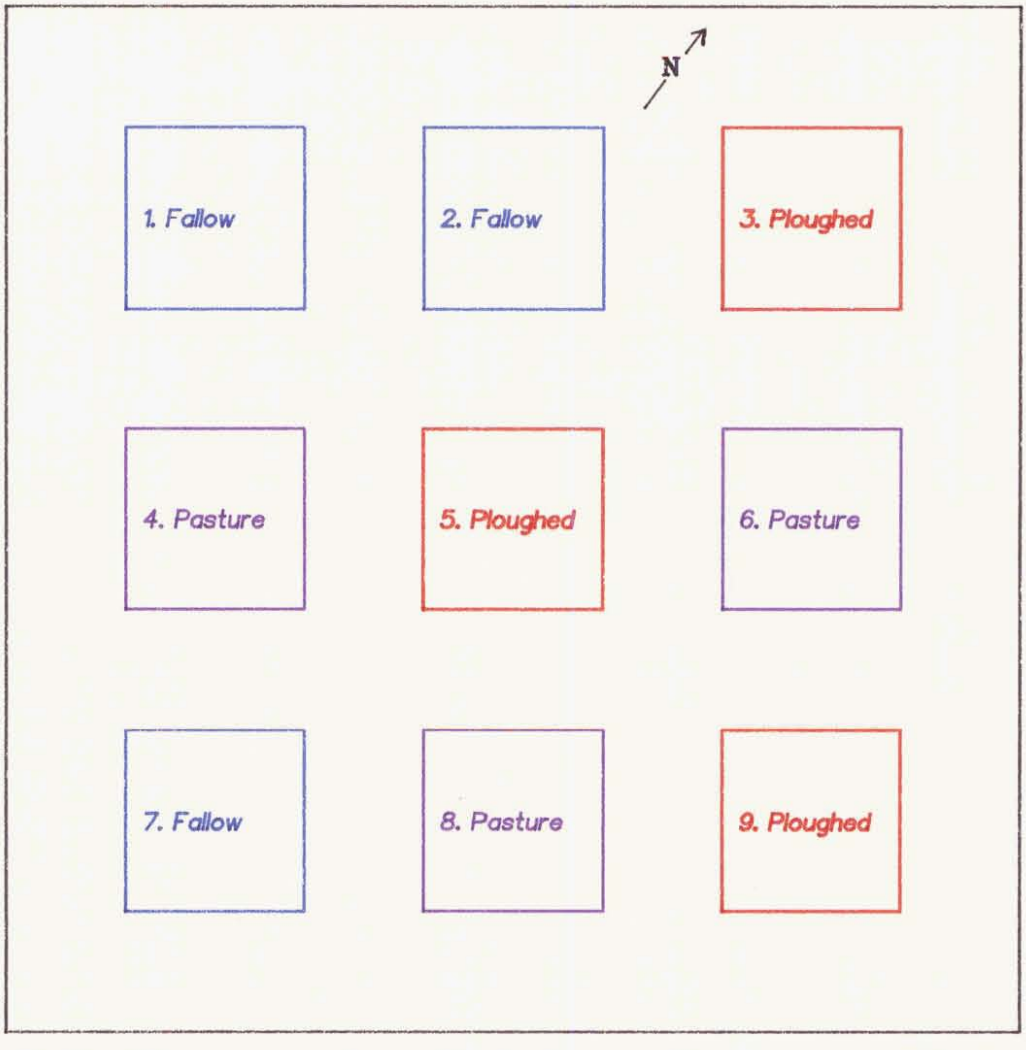


Figure 10.1 Digramatic representation of the field trial conducted at Lincoln College Field Service Centre in 1986–1987.

10.2.2 Sample collection and analysis

Soil samples were collected from 0-7.5 cm soil depth. Three cores (2.5 cm) were augered from each plot at monthly intervals between December, 1986 and December, 1987. The cores from individual plots were bulked together and screened through a 2 mm sieve. Screening was aimed to separate coarser plant residues which otherwise might interfere with the sulphur analysis. In cases where soils were excessively wet then soil samples were air-dried to 50% of the F.C. prior to screening. Soils from each plot were analysed in triplicate for KH_2PO_4 extractable S and biomass-S. Details of these methods are described in section 3.5.2 and 3.7 respectively. These analysis were carried out on moist samples. Results were corrected for moisture content.

Pasture plots were harvested from time to time and the total sulphur content in the herbage was determined using the oxidation/combustion method (Steinbergs *et al.*, 1962). Data for soil temperature at 10 cm depth and rainfall were obtained from the Lincoln College Meteorological Observation Station.

10.3 RESULTS AND DISCUSSION

10.3.1 Seasonal fluctuation in sulphate-S and biomass-S

Seasonal fluctuations are in sulphate-S caused by microbial activity, leaching, run-off, atmospheric input and plant uptake. The balance between these five factors determines the level of sulphate-S in soils. Interactions between these factors vary from season to season. The seasonal effects on sulphate-S fluctuations in this study are shown in Fig. 10.2a. and can be divided into three periods; fluctuations during the summer of 1986, fluctuations during the winter of 1987 and fluctuations during the spring and beginning of summer 1987. At the beginning of the trial i.e. during the summer of 1986/87, there were no significant differences in sulphate-S levels between the pasture, ploughed and fallow plots. During this period of time sulphate-S levels remained between approximately 6 and 7.5 $\mu g\ S\ g^{-1}$ soil. In May, 1987, the sulphate-S level decreased by more than 50%, from 6 to 2.5-3.0 $\mu g\ S\ g^{-1}$ soil. This decrease occurred in soil from the plots of all three treatments.

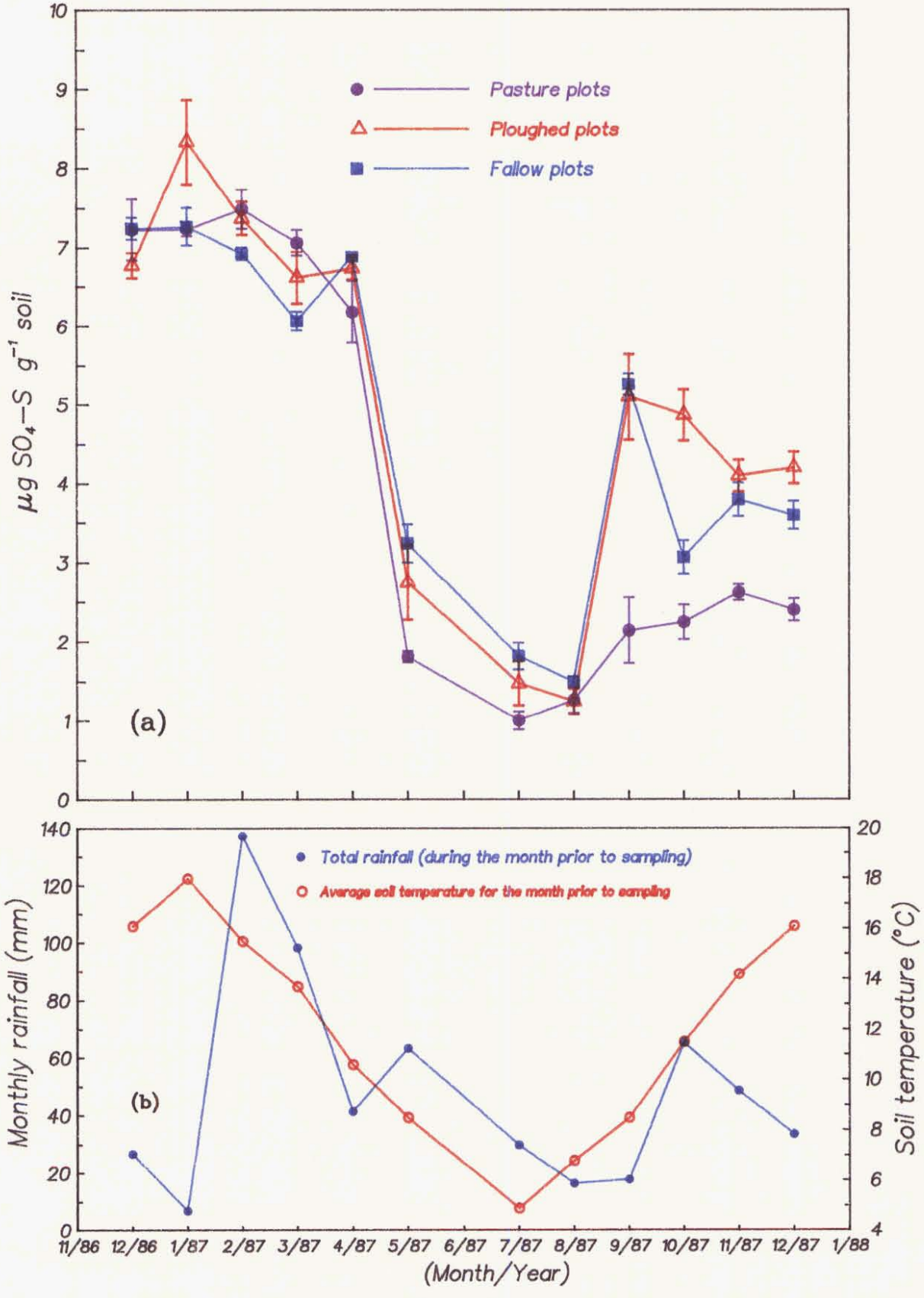


Figure 10.2 Effect of monthly rainfall and average soil temperature at 10 cm soil depth on sulphate–S fluctuations during the year.

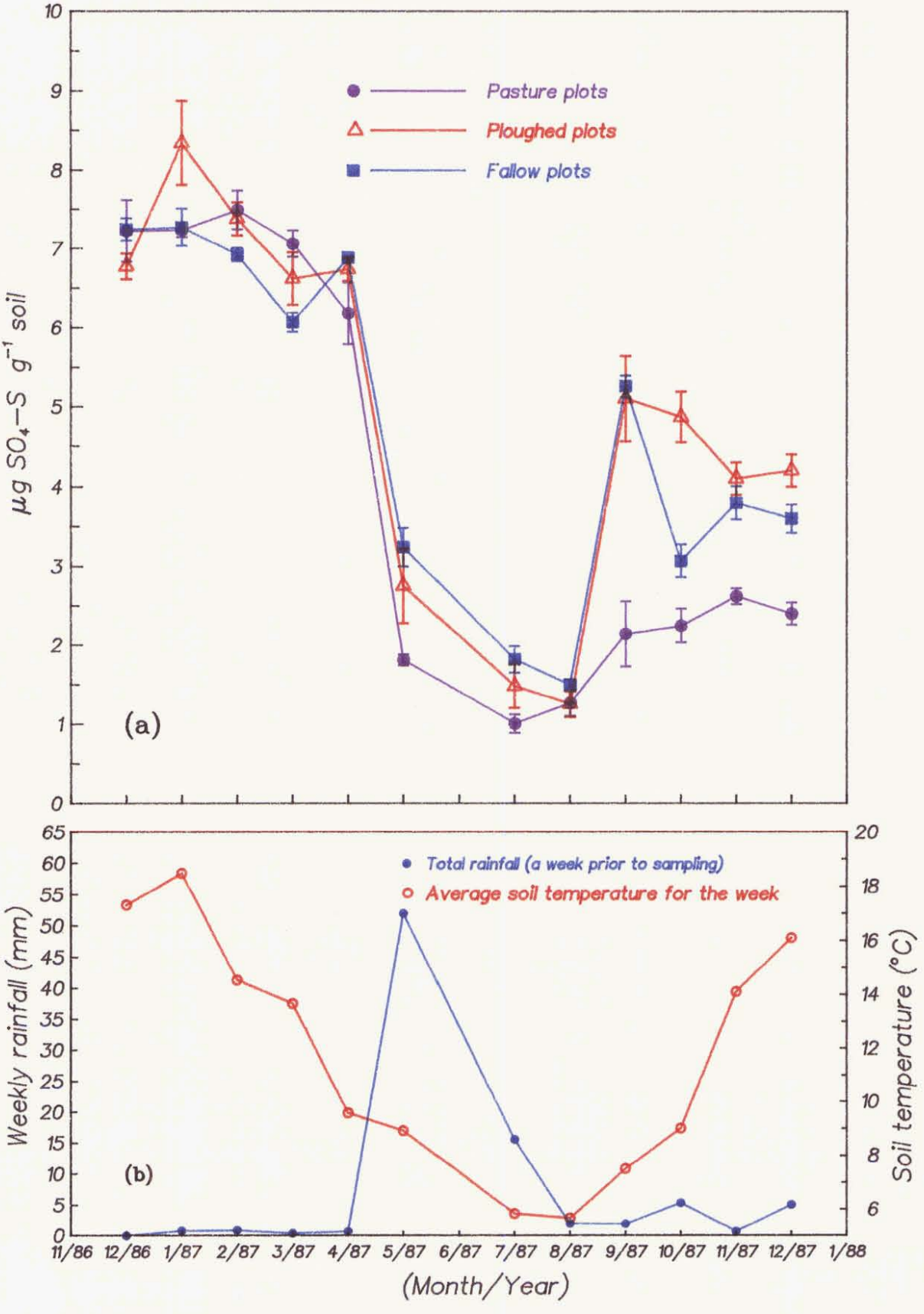


Figure 10.3 Effect of rainfall and average soil temperature at 10 cm soil depth on microbial biomass–S during the year.

Table10.1 Total sulphur concentration in plant tissues harvested from the pasture plots during the year. First cut was in December, 1986 and six cut was made in November, 1987.

Harvesting number	Harvesting time (month/year)	Total concentration of S (% of dry matter)*
First cut	12/86	0.27±0.05
Second cut	2/87	0.25±0.03
Third cut	3/87	0.26±0.08
Fourth cut	8/87	0.27±0.06
Fifth cut	9/87	0.23±0.06
Sixth cut	11/87	0.24±0.03

* mean ± s.e. of three determinations.

The levels of sulphate-S decreased further during the winter months (May-August). The reasons for these decreases are discussed in the following section. From August 1987, sulphate-S levels started increasing again as the temperature increased. The plot treatments had a significant effect on the amounts of sulphate-S present during this period (August-December 1987). In particular the pasture plots had significantly lower amounts of sulphate-S than the other two treatments. This was possibly due to uptake of mineralised sulphur by growing plants, leaving less sulphate-S in the soil solution. Levels of sulphate-S in the last two months of 1987 were lower than they were in 1986 at the same time. During the period when soil sulphate-S levels were adequate (December 1986 to April, 1987) , plants showed higher concentration of sulphur, containing between 0.26 and 0.27% S on the plant dry matter weight basis (Table 10.1) and when sulphate-S in soil reached towards deficient levels in the summer 1987 (between 3 and 4 μg sulphate-S g^{-1} soil), plant sulphur concentration also showed a corresponding decrease. Sulphur concentration in plants during this period ranged between 0.23 to 0.24%. (Table 10.1). According to the values suggested by Cornforth (1981), total sulphur in mixed pasture herbage should be above 0.27% of the dry matter. The plants in the latter stages of this study contained below this optimum concentration and hence could be considered sulphur deficient.

Seasonal fluctuations in microbial biomass-S levels in the soil are shown in Fig. 10.3a. The amounts of biomass-S were lower in the summer of 1986/87 compared to the spring/summer of 1987/88. It is known that soil microbial activity is higher at higher temperatures (Williams, 1967). However, despite the high temperature during the 86/87 summer, biomass-S remained relatively low. The reason for this could be due to low moisture content during that period. There were at least three months (Dec. 1986, Jan. and April, 1987) when plants were suffering from acute water stress, where soil moisture content was approximately at wilting point (Table 10.5). Under these conditions micro-organisms would have had to compete for moisture with plant roots and therefore may have suffered moisture stress. This would have reduced the microbial biomass in soils resulting in lower biomass-S and possibly, dead microbial cells may have contributed to the higher levels of sulphate-S recorded during that period of time.

There were significant effects of soil treatments on the amount of biomass-S. The effects of treatments were clearly observed between April and December, 1987, where biomass-S levels in the ploughed and fallow plots were significantly lower than those in the pasture plots. The lower amounts of biomass-S in the ploughed and fallow plot may have been initially caused by the herbicide treatment, but later affect may have been due to seasonal variation caused by lower temperature, moisture and possibly lack of metabolisable nutrients. Biomass-S as a percentage of the total sulphur in these plots ranged from 0.37 to 1.42 (Table. 10.2). In general, the amount of biomass-S was lower in the winter and increased in the summer. Biomass-S in the pasture plots varied between 0.59 to 1.79% of the total sulphur. These variations are not only dependent on soil temperature and nutrients but also soil moisture, which plays an important role in the build-up of microbial population. Soil moisture at the time of sampling shown in Table 10.5 indicates at least three occasions when soil moisture was below the wilting point. When moisture reaches that level then competition between plant roots and micro-organisms for the moisture remaining in the soil increases. Plant roots having large surface area would probably utilise most of the soil moisture and this would have adverse effects on the microbial population. Moisture may have had some effect on lowering biomass-S during December 1986 to April 1987.

10.3.2 Effect of rainfall and soil temperature on sulphur fluctuations

There are a number studies which have shown that losses of SO_4^{2-} in field conditions are associated with the amount of rainfall (e.g., Goh and Gregg, 1982; Kuhn and Weller, 1977). The higher the rainfall the higher is the loss of sulphate-S from surface soil. There is also sufficient evidence to suggest that soil temperature is an important factor in sulphur mineralisation (e.g., Tabatabai and Al-Khafaji, 1981) sulphate-S concentrations increasing with temperature. Therefore, an attempt was made to relate the changes in sulphate-S levels observed in this study with the monthly rainfall and average monthly temperature at 10 cm soil depth. The relationship between these two factors and the seasonal fluctuation in soil sulphate-S levels is shown in Fig. 10.2b. Clearly, monthly rainfall showed no relationship with the loss of SO_4-S. However, soil temperature shows a much better relationship with the changes in sulphate-S observed during the year. An increase or decrease in soil temperature is followed by an increase or decrease in sulphate-S in soil.

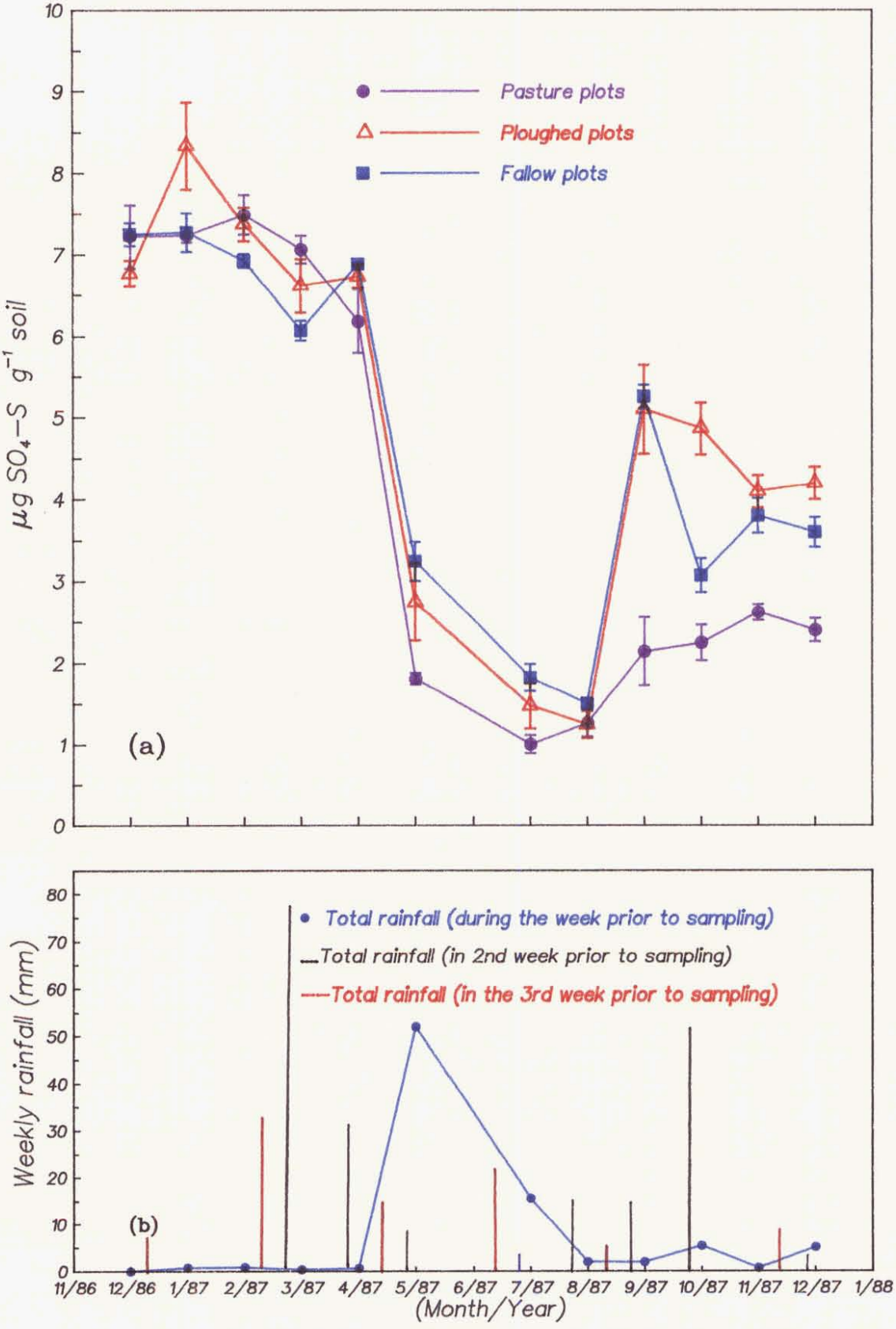


Figure 10.4 Relationship between sulphate–S in soil samples and rainfall prior to sampling period.

Table10.2 Percent of total sulphur held in soil microbial biomass in pasture, ploughed and fallow plots during the year.

Sampling time	(Biomass-S as percent of total S* in soil)		
(month/year)	Cultivation practice		
	Pasture	Ploughed	Fallow
12/86	1.30	0.75	0.99
1/87	1.24	0.99	0.76
2/87	0.92	0.94	0.71
3/87	0.68	0.82	0.69
4/87	0.59	0.47	0.38
5/87	1.20	0.77	0.48
7/87	1.72	0.75	0.37
8/87	1.43	0.44	1.28
9/87	1.79	1.46	0.98
10/87	1.76	0.72	1.10
11/87	1.64	1.12	1.19

* total sulphur in these soils was 250 $\mu g\ g^{-1}$ soil.

Table10.3 Rainfall required to cause leaching in relation to water holding capacity of the Wakanui silt loam soil (Greenwood, personal communication).

soil depth (cm)	available water capacity (AWC)	75% of the AWC	rain required to cause leaching
		(mm)	
0-5	6.6	4.9	4.9
5-10	7.7	5.8	10.7
10-15	7.1	5.3	16.0
15-20	7.4	5.6	21.6
20-25	8.4	6.3	27.9
25-30	8.2	6.2	34.1
30-35	6.6	4.9	39.0
35-40	6.0	4.5	43.5
40-45	6.0	4.5	48.0
45-50	6.6	5.0	53.0
50-55	7.3	5.5	58.5
55-60	7.4	5.6	64.1

* assuming that soil contained 25% of the moisture of the AWC prior to rainfall.

Possibly this is a direct affect of increased microbial activity which is controlled by temperature. Studies by Tabatabai and Al-Khafaji (1981) and Pirela and Tabatabai (1988) clearly show that an increase in the temperature increases the release of sulphate-S from the soil organic sulphur fraction. However, temperature alone cannot be taken as an indicator for the amount of sulphate-S in soils. The soil micro-organisms have a minimum requirement for moisture as well as heat.

Changes in soil temperature during a month are generally gradual (and some-what predictable). However, the amount of rainfall, its frequency and intensity can be extremely variable. If the bulk of the rainfall occurs in the early part of the month and soil samples are collected at the end of the month the effect of rainfall, calculated on a monthly basis, on sulphate-S levels might not show any meaningful relationship. Therefore, despite heavy rainfall recorded during a month soil sulphate-S levels may recover and give a high value. In a 1-2 week period after a rainfall event, more S may be mineralised by soil micro-organisms thus returning soil sulphate-S to approximately the same level as prior to the rainfall. This assumption would only be true if the soil has sufficient mineralisable organic sulphur.

A more detailed examination of rainfall in relation to soil sulphate levels is shown in Figure 10.4. Total rainfall a week prior to soil sampling showed a much better relationship with sulphate-S levels compared to the monthly rainfall data. The decline in sulphate-S levels in May 1987 appears to be related to the high rainfall in the week preceding the sampling (52.0 mm). Although rainfall was not particularly high after this event, soil sulphate-S levels remained low, presumably as a result of low temperatures (4-5 °C) restricting mineralisation. Laboratory studies have shown that mineralisation is very poor at 5 °C (Swift, 1983). A number of studies have suggested that a temperature between 4-5 °C would arrest microbial activity and that is why this temperature is widely used for storing field samples. As soil temperature increased from September onwards so did soil sulphate-S levels. Since total rainfall prior to soil sampling during these months was relatively low most of the mineralised sulphate-S remained within the sampling zone.

Another conclusion can be drawn from Fig. 10.4, where rainfall 2 and 3 weeks prior to sampling showed very little effect on sulphate-S measured in soils. Even after a substantial

rainfall (Feb. and Oct. 1987) two weeks prior to soil sampling, which is likely to have caused leaching of soil sulphate, the level of sulphate-S remained relatively high. This would suggest that significant amounts of sulphate-S were subsequently released through mineralisation. This perhaps shows the magnitude and speed of this process operating under field conditions.

Biomass-S fluctuations showed a stronger relationship with soil temperature than with soil moisture (Fig. 10.3). At lower temperatures (May to September), biomass-S was lower in the fallow and ploughed compared to the pasture plots. Biomass-S in the pasture plots remained relatively high even in the winter months. This is largely due to the rhizosphere effect where increased microbial activity in the root zone results from the increased level of organic compounds in that region, originating from the exudation of soluble C-compounds, lysis of root cap cells, root hairs and epidermal cells (Sparling, 1985). Presumably, when plant growth increases during the spring and summer seasons, root exudates also increase which giving a corresponding increase in soil biomass and consequently increases the biomass-S (Fig. 10.3).

10.3.3 Sulphate-S leaching caused by rainfall

Although, total rainfall a week before sampling appears to be effective in explaining the cause of sulphate-S losses through leaching, the quantity of water from the rainfall may not be enough to cause the downward movement of solutes. Therefore, it is essential to have an understanding of how much water is required to cause leaching. Such assessments can be made by using a water balance approach in the soil profile. If the water input from the rainfall is more than the available water capacity (AWC) then the excess water is likely to cause leaching of soluble sulphate-S. Available water content is the amount of water retained between field capacity (F.C.) and wilting point (W.P.). Using the data from P. Greenwood (personal communication) for available water content in the Wakanui soil (Table 10.3), it was determined that this soil could retain approximately 11 mm of rain-water within the 7.5 cm sampling depth. Without accounting for evaporation, and assuming that the soil had 25% of the AWC moisture before rainfall occurred, then any rain more than 75% of the AWC as shown in Table 10.3 would cause

Table10.4 Depth of sulphate-S movement caused by rainfall estimated from Table 10.3.

Month/Year	Rainfall* (mm)	Estimated depth of SO_4^{2-} movement (cm)
12/86	0.0	0-5
1/87	0.8	0-5
2/87	0.9	0-5
3/87	0.4	0-5
4/87	0.7	0-5
5/87	**52.0**	45-50
7/87	**15.6**	10-15
8/87	2.0	0-5
9/87	2.0	0-5
10/87	5.4	0-5
11/87	0.7	5-10
12/87	5.1	0-5

*. total rainfall which occurred during the week prior to soil sampling

Table10.5 Moisture content in the soil samples at the time of sample collection*.

Month/year	Pasture	Fallow	Ploughed
	(% moisture w/w)		
12/86	**11.2**	**11.4**	**11.7**
1/87	**9.8**	**9.3**	**10.0**
2/87	14.2	13.6	13.0
3/87	13.0	12.2	12.0
4/87	**11.6**	**9.6**	**9.4**
5/87	22.4	21.2	20.2
7/87	22.0	20.0	19.0
8/87	15.4	13.0	13.8
9/87	19.5	16.5	18.0
10/87	15.0	14.5	15.0
11/87	-	-	-
12/87	14.8	13.0	13.4

* soil contains 9.4% moisture at the wilting point and 19.8% moisture at field capacity. values close to the wilting point (**bold**) may affect the microbial biomass-S.

depth, more than 7.8 mm rainfall is required. As shown in Table 10.4 there were only two rainfall events greater than 7.8 mm which could have leached the SO_4-S beyond 7.5 cm soil depth. Rainfall in May/87 would have leached soil SO_4-S to 45-50 cm depth while rain in the month of July would have moved it to 15 cm of the soil depth. It is clear that leaching which occurred in May would seriously affect the plant-available sulphur because most of the plant roots in pasture are within 20-30 cm of the soil surface. However, leaching in the month of July would have moved the sulphate-S beyond 7.5 cm depth, but it would still be retained within the rooting zone. Therefore it would not affect plant uptake of sulphate-S. The estimated movement of SO_4-S calculated in Table 10.4 has not taken into account the by-pass flow which can cause rapid downwards movement of water (White, 1985). Therefore leaching losses in sub-surface soils would be of greater magnitude than those given in Table 10.4.

10.4 CONCLUSIONS

It can be concluded from this study that using monthly data to explain sulphate-S leaching is not satisfactory. Total rainfall during the week prior to sampling is likely to give a much better indication of leaching losses in these soils. Soil temperature is also an important factor in determining the extent of sulphur mineralisation. These studies have a direct implication in soil testing and fertilizer recommendations for sulphur. An improved understanding of these soil factors (rainfall and temperature) could help to avoid errors in fertilizer recommendations. At the time of the interpretation of the soil test, one should also consider the soil temperature and rainfall occurring within the week prior to sampling. One should also consider sampling the soil beyond 7.5 cm, perhaps to 20 to 30 cm soil depth. It is likely that sampling of the surface soil may show a deficiency but in fact soils below that depth and within the plant root zone may have adequate amounts of sulphate-S. Hence, recommendations made on the basis of surface soil samples may give inconsistent results.

CHAPTER ELEVEN

GENERAL SUMMARY, CONCLUSIONS AND SUGGESTIONS FOR FURTHER WORK

This chapter of the thesis summarises the results and also conclusions of the study. Summary has been arranged in an order compatible with the objectives outlined in the introduction (see section 1.2). After the conclusions, some suggestions also are given for future research work aimed at improving the understanding of the sulphur cycling in soil system.

11.1 SUMMARY AND CONCLUSIONS

11.1.1 Methods for measuring sulphur mineralisation in soils

The closed incubation system which has been used in the past by many workers to measure sulphur mineralisation was also examined in this present study. Results obtained from this type of incubation showed variable pattern of sulphur mineralisation and immobilisation (Fig. 4.2). Limitations of this method have been discussed in section 4.3. As it is stated in the objectives (section 1.2) a method which measures sulphur mineralisation should show consistent results so that mineralisation can be correlated with soil properties. Hence, closed incubation method was not considered for measuring mineralisation in soils.

An open incubation system was adopted for studying mineralisation of sulphur in New Zealand soils. The main features of open incubation system have been explained in section 4.3.1. The amount of sulphur mineralised as SO_4^{2-} is periodically removed from the soil system. The removal of mineralised-SO_4^{2-} in this type of incubation resembles field conditions, where mineralised-SO_4^{2-} is continuously removed either by plant

uptake or by leaching down the profile. This method of determining the mineralisable sulphur gave consistent pattern of sulphur mineralisation (Fig. 4.2, 4.4 and 4.8).

Soil temperature has shown to have a direct influence on the amount of sulphur mineralised (Fig. 4.5). These results are consistent with those reported by Williams (1967) and Tabatabai and Al-Khafaji (1980), who have reported similar increase in sulphur mineralisation with the increase in temperature.

A comparative study of sulphur mineralisation in eighteen New Zealand soils (described in Table 3.1) was carried out using the open incubation method at 30 °C for 10 weeks. A minimum of 1.13% and a maximum of 6.58% of the total organic sulphur was mineralised from the Waimakariri and Teviot (limed) soils respectively (Table 4.6). These amounts represent approximately 3.4 and 32.2 kg sulphur ha^{-1}. The magnitude of mineralisation reported in this study is in agreement with the findings of other workers who used similar system of incubation e.g. Tabatabai and Al-Khafaji 1980; Pirela and Tabatabai, 1988. A prolonged incubation (28 weeks) on six soils showed even higher level of mineralisation (Table 4.3) where the release of SO_4^{2-} ranged between 19.6 to 49.9 kg sulphur ha^{-1} (representing between 5 and 11% mineralisation of total organic sulphur in soils). Studies by Tabatabai and Al-Khafaji (1980) and Pirela and Tabatabai (1988) have shown that mineralisation of sulphur is linear; in other words sulphur is mineralised at a constant rate. However, in this present study, the rate of mineralisation of sulphur decreased with an increase in the incubation period which would suggest a more curvilinear relationship rather than linear one, particularly in the long-term. A clear illustration of curvilinear nature of sulphur mineralisation is shown in the prolonged incubation studies (Figure 4.7). The rate of sulphur mineralisation during the later period (26-28 week) was almost 4 to 10 times less than the initial rate of mineralisation.

11.1.2 Correlation between sulphur mineralisation and soil properties

A single factor correlation between total mineralised-SO_4^{2-} measured in the open incubation and the soil chemical properties showed non-significant relationship with the total nitrogen, C:N and C:S ratio (Table 4.10). There was a weak relationship between the mineralised sulphate and water-soluble SO_4^{2-}, HI-reducible S, soil pH and N:S ratio

(significant at 5% level of significance). These soil properties could account for only 25-30% of the variation in the mineralised sulphate between soils. Other soil properties such as phosphate-extractable SO_4^{2-} and total organic sulphur in soils were slightly better indicators of potentially mineralisable sulphur in soils (significant at 5% level of significance). These soil properties were able to account for between 35 and 45% of the variation of sulphur mineralisation between soils. The correlation between mineralised-SO_4^{2-} and phosphate-extractable sulphate is of particular interest especially for computerized fertilizer advisory service (CFAS), where the amount of extractable sulphate is used as an indicator for recommending sulphur fertilizer. The results from this study suggest that extractable sulphate is a poor indicator for estimating sulphur mineralisation potential of the soil. The best single factor correlation for mineralised-SO_4^{2-} was found with C-bonded forms of S. The amount of C-bonded sulphur accounted for upto 63% of the variation in sulphur mineralisation between soils. Soils having higher amounts of C-bonded sulphur mineralised higher amounts of sulphur during the incubation. Since soil chemical properties are somewhat inter-related with each other (Table 3.4), therefore a multiple regression was attempted with all possible combinations of soil properties to achieve an improved correlation. The best correlation was obtained from the combination of C-bonded sulphur and C:N ratio (see equation 4.7), which accounted for up to 71% of the variation in sulphur mineralisation between soils. The weakness of the correlation could be due to the variation in the microbial population and their species. Variations in the soil physical characteristics may also contribute to the overall variation in the mineralisation of sulphur in soils.

11.1.3 Mineralisable forms of organic sulphur

A comparison of organic sulphur fractions before and after the incubation has helped in identifying the forms of sulphur which contributed to the release of sulphate during the incubation studies. Most of the soils showed a significant decrease in the C-bonded forms of sulphur during the incubation and at the same time, the HI-reducible forms of sulphur either remained unchanged or increased (Fig. 5.2 and 5.3). This affirms that the organic sulphur held as C-bonded sulphur represents the mineralisable forms of sulphur in soils. These findings are consistent with the studies by Freney *et al.* (1975); McLachlan and DeMarco, (1975) and McLaren and Swift, (1977), all of these workers have reported

considerable mineralisation of C-bonded forms of sulphur both in field studies (McLachlan and DeMarco, 1975; McLaren and Swift, 1977) and in glass-house studies (Freney *et al.*, 1975). Identification of C-bonded form of sulphur as a mineralisable pool of sulphur is also consistent with the single factor correlation coefficient values, where the amount of C-bonded sulphur in soil gave the most significant correlation with the amount of mineralised-SO_4^{2-}. It is difficult to quantify as to how much of C-bonded sulphur is mineralised. In order to identify the fraction of C-bonded sulphur which are mineralised during the incubation, a detailed characterization may help to estimate the fraction of mineralisable S.

11.1.4 Estimation of potentially mineralisable sulphur

The usefulness of Pirela and Tabatabai (1988) methods of estimating the potentially mineralisable sulphur (S_0) in soils and time required to mineralise at least 50% of the potentially mineralisable sulphur (K_f) were examined for data obtained in this study. The two techniques used by these workers i.e. a reciprocal-plot technique and exponential method of estimating the S_0 and K_f were examined in detail (see section 4.6). It was found that the estimation of these values depended upon the temperature and the length of incubation. In some cases, the values estimated for S_0 either by reciprocal plot technique or exponential method gave a negative value although significant amounts of sulphur were in fact mineralised during the incubation (Table 4.8a and 4.8b). Therefore, it is concluded that such estimations of S_0 and K_f by either technique is unsatisfactory.

11.1.5 Interaction of nutrients on sulphur transformations

Effects of adding carbon, nitrogen and sulphur on the mineralisation of sulphur were investigated. Effects of these nutrients were also examined on the transformation of sulphur from one organic form to the other (see section 6.3-6.5). Addition of sulphate-S significantly decreased the amounts of sulphur mineralised. Addition of nitrogen as NO_3^- to soil showed inconsistent effects on sulphur mineralisation. In some cases it increased sulphur mineralisation (Table 6.1a) and other cases showed no effect (Table 6.1b). Addition of carbon had the strongest influence on sulphur mineralisation where it

generally decreased the amount of sulphate in soil solution. It is well known that addition of carbon encourages microbial growth. Such a growth was also noted (increased CO_2 evolution) in these experiments which would have encouraged the assimilation of sulphate-S into microbial tissues.

The dichotomous model of sulphur cycling as proposed by McGill and Cole (1981) (see section 2.7) was tested by manipulating the nutrient availability (C,N and S) in soil (see section 6.3). It was found that deficiency of SO_4^{2-} in soils with an increased microbial activity (where C was added) did not necessarily encourage mineralisation from the HI-reducible forms of S. In most cases microbes selectively mineralised more C-bonded sulphur than HI-reducible sulphur (see Fig. 6.4 and 6.5). It was also noted that micro-organisms synthesised considerable amounts of HI-reducible forms of sulphur even when soils were deficient in sulphate-S. Addition of carbon to soils resulted in a significant decrease in the mineralisation of C-bonded S. This would suggest that mineralisation of C-bonded forms of sulphur is controlled by the availability of metabolisable C in soils.

It can also be concluded that such a mineralisation is occurring mainly to obtain carbon. However, this present study also showed that C-bonded forms of sulphur could be mineralised to require sulphur or possibly N. These finding show that the dichotomous model requires further refinement and that work is needed to assess this model.

11.1.6 Sulphur cycling in soils

Sulphur-35 was used as a tracer both as a carrier-free and with sulphate as a carrier to examine the cycling of sulphur in a closed incubation system (see section 7.3.1 and 7.3.2). Soil treatment prior to the addition of sulphur-35 i.e. preconditioning, air-drying and treatment of air-dried soil with 1% glucose-C had a marked effect on the rate of ^{35}S incorporation into the soil. These treatments also affected the nature of ^{35}S incorporation into organic sulphur fractions (Figures 7.2, 7.3 and 7.4).

Preconditioned soil showed comparatively slower rate of incorporation than the air-dried and glucose-treated soils. On average between 8 and 19% ^{35}S was incorporated within a short-term (1-5 days). A maximum of 48% of the added ^{35}S was incorporated between 90 and 120 days. Air-dried soil showed slightly faster rate of incorporation in the short-term, incorporating between 14 and 28% of the added sulphur-35. A maximum of 46% of the was incorporated within 60 to 90 days of incubation. The rate of ^{35}S incorporation was extremely high in the glucose-treated soils, where 77% of the ^{35}S was incorporated within 5 days. A maximum of 84% incorporation was reached within 32 days. After maximum incorporation in air-dried and glucose-treated soil, some of the recently incorporated ^{35}S was mineralised to sulphate-^{35}S. This was particularly obvious in glucose-treated soil (see Fig. 7.4). It was noted, the mineralised-SO_4^{2-} had come from the mineralisation of C-bonded forms of sulphur because the amount incorporated in this fraction decreased towards the end of the experiments.

Results have shown that the nature of sulphur cycling varied depending on the soil treatments. In the preconditioned soil, in the short-term, incorporation of added ^{35}S was mainly into HI-reducible forms of sulphur (80-100% of the total incorporated ^{35}S). During the same period, in the air-dried soil, the proportion incorporated as HI-reducible sulphur-35 was 60% and up to 40% was incorporated into C-bonded forms of S. In glucose-treated soil the proportion of HI-S decreased further and only 11% of the incorporated ^{35}S remained into this fraction. In other words, in the glucose-treated soil ^{35}S incorporation was predominantly into C-bonded forms of sulphur (89% of the total incorporated ^{35}S). In the long-term (60-120 days), the proportion of labelled HI-S in the preconditioned soil decreased to 65% while in the air-dried and glucose-treated soil the proportion of labelled HI-S increased to 50% and 30% respectively.

Soil microbial biomass-S was determined using the biocidal fumigation technique as suggested by Saggar *et al.* (1981a). Radio-activity (^{35}S) measured in the biomass-S gave an idea about their involvement in the sulphur cycling. Results from this study showed considerable amounts of ^{35}S are cycled through the microbial biomass. Particularly in the glucose-treated soil, at times up to 90% of the incorporated ^{35}S appeared to be present in the microbial biomass. The amount of biomass-^{35}S varied from treatment to treatment. Glucose-treated soil had maximum followed by air-dried

and preconditioned soil. The amount of biomass in all treatments fluctuated considerably during the incubation, probably reflecting the transitory nature of the sulphur held in the microbial tissues. Considerably higher microbial activity in the short-term in air-dried and glucose-treated soil seemed to be responsible for incorporating the ^{35}S predominantly into C-bonded forms of S. Early incorporation of ^{35}S in the preconditioned soil is believed to be carried out by extracellular enzymes which did not involved the direct participation of soil microbes (Fig. 7.7) and that is why there was relatively little incorporation into C-bonded sulphur.

Addition of sulphate seemed to have retarded cycling of sulphur. Results from sulphate-^{35}S showed considerably lower incorporation compare with carrier-free ^{35}S (Table 7.8). Presence of sulphate-S appeared to have encouraged incorporation mainly into HI-reducible sulphur.

11.1.7 Mineralisation of recently incorporated sulphur-35

A study of the mineralisation of recently incorporated sulphur-35 was carried out in an open incubation system for 10 weeks. Soils were preincubated in a closed incubation system with sulphur-35 for 32,60,90 and 120 days, where different amounts of added sulphur-35 were incorporated into organic sulphur fractions (see section 7.3.1.2). It was found that the longer the sulphur-35 remained in the organic matter the less of it was remineralised as sulphate (Figures 8.1 and 8.2). A comparison between the amounts of sulphur mineralised from the native sulphur and recently incorporated sulphur-35 further confirms this phenomenon, where between 15-55% of the total incorporated sulphur-35 was mineralised compared with 3-5% mineralisation from the native sulphur pool.

Unlike the unlabelled experiments, where mineralisation of organic sulphur was measured mainly from C-bonded forms of sulphur, this experiment showed that both forms of organic sulphur i.e. HI-reducible and C-bonded mineralised to sulphate during the 10 week open incubation (Figure 8.4). However, in some cased, mineralised-SO_4^{2-} appeared to have come mainly from C-bonded forms of sulphur (Figure 8.5).

11.1.8 Mineralisation of C-bonded forms of sulphur

A technique was developed whereby HI-reducible forms of sulphur were removed from the soil organic matter (details in section 9.2). Such a separation allowed a study of the mineralisation characteristics of C-bonded sulphur (see section 9.3) and also established a better understanding of the pathways of sulphur transformations in the soil system.

Organic sulphur retained as C-bonded forms of sulphur in soil was mineralised to sulphate and transformed to HI-reducible forms of sulphur. Approximately 10% of the total C-bonded sulphur was mineralised/transformed during a 40 day period of incubation. Since SO_4^{2-} and HI-reducible sulphur appeared simultaneously in the incubated soil, it was difficult to determine whether sulphate was a precursor of HI-reducible sulphur or HI-reducible sulphur was directly formed from C-bonded forms of sulphur. However, this present study confirms that C-bonded forms of sulphur represents a mineralisable pool of organic sulphur and it can be transformed to HI-reducible forms of sulphur.

11.1.9 Seasonal variations in sulphate-S and biomass-S

Under field conditions, seasonal changes have considerable impact on the amounts of sulphate retained in the upper horizon (in this case 75 mm soil depth). During the winter period when rainfall is high, the amounts of sulphate-S decreased considerably (Fig. 10.2). The reason for this loss is mainly attributed to sulphate leaching, where excess rain-water transports water-soluble sulphate down the profile.

In the past, workers have attempted to explain sulphate losses by using the average monthly rainfall prior to sampling (e.g. Cornforth *et al.*, 1983). However, this present study found a poor relationship between monthly rainfall and the amounts of sulphate measured in soils (Fig. 10.2). It was found that the amounts of rainfall a week prior to sampling relate better to the amounts of sulphate in soils (Fig. 10.4).

Biomass-S fluctuates throughout the year. When moisture is adequate and temperature is high (autumn and summer), the amount of sulphur held in microbial tissues is also high compared with the winter period (Fig. 10.3). A direct comparison of biomass-S values between fallow and pasture plots shows that biomass-S is always higher in the pasture plots (Fig. 10.3). This could be due to a rhizosphere effect which encouraged higher microbial growth in pasture plots.

The present study concludes that use of monthly rainfall data to explain sulphate leaching losses in soils is unsatisfactory. Instead, total rainfall a week prior to sampling is likely to give much better indication of leaching losses in soils. During the time of interpretation of soil sulphate results for sulphur fertilizer recommendation, due consideration should also be given to soil temperature, which is likely to effect the supply of sulphur for plant growth.

11.2 SUGGESTIONS FOR FUTURE RESEARCH

The results presented in this thesis have shown the need for further research in several areas related to sulphur cycling in the soil.

(a) The open incubation system developed during this study need further investigation to test its general applicability, for instance, measuring sulphur mineralisation in soils from different climatic zones and cultivation regimes. For practical purposes it is also necessary to correlate sulphur mineralisation data with plant uptake of sulphur and responses to sulphur measured under field conditions. It may then be possible to integrate such information in the existing CFAS model.

(b) It has been shown in Chapter six of this thesis that dichotomous model proposed by McGill and Cole (1981) does not satisfactorily explain some of the results obtained in this study. Since this model is gaining acceptance by scientists, it is necessary to examine this model more critically.

(c) Procedures for determining biomass-S need improving. In Particular, K_S values need to be determined for soils from different cultivation and climatic regimes.

(d) Organic sulphur in soils is presently characterised rather crudely as HI-reducible and C-bonded sulphur. This aspect of sulphur research needs further investigation and the forms of organic sulphur need to be further refined.

(e) Having characterised the forms of organic sulphur in soils in more detail, the contribution of a specific fraction can then be studied. This would help in understanding the sources and pathways of sulphur mineralisation in soils.

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Appendix 1

Derivation of Equation 4.5

$$S_m = S_o (1-e^{-kt}) \quad \ldots\ldots \quad (4.4)$$

Where S_m = S mineralised (measured)
S_o = Potentially mineralisable S
k = First order rate constant

Therefore for half of the S_o to be mineralised, t can be determine as follows;

$$1/2\ S_o = S_o (1-e^{-kt})$$

$$1/2 = 1 - e^{-kt}$$

$$e^{-kt} = 1/2$$

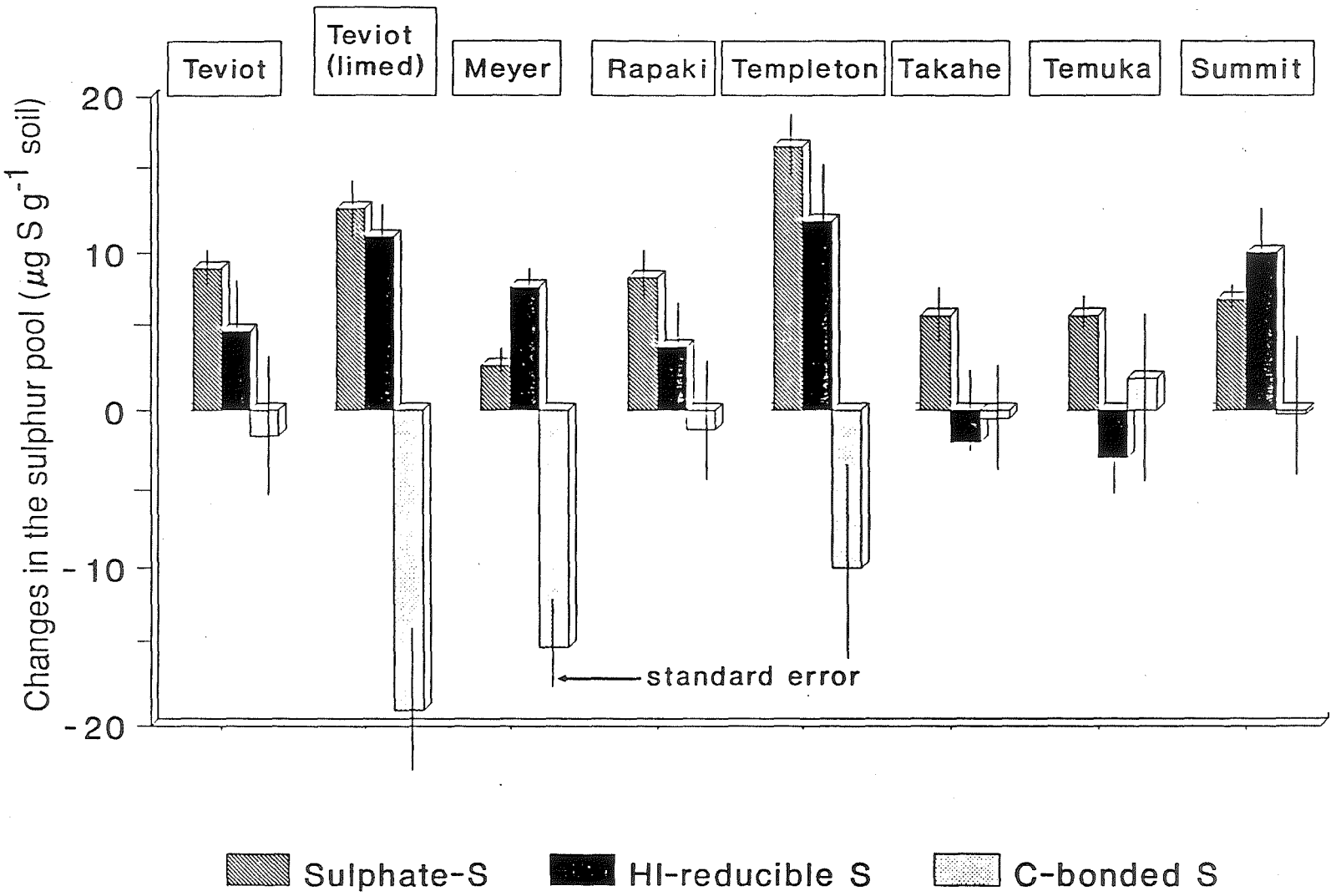
$$kt = - \log_e 2$$

$$t = - \frac{\log_e 2}{k}$$

$$t = \frac{0.69315}{k}$$

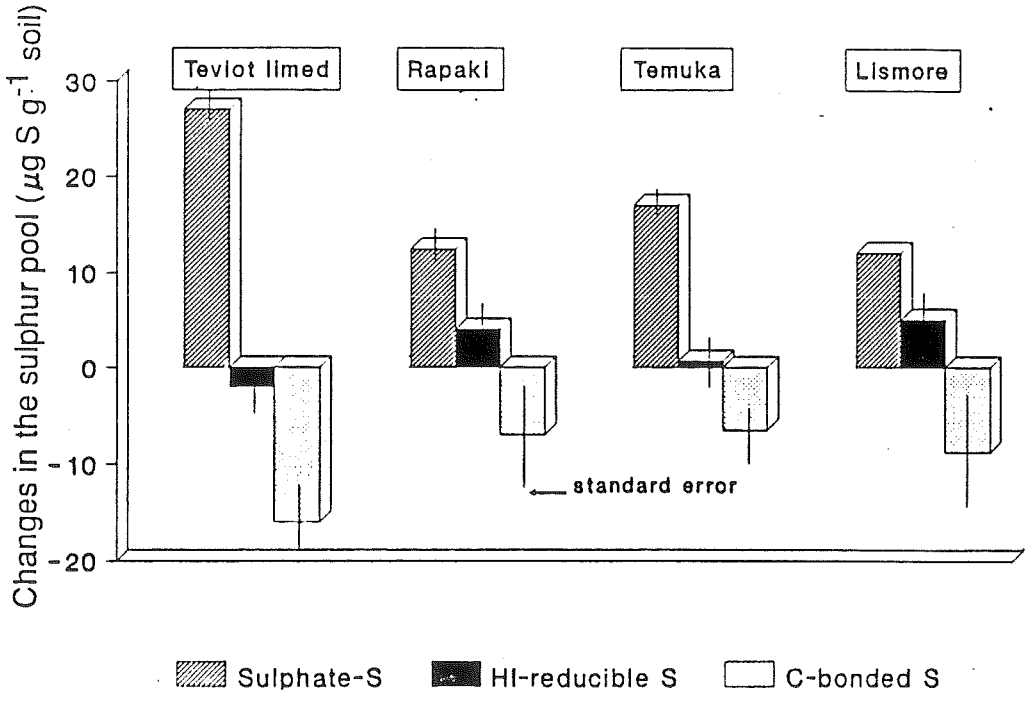
Note; t is equal to k_t when $S_m = 1/2\ S_o$

Appendix 2a



Changes in the individual sulphur fractions after 18 weeks of open incubation at 10 °C.

Appendix 2b



Changes in the individual sulphur fractions after 18 weeks of open incubation at 20 °C.

Appendix 3

Amounts of sulphur held in soil organic fractions during the sulphur-35 incubation study.

Treatment/ Sulphur fractions	Incubation Period (days)											
	0*	1	2	3	5	10	16	24	32	60	90	120
	Sulphur concentration (μg S g^{-1} soil)[1]											
Preconditioned												
Total organic S	400±4	398±6	394±4	405±6	403±4	398±6	400±2	420±6	415±5	402±3	406±6	403±3
HI-reducible S	185±3	188±3	176±5	180±3	176±2	190±5	186±3	183±4	172±4	175±1	185±4	185±5
C-bonded S	215±7	210±9	218±9	225±9	227±6	208±11	214±5	237±10	233±9	227±4	221±10	218±8
Air-dried												
Total organic S	406±6	408±2	403±3	400±2	401±3	404±2	398±5	400±3	396±4	408±2	410±4	400±3
HI-reducible S	176±3	190±5	188±3	194±7	178±4	186±3	188±4	176±4	183±4	190±4	180±4	178±3
Carbon-bonded S	230±9	218±7	215±6	206±9	223±7	218±5	210±9	224±7	213±8	218±6	230±8	222±6
Glucose-treated												
Total organic S	406±4	401±3	405±1	410±3	410±4	398±6	408±4	410±5	405±5	408±4	406±3	n.d
HI-reducible S	180±4	186±5	180±4	176±4	188±5	179±4	190±5	190±6	182±5	178±2	180±4	n.d
Carbon-bonded S	226±8	215±8	220±5	234±7	222±9	219±10	218±9	220±11	223±10	230±6	226±7	n.d

* amount of sulphur held in organic sulphur fraction prior to incubation.

1. mean ± s.e. of six determinations, n.d = not determined.

Appendix 4

Amounts of total, HI-reducible and C-bonded sulphur in organic fractions before and after the reincubation.

Soil treatments	Incubation period (days)							
	32		60		90		120	
	sulphur concentration (μg S g^{-1} soil)							
	before	after	before	after	before	after	before	after
Preconditioned								
Total organic S	415±5	410±5	402±3	398±4	406±6	400±2	403±3	400±4
HI-reducible S	186±4	175±2	180±1	185±3	185±4	185±3	185±5	174±4
C-bonded S	232±9	227±7	218±4	221±7	221±10	215±5	218±8	226±5
Air-dried								
Total organic S	396±4	397±6	408±4	400±5	410±4	404±4	400±3	396±4
HI-reducible S	183±4	178±4	190±4	184±3	180±4	183±3	178±3	174±3
C-bonded S	213±8	219±10	218±8	216±8	230±8	217±7	222±6	222±7
Glucose-treated								
Total organic S	405±5	402±5	408±4	400±4	406±3	400±3	n.d	n.d
HI-reducible S	182±5	192±3	178±2	180±4	180±4	184±4	n.d	n.d
C-bonded S	223±10	210±8	230±6	220±8	226±7	216±7	n.d	n.d

n.d = not determined