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FACTORS AFFECTING SULPHUR NUTRITION OF SUBTERRANEAN CLOVER
ON SANDPLAIN SOILS OF SOUTH-EASTERN SOUTH AUSTRALIA.

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Thesis submitted for the degree of
Doctor of Philosophy
in the Faculty of Agricultural Science.

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March 1970.

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Summary

Sulphur availability and plant yields on Laffer Sand were examined in a field experiment. Fine gypsum (<300 mesh) applied at the rate of 224 kg per ha in autumn or late winter disappeared from the top 20 cm of soil before subterranean clover (Trifolium subterraneum, L.) could respond. Downward movement of gypsum following rain caused increases in the $\text{SO}_4^{=}\text{-S}$ content of the 10-20 cm layer of soil, showing that leaching was occurring. Plant yield responses to the autumn application of gypsum were small, and no responses to the winter application occurred. No residual effects from previous applications of gypsum were evident.

Soil $\text{SO}_4^{=}\text{-S}$ content of the top 10 cm of unfertilized plots remained close to 1 ppm throughout the growing period, but the corresponding plant tops were only moderately deficient, suggesting that mineralization was supplying S for plant uptake.

In an incubation experiment, soil water contents between 6% and 18% had little effect on $\text{SO}_4^{=}\text{-S}$ accumulation, but at 3%, mineralization was limited. At a soil water content of 24% in the presence of 30 ppm of added $\text{SO}_4^{=}\text{-S}$ low recoveries of mineral N and S were recorded, suggesting that losses of volatile forms of S had occurred. The addition of $\text{SO}_4^{=}\text{-S}$ lowered $\text{SO}_4^{=}\text{-S}$ accumulation at all soil water contents.

In a second incubation experiment, intermittent leaching of soil samples increased recoveries of $\text{SO}_4^{=}\text{-S}$ in the leachates compared with those from samples unleached until the end of the experiment. Increases in incubation temperatures from 8.5°C to 32.5°C led to increased $\text{SO}_4^{=}\text{-S}$ accumulation in leached and unleached soils.

Two pot experiments tested the effects of soil temperature, plants, added $\text{SO}_4^{=}\text{-S}$ and soil organic matter accumulation on S mineralization. Failure to recover significant quantities of S originally present in the soil and soil/plant systems provided evidence of volatile S losses. Less S was recovered from the bare pots than from planted pots.

Differences in organic matter contents and C/N/S ratios of Laffer Sands after periods of topdressing ranging from 0 to 27 years had almost no influence on net S mineralization. As the level of organic S rose a decreasing proportion was mineralized. The pattern of organic matter accumulation in the six soils examined suggested that in Laffer sands, under existing cultural practices, an equilibrium may be nearly reached as early as five years after development.

One soil in pot experiment II was fertilized with ^{35}S sulphate and increases in the proportion of unlabelled S in plant tops between harvests I and II, and with increasing soil temperatures, showed that mineralization was supplying S for plant uptake. Radiochemical estimates of S in soil $\text{SO}_4^{=}\text{-S}$ extracts were higher than those made

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by reduction with hydriodic acid, showing that some of the added ^{35}S - sulphate had been converted to reduced forms.

The experiments led to the conclusion that in spite of evidence of S mineralization on Laffer Sands there is a need for regular S fertilization using material such as fragmented rock gypsum, that will maintain S supplies for an extended period.

Declaration

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

A.J. NICOLSON

Preface

A section of this work entitled: "Soil sulfur balance studies in the presence and absence of growing plants" by A.J. Nicolson has been accepted by Soil Science for publication in 1970.

Acknowledgements

These studies were made possible by a generous grant from the Sulphur Institute, Washington, D.C., and I appreciate the keen interest shown by Dr. S.L. Tisdale of that organisation. The work was carried out in the Agronomy Department of the Waite Agricultural Research Institute through the kind permission of Professor C.M. Donald whose help and advice is acknowledged.

The guidance of my first supervisor, the late Mr. J.K. Powrie, who died tragically before the project was completed, was particularly valuable during the experimental phase. I wish to thank my supervisors, Mr. K.P. Barley, Agronomy Department, and Dr. R.J. Swaby, C.S.I.R.O., Division of Soils, for advice, guidance and constructive criticism freely given, especially in the latter part of this study.

Appreciation is also extended to Dr. R.F. Sealark, Department of Animal Physiology, for assistance and advice in the radioisotope studies. To other members of the Waite Institute staff, thanks are extended for their help and advice; to Messrs. D.J. Williams and J. Scarvelis for assistance with experimental work; to Messrs. E.J. Leaney and M.J. Barge, for assistance with apparatus; to Mr. R. Lamacraft, for statistical analyses; to Mr. B. Palk for photography and reproduction of figures; and to Mrs. V. Matheson for

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typing the draft and final manuscripts.

Special acknowledgement is due to my wife, Mrs. J.R. Nicolson for assistance with statistical analyses, preparation of figures, the reading and checking of the manuscript and unfailing support throughout.

I Introduction

Sulphur is an essential macronutrient for plant growth. In plants it is a component of many proteins, S-amino acids, some vitamins, and inorganic sulphates. The importance of S in plant and animal nutrition has slowly been recognized, and throughout the world widespread areas of deficiency are now known to exist (Coleman, 1966).

In the agricultural areas of South Australia where most soils are phosphorus deficient, the use of superphosphate containing up to 12% S as gypsum ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$) has masked S deficiency. Experiments to study the residual effect of superphosphate (Powrie, unpublished) on subterranean clover (Trifolium subterraneum, L.) pastures in the upper south east of South Australia revealed that the sand plain soils (mainly solodized solonetz soils) of the region were S deficient. The incidence and severity of the deficiencies appeared to vary between sites, and with seasonal conditions, therefore it was decided to investigate the factors affecting S availability on these soils.

The experiments described were designed to examine aspects of the S-cycle affecting the supply of soil $\text{SO}_4^{=}\text{-S}$ for plant uptake. The field experiment was the first step in the investigation and followed changes in the level of soil $\text{SO}_4^{=}\text{-S}$ at

2.

intervals over a period of one year. Soil water and temperature measurements made during this experiment were used to select the ranges of the environmental treatments for the subsequent incubation studies and pot trials. These experiments examined the significance of S released from soil organic matter by mineralization.

II Literature Review

1. Development of Knowledge of the S-Cycle

In the early days of plant nutrition studies, Liebig (cit., Russell 1962) recognized that S was an element necessary for plant growth. The full extent of S requirements was, for many years, masked by poor analytical methods and the use of superphosphate fertilizer.

Dry combustion methods of plant analysis used until the late nineteenth century, gave low recoveries of S and led to low estimates of plant requirements. Superphosphate was patented by Lawes in 1842 (cit. Jacob 1964) as a phosphorus fertilizer. Widespread use of this fertilizer following its introduction led to incidental application of gypsum, and in some parts of the world delayed the recognition of S deficiencies for nearly a century.

Early measurements of S additions in rainfall were made by Smith (cit. Lawes et al. 1881) and by Lawes, Gilbert and Warrington (1883) who, in view of then current estimates of S requirements, concluded that such additions were sufficient for the demands of ordinary cereal crops. These views persisted until 1905 when Dymond, Hughes and Jupe published the results of experiments conducted in Essex, England, that demonstrated the true extent of plant S

requirements. They obtained yield responses in crops to the application of S both as ammonium sulphate and as gypsum. These results stimulated interest in S in the United States of America.

Swanson and Miller (1917) examined some soils in the U.S.A. and recorded disproportionate losses of S compared with other elements, after cultivation and cropping. They examined S, N, P, K and Ca on a number of cultivated and virgin soils and suggested that the loss of S from the soil was proportional to the loss of soil organic matter. In more detailed work Swanson and Latshaw (1922) confirmed the relationship between changes in S, N and C content of soils. They noted that more S was leached from soils under cultivation than under grass.

Evidence that reserves of soil S were organic in nature was mounting, and in 1926, by means of pot experiments and incubation tests Johnston studied some of the factors affecting $\text{SO}_4^{=}$ -S release in soils. Johnson^t used sodium bicarbonate fusion to recover total S from soil, followed by turbidimetric determination as barium sulphate; this is similar to some methods of soil S determination currently used today.

At the same time as these studies were being conducted, Lipman and his associates (Lipman, McLean and Lint 1916a, Lipman, McLean and Lint 1916b, Lipman and Joffe 1920, Lipman, Waksman and Joffe 1921) were conducting experiments on biological oxidation of S in composts and soils for the purpose of producing sulphuric acid to render mineral

phosphates available for plant growth. Although their experiments did not achieve commercial success they provided an insight into the mechanisms, organisms, and optimum conditions necessary for S oxidation in soils. Isolation of Thiobac¹illus thiooxidans (Lipman, Waksman and Joffe 1921) was one significant achievement which emerged from their investigations.

Incubation experiments conducted by Shedd (1928) revealed that in certain soils immobilization of S could occur. By 1928 much evidence of the importance of S in agriculture existed, but interest in the element was spasmodic over the next 20 years. The use of superphosphate still continued to mask S deficiency. In an address to the American Society of Agronomy, Alway (1940) described S as "A nutrient element slighted in agricultural research" and may have stimulated interest in the subject.

Definite evidence that most S present in surface soils was in the organic form came when Evans and Rost (1945) fractionated the organic matter from a number of Minnesota soils and measured the S content of the fractions. They established a relationship for the C:N:S ratio of soil organic matter. Determinations of C:N:S ratios, carried out by subsequent workers for different soils, have been similar to those of Evans and Rost.

S deficiencies occur for various reasons in different localities. The decline in the use of solid fuels, including coal, for industrial purposes has reduced the SO_2 content of air and rain. Rising freight costs have forced manufacturers to eliminate unnecessary elements in fertilizer by introducing high analysis phosphates and anhydrous ammonia to replace superphosphate and sulphate of ammonia. There has been a resultant reduction in the incidental application of $\text{SO}_4^{=}\text{-S}$. The desire to use fertilizers more efficiently has led to studies of residual effects, and in Australia such studies on superphosphate have frequently revealed S deficiencies.

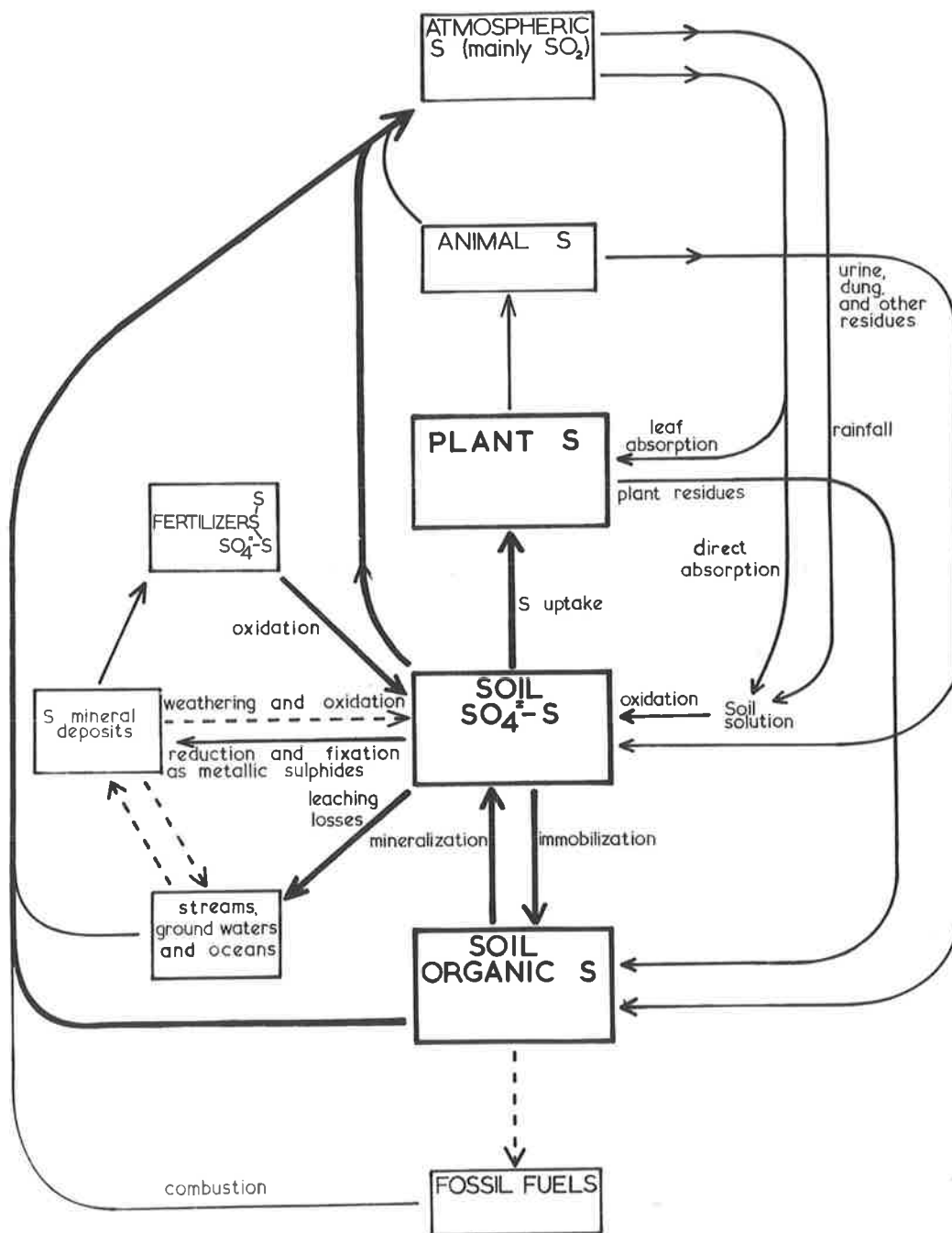
It is now accepted that a sulphur cycle analogous to the N cycle exists in nature (Walker, 1957, Jordan and Ensminger 1958). A diagram of the cycle, summarizing possible S transformations is shown in Figure 1.

Since plants mainly absorb S from the soil as $\text{SO}_4^{=}\text{-S}$, the transformations and factors affecting the size of this pool are of prime importance to agriculture. Apart from the placement of fertilizer, fixation by sesquioxides and the effect of leaching, the transformations affecting soil $\text{SO}_4^{=}\text{-S}$ availability are largely microbiological.

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Figure 1

The "Sulphur Cycle" in nature - summarizing S transformations. The pathways shown by heavy black lines were examined in this project.



2. Forms of S in Soils.

(a) Inorganic $\text{SO}_4^{=}\text{-S}$

S exists in soils in inorganic and organic forms. The main inorganic form present is usually $\text{SO}_4^{=}\text{-S}$, which represents the stable oxidation state of S under aerobic conditions. Under anaerobic conditions reduced forms of S, including metallic sulphides may accumulate. Sulphates are susceptible to leaching losses and S is retained in the surface of humid agricultural soils largely in organic matter (Evans and Rost 1945).

Inorganic S is often present in parent materials. Igneous rocks may contain sulphides and these are released and oxidised to sulphates on weathering (Whitehead 1964). Sedimentary rocks such as limestones may contain insoluble sulphates which have been co-precipitated with calcium carbonate (Williams and Steinbergs 1962). Sulphates associated with calcium carbonates in this way form a significant part of the $\text{SO}_4^{=}\text{-S}$ fraction of some soils, and are not readily available for plant nutrition. Other insoluble forms of S are strontium and barium sulphates. Williams and Steinbergs (1962) showed the presence of barium sulphate in some Australian soils, but of all the samples they examined none contained significant quantities of strontium.

On acid soils, sulphates are frequently retained in association with hydrated oxides of iron and aluminium (Ensminger 1954, Kamprath, Nelson and Fitts 1956, Berg and Thomas 1959, Chao Harward and Fang 1962b). Hydrated iron and aluminium oxides ~~from~~ ^{form} coordination complexes with electron-donating substances such as sulphate (Harward and Reisenauer 1966). The amount of $\text{SO}_4^{=}\text{-S}$ retained by this mechanism is dependent on soil pH, the nature of the soil colloids and the presence of competing anions such as phosphate (Ensminger 1954, Kamprath, Nelson and Fitts 1956, Berg and Thomas 1959, Chao, Harward and Fang 1962b). Where the soil pH exceeds 6-7 little or no $\text{SO}_4^{=}\text{-S}$ is retained by this mechanism (Williams and Steinbergs 1962, Harward and Reisenauer 1966).

$\text{SO}_4^{=}\text{-S}$ held to clay minerals by anion adsorption may be readily displaced by the presence of phosphate in quantities of agricultural significance (Chao, Harward and Fang 1962a). It is evident that organic matter is also responsible for the retention of some $\text{SO}_4^{=}\text{-S}$ by adsorption, but this effect depends on the form of the organic matter rather than the quantity (Chao, Harward and Fang 1962c). Evidence also exists that $\text{SO}_4^{=}\text{-S}$ can be covalently bound to soil organic matter as ester sulphates (Freney 1961).

The ability of phosphates to displace $\text{SO}_4^{=}\text{-S}$ from exchange sites results in accumulation of phosphate in surface soil and

removal of $\text{SO}_4^{=}\text{-S}$ to subsoils by leaching. Where acid subsoils exist retention of $\text{SO}_4^{=}\text{-S}$ may be high in the presence of iron and aluminium hydroxides. The main influence on the retentive power of subsoils is soil pH (Williams and Steinbergs 1962), and in the presence of free calcium carbonate, as in many temperate Australian subsoils, $\text{SO}_4^{=}\text{-S}$ retention is likely to be low.

(b) Reduced forms of S

Under ^aanerobic conditions microbiological reduction of $\text{SO}_4^{=}\text{-S}$ leads to the accumulation of sulphides. Conditions favouring reduction of $\text{SO}_4^{=}\text{-S}$ include waterlogging in the presence of a source of decomposable organic matter (Janitsky and Whittig 1964), and frequently occur in paddy soils (Vamos 1964). Sulphides formed may accumulate as insoluble iron or other transition metal sulphides. The soil pH rises due to transformation of $\text{SO}_4^{=}\text{-S}$ to sulphide and of organic anions to bicarbonate while the balancing cations remain practically unaltered (Abd-El-Malek and Rizk 1963).

In addition to the fully oxidized and reduced forms, small quantities of S may exist in intermediate states of oxidation as elemental S, thiosulphate, and polythionate. Such compounds are transitory intermediates in the redox sequence. In normal agricultural soils little inorganic S is present in reduced forms (Freney 1961), since these are rapidly reoxidized chemically and

biologically. Even so the loss of H_2S from soils during temporary waterlogging has hardly been studied.

(c) Organic S

Organic S is deposited in soils in plant, animal, and microbial residues and accumulates in various humus fractions. Due to the complex nature of soil organic matter, analysis of the S compounds present therein has proved difficult. Evans and Rost (1945) were the first workers to demonstrate the significance of the S retained in soil organic matter. They showed that of the total S present in 39 Minnesota soils of various types, from 16-81% was present as organic S. Most of the soils contained more than 45% of their S in organic form and the average percentage was higher for chernozems and black prairie soils than for podzols.

Indirect evidence has also been collected which demonstrates the organic nature of S compounds in surface soils. It is well known that the amount of S in a soil is closely related to the amounts of carbon and nitrogen present. Some of the earliest evidence of this was obtained by Swanson and Miller (1917) and Swanson and Latshaw (1922) who determined the S, N, C and P contents of three types of soils after differing cropping treatments. The mean ratio of C : N : S calculated from their results is 223 : 10 : 2.03; and, although they did not express their results as ratios, they noticed that

losses of S, N and C were proportional from most soils under cultivation. Following more recent work (Evans and Rost 1945, Williams and Donald 1957, Walker and Adams 1958a) the C : N : S ratio has become a widely used criterion when assessing the potential S supplies of soils. Tables showing the remarkable similarity of C : N : S ratios from soils in various parts of the world have recently been compiled (Whitehead 1964, Freney and Stevenson 1966), and emphasize the organic nature of the S retained in surface soils.

Determinations of organic S in soils are usually made using indirect methods. Bardsley and Lancaster (1960) proposed using the term "reserve sulphur" to describe the fraction of soil S determined by the difference between SO_4^{2-} -S and total S analyses. Such S may be regarded as being almost wholly organic in nature, since in most soils the levels of reduced inorganic S compounds, (which would be included in the "reserve sulphur" fraction) are low (Freney 1960). The highly significant correlation ($R = 0.971$) between reserve S and soil N obtained by Bardsley and Lancaster confirms the organic nature of this fraction.

Some recent studies on the nature of organic S compounds in soil have been made by Freney (1960), Lowe and de Long (1961), (1963), Lowe, (1964) and Freney, Melville and Williams (1969). It is

now known that a large proportion of organic S is readily reducible and may exist as organic sulphates, while other organic S may be directly bonded to C. Compounds in which C is directly bonded to S include the amino acids methionine and cysteine. Whitehead (1964) estimated that, based on the results of Stevenson (1956), 11-19% of the total S present in four soils examined was in amino acid form. Freney and Stevenson (1966) obtained similar estimates from other data which supported this conclusion. Since estimates of organic S bonded to C are as high as 58% (Lowe and de Long 1963, Lowe (1964) much of this must occur in organic compounds other than amino acids.

Only a limited number of organic S compounds have been isolated from soils and identified. A frequently quoted one is trithio-benzaldehyde (Shorey 1913) that accounted for approximately one third of the total S present in the California soil from which it was recovered. This may not have been important since a significant quantity of this compound was found in only one other soil of many examined by Shorey. More recently the S-amino acids cysteine (Putnam and Schmidt 1959) and methionine (Gilbert and Altman 1966) have been isolated from soils. Even so the levels of free amino acids in soils are likely to be low since they provide a readily available substrate component for microorganisms. Root exudates may be one source of amino acids in the rhizosphere zone (Paul and Schmidt 1961), although some may be released during the

breakdown of organic matter. It would appear that there is scope for studies using ^{35}S labelled material, coupled with chromatographic techniques to clarify these points. Acid hydrolysis of soil is known to liberate S-amino acids (Sowden 1955, Stevenson 1956), and since thioester bonds play an important role in nature it is likely that they too could be hydrolysed.

Speculation about the formation of other organic S compounds in soils (Whitehead 1964, Freney and Stevenson 1966) has been based on biochemical reactions known to occur elsewhere. Suggested compounds include dihydrothiazine and thio-derivatives of aldehydes including reducing sugars. It has been suggested that such products forming part of the S fraction of soil humus may be quite stable and resistant to microbial attack. In spite of their postulated stability no such compounds have been isolated and treatments designed to fractionate soil organic S are so severe that drastic chemical changes occur during extraction (Freney, Melville and Williams 1969). It seems that speculation must continue until a satisfactory method of fractionation is found.

Although the level of organic S may be high in many soils this fraction is not at all readily available for plant nutrition. Estimates of total S, which include organic S, are not well correlated with plant growth (Williams and Steinbergs 1959). It is

evident that the value of organic S for plant nutrition depends on factors that effect the decomposition of the soil organic matter and the resultant mineralization of S compounds.

3. S Balance in Soils

The S content of surface soils changes slowly depending on cultural practices. Under sound agronomic practices organic matter slowly accumulates and is accompanied by a corresponding rise in total N, P and S, (Donald and Williams 1954, Williams and Donald 1957). Under exploitive systems the reverse occurs. Superimposed on the gradual changes are short term changes due to fertilizer additions, leaching losses, and utilization of mineral nutrients. The balance of S present in a soil is the resultant of the above factors, and depends on the various inputs and outputs in a given situation.

(a) S Addition

S is added to soils from the atmosphere in rainfall, in fungicides, insecticides, fertilizers, animal excreta and plant residues. Returns of animal excreta and plant residues are usually part of a recycling process rather than direct inputs.

(i) Rainfall - Accession in rainfall has long been recognized as a source of S for plant nutrition, and early evidence (Lawes, Gilbert and Warington 1881) showed the variations which could occur depending on proximity to towns. Increments recorded at Rothamsted

varied with the season where up to 9 kg/ha of S was added in rainfall in a six monthly period (Lawes, Gilbert and Warington 1883). Erdman (1922) obtained evidence of wide variations in the amount of S available from rainfall depending on the locality.

In the United States amounts of S recorded in precipitation range from greater than 100 kg per ha per year in industrial areas (Bertramson, Fried and Tisdale 1950) to mean values as low as 5 kg per ha per year in south eastern rural areas (Jordan and Bardsley 1958). Most measurements made in agricultural areas of Australia have revealed that only small quantities of S are added in rain, ranging from less than 1 kg per ha per year in inland Western Australia (Rossiter 1952) to 8 kg per ha per year in coastal Victoria (Hutton and Leslie 1958). Whitehead (1964) has tabulated results from rural and industrial areas in widely separated parts of the world, and it is apparent that in many localities accession of sulphur in rainfall can provide significant quantities for plant growth.

(ii) Atmospheric S - In addition to direct accession in rainfall S may be absorbed by plants and soil directly from SO_2 in the atmosphere (Thomas and Hill 1935, Alway, Marsh and Methley 1937). Even when adequately supplied with SO_4^{2-} -S in solution plants can absorb SO_2 through the leaves from the atmosphere (Olsen 1957). This was demonstrated by growing plants in a sealed nutrient solution

containing ^{35}S labelled sulphate and measuring the dilution of labelling in the plant material due to intake of SO_2 by aerial parts of the plants. The amount absorbed depended on the size of the plant suggesting that intake was a function of leaf area. Where plants were grown on S-deficient solutions up to 50% of the S could be supplied in this way. Absorption of SO_2 by soils was demonstrated by Alway, Marsh and Methley (1937). Comparisons with lead peroxide absorbers showed that soils were less effective and absorption depended on soil type. Direct absorption of SO_2 from the atmosphere by soil may not be very important when compared with the amount of S added in rain, and it has been claimed (McKell and Williams 1960) that less than 0.1 kg per ha of S per annum is contributed by this method.

The three main sources of atmospheric S are H_2S from the decomposition of organic matter and reduction of S in marshy areas and coastal regions (Miller 1961), cyclic sulphates from sea spray, and products from the combustion of fossil fuels in industrial plants. The significance of S provided for crop growth in rainfall or as atmospheric SO_2 depends on proximity to industrial areas and to the sea coupled with the direction of prevailing winds (Coleman 1966). With a decrease in the use of solid fuels and the introduction of air pollution controls there is likely to be a fall in the atmospheric S content in industrial areas.

Since the major industries in Australia occupy comparatively few localities they contribute little to atmospheric S supplies likely to be of agricultural importance, and the main factor influencing S content of the atmosphere is nearness to the sea.

(iii) S pesticide residues - The use of insecticides such as nicotine sulphate may add appreciable quantities of S to plants and soil. For many years fungicides including "lime-sulphur" and "Bordeaux mixture" containing high S contents have been widely used on horticultural crops. Recently the use of these traditional inorganic pesticides and consequent incidental application of S has declined as newer organic formulations have been introduced. Some of the newer pesticides also contain S (e.g. dithiocarbamates) but due to their higher toxicities are used in much smaller amounts. Hartley and West (1969) state that dithiocarbamates can undergo oxidation to thiuram disulphides which on exposure to air yield mono-sulphides and elemental S. In soils, however, oxidation to sulphate would seem more logical and these compounds could supply limited amounts of S to plants. Many of these compounds however, act against soil fungi and may adversely affect organisms involved in soil S transformations. Metham sodium (sodium monomethyldithiocarbamate) one such compound is used at rates of from 50 to 200 kg per ha for soil sterilization prior to planting high value crops and would therefore be capable of supplying from 25 to 100 kg per ha of S. Much of this could be lost for these substances depend for their action on the production of volatile S compounds.

Many new pesticides contain no S at all and their use could result in a fall of available S. The change to organic pesticides is likely to have more serious effects because of their persistence and toxicity, rather than because of smaller additions of S.

(iv) Fertilizer S - Fertilizer additions are used to deliberately control or alter the S balance of soils for agricultural production. Additions are governed by the economics of the particular situation, and are aimed at providing sufficient available S in soils for maximum profit from crop or pasture production. The main forms of S fertilizers are superphosphate, ammonium sulphate, calcium sulphate, and elemental sulphur. High analysis fertilizers such as triple superphosphate, urea, and anhydrous ammonia contain no S, and their use has necessitated the introduction of specific S containing fertilizers (Coleman 1966). The amount of S fertilizer required to maintain a desired balance in soils depends on the amount received from the atmosphere and the amount removed by leaching and crop removals. Details of fertilizer usage are discussed in section II, 7.

(v) Plant and animal residues - Additions of S to soils will occur with the return of plant residues and animal excreta. Fresh plant residues contain a proportion of S that is easily released in available form provided the S content is high and the C/S ratio is low enough (Stewart, Porter and Viets 1966).

Barrow and Lambourne (1962) estimated by means of balance studies that half of the S intake of animals is excreted in faeces and slightly less than half in urine, the latter fraction being more readily available for plant growth. Bray (1969) using ^{35}S labelled sulphide and sulphate demonstrated that the form in which S was absorbed by the animal from the rumen and duodenum influenced partitioning between faeces and urine. When sulphide was injected directly into the duodenum there was a rapid rise in excretion of urinary S whereas injection of $\text{SO}_4^{2-}\text{-S}$ led to a rise in faecal output of ^{35}S . Since most S compounds are reduced in the rumen prior to absorption these effects may not be as noticeable in partitioning S between faecal and urinary outputs from animals under normal grazing conditions. Animals may excrete S as volatile etherial sulphates that are lost from the grazing system.

A positive approach to the study of nutrient recycling in grazed pastures using ^{35}S -labelled gypsum has recently been made (May, Till and Downes 1968). Results showed that recycling of applied S through sheep continued over a long period (600 days) and that some redistribution of S occurred with higher concentrations being excreted on camp sites. A surprising finding was that although the experiment was sited on a podsollic soil there were no significant leaching losses and the application of S had a long residual effect with most of the applied S remaining in the top 10 cm of the soil over the first 200 days. There may have been three reasons for this that

these workers omitted to point out. Firstly, the rainfall of 26 cm measured over the first 215 days of the experiment would seem to have been very much below the average of "80 cm uniformly distributed throughout the year" and leaching intensity would therefore have been lower than normal. Secondly, the stocking rate of "about 20 adult sheep per hectare" appears to have been abnormally high (under high rainfall this could have the reverse effect). Thirdly the root systems of the perennial species (e.g. Phalaris tuberosa, and Lolium perenne, in the pasture might have been more effective than annuals in absorbing $\text{SO}_4^{2-}\text{-S}$, thereby further limiting leaching. Facts one and two are supported by the explanation that sheep had to be removed to avoid supplementary feeding due to adverse seasonal conditions.

Under such a high stocking rate it is not surprising that much S continued to be recycled since the greater proportion of plant S passing through a ruminant is likely to become reavailable more quickly after excretion than by the process of plant decay in the soil. The effectiveness of recycling through animals in this experiment demonstrates the value of the process in maintaining S availability.

(b) S Losses from Soil

Losses of S from an agricultural system also affect the net balance in soils. The three main reasons for loss are leaching, removal in plant and animal products and volatilization.

(i) Leaching - Swanson and Miller (1917) were among the first to recognize that S could be lost from surface soils by leaching when they examined the effect of cultivation and cropping on a number of Kansas soils. Erdman (1923), in a pot experiment, and Joffe (1933) in a lysimeter experiment, recorded $\text{SO}_4^{=}\text{-S}$ losses in leachates. In recent years more extensive studies on leaching losses have been made.

Losses of $\text{SO}_4^{=}\text{-S}$ applied as gypsum can be high from the surfaces of coarsely textured soils under moderate rainfall conditions. McKell and Williams (1961) recorded losses of nine tenths of applied gypsum from lysimeters containing sandy soil after 480 mm of rain. The gypsum had been applied at rates from 112 to 336 kg per ha, and the percentage leached was similar for all rates of application. Even in drier years significant losses of $\text{SO}_4^{=}\text{-S}$ applied as gypsum occurred (Williams, McKell and Reppert 1964), but were more dependent on rainfall distribution and consequent intensity of leaching.

Laboratory experiments in New Zealand revealed that substantial leaching of S could occur from sandy and pumice soils where $\text{SO}_4^{=}\text{-S}$ retention was low (Hogg and Cooper 1964, Hogg and Toxopeus 1966, Cooper and Hogg 1966), but losses were lower from the loamy soils of higher clay mineral content (Hogg 1965).

Field experiments in south western Western Australia (Barrow 1966) demonstrated that severe S deficiencies could occur in pastures due to leaching of $\text{SO}_4^{=}\text{-S}$. Heavy opening rains were capable of removing available S from sandy soils before significant plant uptake could occur.

It is clear that S losses by leaching are affected by a number of factors. The retention of $\text{SO}_4^{=}\text{-S}$ in soils of low pH containing hydrated oxides of iron and aluminium has already been discussed. Among other factors which affect leaching losses are rainfall intensity and seasonal distribution, soil texture, and the forms of S used for fertilizer application. Phosphate tends to displace $\text{SO}_4^{=}\text{-S}$ from exchange sites and its application enhances leaching losses from potentially retentive soils.

(ii) Volatilization - S losses may occur from soils by volatilization. Such losses are thought to be significant only under anaerobic conditions where liberation of H_2S takes place. This effect has been studied in rice paddies (Vamos 1958, 1964, Ezhov 1962). Production of H_2S has also been recorded under aerobic conditions (Bromfield 1953) but this occurred in the laboratory in a soil which had been partially sterilized. Frederick, Starkey and Segal (1957) found that when methionine decomposed in soil under aerobic conditions methyl mercaptan and dimethyl disulphide which are both volatile were released. Losses from

this source are likely to be low since the amount of free methionine in soils is small. Volatile loss of organic S as mercaptan under water-logged conditions is possible but has hardly been studied, and little is known about the size of losses of volatile S from animal faeces and mown pasture during drying.

(iii) Plant uptake -- Plant uptake normally removes significant quantities of S from soils, but as already discussed, much of this is returned as organic S in plant residues or animal excreta. Hay cutting, where complete removal of top growth occurs, causes the biggest loss. The amount of S taken up by a crop will depend on the soil $\text{SO}_4^{=}\text{-S}$ level, the type of crop, the growth rate and total yield. Measurements of plant S uptake have been made in balance studies, and in attempts to establish diagnostic tests for S deficiency. These will be discussed in more detail in section II-6.

The S contents of plant tissues vary depending on available supply and competition between species. On mixed clover grass pastures where nitrogen supply is adequate grasses remove more S from the soil than clover (Walker, Adams and Orchiston 1956; Walker and Adams 1958b). When the S requirement for protein synthesis has been exceeded $\text{SO}_4^{=}\text{-S}$ accumulates in plant tissue (Dijkshoorn, Lampe and Van Burg 1960), and is indicative of adequate supply provided N is non-limiting. S uptake by plants

may frequently exceed requirements, and where maximum yields have been obtained the actual amount of S removed from the soil may be variable depending on the extent of "luxury uptake". Figures for S uptake based on S requirement are therefore only a guide in estimating the effect of plants on soil S balance.

S removed in grain by small grain cereals yielding 260 kg per ha may be between 10 and 13 kg per ha (Beaton 1966), whereas the quantity removed in clover pasture cut for hay yielding from 5,000 to 7,000 kg per ha is likely to be higher at 17 to 20 kg per ha. Removal of S by lucerne may be higher still at 22 to 27 kg per ha for a stand yielding from 6,000 to 8,000 kg per ha (Whitehead 1964). From the values quoted it can be seen that the species of plant grown and the parts of the plant harvested, influence the quantity of S removed.

4. Factors Affecting Soil S Transformations

(a) The Role of Microorganisms, Plants and Animals

The various S fractions in soil exist in a state of dynamic equilibrium. Transformations between fractions influence the amount of available S maintained in a soil from less available reserves. The rates and directions of transformations are important in assessing the availability of S on a particular soil.

Ample evidence has been amassed to show that micro-biological activity is largely responsible for S transformations within soils (Starkey 1950). The earliest studies on microbial transformations in soil were concerned with oxidation of S by sulphur-bacteria and much work carried out at the New Jersey Agricultural Experiment Stations was summarised by Joffe (1922). S is important in the metabolism of all organisms and other microorganisms besides the sulphur-bacteria are also involved in soil S transformations.

The main pathway for the incorporation of S into soil organic matter is through plants (Donald and Williams 1954). Animals, as previously discussed, also play a part in this pathway (Barrow 1961b, Barrow and Lambourne 1962), but tend to obtain much of their S in organic forms prior to digestion. Ruminants are capable of using inorganic S and converting it into organic forms (Bray 1969b).

Evidence has been obtained (Freney and Spencer 1960) which suggests that the presence of plants stimulates S mineralization. The effect was more pronounced where plants had received added S. It is possible that plants may excrete enzymes capable of catalyzing the decomposition of organic matter or that root exudates provide a better environment for rhizosphere organisms (Rovira 1962). Plant roots may even excrete S compounds which quickly oxidize to sulphate.

(b) Influence of C / N / S Ratios

It has already been pointed out that there is a close relationship between the C, N and S content of soils, numerous examples of which have been tabulated by Whitehead (1964). Deviations from the normally accepted range of stable C / N / S ratios may frequently influence the availability of all three of the elements. Extensive investigations have been made into the influence of C / N / S ratios on sulphur mineralization.

Mineralization of S from mixtures of decomposing organic substances in sand cultures (Barrow 1960a) depended mainly on the S concentration of the mixture, variations of which had a large effect on the amount of N mineralized. Low S levels did not prevent rapid mineralization of N even at wide C / N ratios, and this tended to lower the N / S ratio of the residual organic matter. A similar experiment (Barrow 1960b) using a wide range of plant materials

showed that the S mineralized depended to a limited extent on the C/S ratio of the organic matter. Where the C/S ratio was less than 200, mineralization of S always occurred. When the C / S ratio was greater than about 400, immobilization of S occurred. Between the two limits results were variable. The results from both experiments were in agreement, and after 12 weeks of incubation at 27°C the C/S ratios of the residual material were between 50 and 60 often with a large proportion of the S still not mineralized. Sheep faeces proved to be more resistant to decomposition, than plant material (Barrow 1961b), but once again the amount of S mineralized, although proportionately less than in plant material, was related to the S content of the faeces.

The influence of C/S ratio on mineralization may vary with differing carbon substrates. Stotsky and Norman (1961) found that S was a limiting factor in the oxidation of glucose in soils when the C/S ratio was greater than 900. They also demonstrated that a number of different S compounds with the exception of two, thiourea and elemental S, could satisfy the S requirement. On this basis they predicted that since most plant residues have C/S ratios of less than 900, S should not limit the rate of microbial decomposition of the residues and hence should be mineralized rapidly. Results such as these obtained using glucose may not be applicable to plant residues where cellulose is the principle C source. Cellulose

decomposes more slowly than glucose and a much longer period of decomposition may be necessary before S is mineralized.

Critical C/S ratios for net S mineralization that have been determined by most other workers are at the most only ~~of~~ half ^{of} this value. The reason for the discrepancy is not obvious but in the presence of the readily available energy source initial rapid recycling of microbial protein may have occurred until the C/S ratio was lowered to about 400 after which rapid release of S may have occurred. Alternatively if the N/S ratio had been very low, then N would have limited the rate of breakdown with a resultant release of CO₂ and surplus S above the limited microbial requirements.

In contrast decomposition of straw with a C/S ratio of 350 lowered dry matter yields of winter wheat (Stewart and Whitfield 1965) when straw was incorporated into a soil in a glasshouse experiment. This result was supported by later experiments (Stewart, Porter and Viets 1966) that showed the critical ratio of C/S for S mineralization lay between 370 and 270 when wheat straw was the substrate for microbial decomposition. Although these results generally agree with Barrow's original measurements, it is possible that Stewart et al. failed to consider the toxic effects of many straws on plant roots, (Kimber 1967) - toxin damaged plants might extract less SO₄⁼-S.

The N/S ratio of organic matter also affects the mineralization of S. Nelson (1964) found that the ratio of N to S mineralized from soil organic matter was approximately constant. By contrast, Barrow (1961a) recorded simultaneous immobilization of S and mineralization of N where the average N/S ratio of decomposing plant material was 14. He had previously noted (1960a) that as decomposition of organic matter proceeded there was a narrowing of the N/S ratio. White (1959) and Williams (1967) also found that more N was mineralized relative to S than would have been expected from the N/S ratio of the soil organic matter.

Such differences may occur because N and S are present in different compounds within the soil organic matter. The incorporation into soils of organic matter with wide N/S ratios may result in immobilization of S similar to that observed when organic matter with wide C/S ratios is added.

The normal N/S ratio of 8 in soil organic matter is lower than the ratio of approximately 15 found in plant protein. Because of this Stewart (1966) suggested that a crop obtaining its N supply solely by mineralization from soil organic matter should obtain an adequate S supply. Although the values for N and S contents of plant material obtained in an experiment where wheat was grown on an unfertilized soil (Stewart and Whitfield 1965) supported the above idea it need not always apply. N/S ratios as low as 8 are usually found in stable

organic matter which is generally resistant to decomposition because it is largely humified. The N and S may occur in different fractions; as demonstrated by Freney et al. (1969) who showed that in a soil with a total N/total S ratio of 9.4 the N/S ratios extracted by different chemical methods ranged from 7.8 to 4.5. The other soils tested also showed wide variations. The distribution of N and S between different fractions of humified organic matter may contribute to its stability by preventing simultaneous supply of the two elements to microorganisms.

It is clear that C/N/S ratio influences the relative release of these elements by mineralization from soil organic matter. The reverse is also true, since a relative shortage of either N or S is likely to slow the accumulation of soil organic matter. This may occur due to reduced dry matter yields coupled with instability of the resultant organic matter produced. Williams and Donald (1957) suggested that insufficient S applied in superphosphate dressings limited organic matter accumulation under subterranean clover (Trifolium subterraneum L.) pastures in the Crookwell district of New South Wales. Previously they had shown that S was still limiting yields on improved soils of the area that had been regularly topdressed with superphosphate for up to 25 years (Donald and Williams 1954).

(c) Heating and Drying of Soils

$\text{SO}_4^{=}\text{-S}$ can be released from soil organic matter by drying the soil (Freney 1958, Barrow 1961a, Williams and Steinbergs 1964), but

measurement of the amount of S released varies with the extractant used. Williams (1967) extracted large amounts of $\text{SO}_4^{2-}\text{-S}$ from soils regardless of the method of drying by using potassium dihydrogen phosphate (KH_2PO_4) extractant rather than calcium chloride (CaCl_2) solution. In all cases he obtained a significant release of $\text{SO}_4^{2-}\text{-S}$ on air drying soils, while oven drying at 100°C released even more $\text{SO}_4^{2-}\text{-S}$. Moistening and redrying of soils also gave small additional increases in the extractable $\text{SO}_4^{2-}\text{-S}$. It was apparent that the manner and frequency of drying affected the amount of S mineralized.

Mechanisms of S release that are affected by wetting and drying are not well understood, but in the series of experiments conducted by Williams it was observed that a drying and wetting cycle prior to incubation enhanced mineralization of S from soils. Drying may result in a breakdown of labile compounds and provide a better substrate for subsequent microbial activity. Partial dessication of the biomass may cause breakdown and release of both labile S compounds and enzymes such as sulphatase that hydrolyse ester sulphates upon rewetting. According to Jenkinson (1966) drying a soil kills most of the biomass which on remoistening mineralizes rapidly releasing C, N, S and P.

(d) Soil Water Content

Williams found that extremes of soil water content restricted S mineralization. At low soil water contents ($<15\%$) the dry conditions

would have reduced microbial activity, whereas high soil water contents (40%) would have reduced air space thereby limiting aeration and creating conditions favouring microbial reduction. The increase in output of ammonium N which accompanied the decline in net S mineralization at high soil water content demonstrated the latter point. Maximum mineralization of S from three podsollic soils examined by Williams occurred at a soil water content of 25% corresponding to a suction of 100 cm. Under such conditions it is quite possible that both aerobic and anaerobic spots occurred in soil so that the net mineralization recorded resulted from a combination of many different transformations. The presence of enzymes such as sulphatase could also have a marked effect on the hydrolysis of ester sulphates under these conditions.

(e) The Influence of Liming and Soil pH

The addition of liming materials to soils influences rates of S mineralization. MacIntyre (1926) in a lysimeter experiment recorded increased leaching of NO_3^- -N and $\text{SO}_4^{=}$ -S from a brown loam of pH 6.4 following the addition of Ca and Mg liming materials. The losses were recorded in the first year of application with no residual effect thereafter and were attributed to increased biological activity following liming. More definite evidence of the effects of liming on mineralization were obtained from soils in lysimeters where organic matter treatments were applied (Ellet and Hill 1929). In

the presence of organic matter, liming increased the amount of S recovered in leachates. White (1959) measured increased $\text{SO}_4^{=}\text{-S}$ levels in acid soils to which $\text{Ca}(\text{OH})_2$ had been added in incubation studies.

The addition of CaCO_3 to soils which contained S mainly in organic form brought about increased S uptake by plants (Williams and Steinbergs (1964)). The soils used were S deficient, and it was concluded that raising the pH increased the availability of S from soil organic matter. If pH was the factor affecting S mineralization, other alkaline materials such as NaOH should show similar effects; this was demonstrated by Barrow (1960b).

Release of $\text{SO}_4^{=}\text{-S}$ at raised pH may be due to a number of factors. Firstly, increased microbial activity at more favourable pH may occur. Secondly, where iron and aluminium oxides are present raising the pH to 7.0 or higher will result in release of $\text{SO}_4^{=}\text{-S}$ bound to these compounds. Thirdly enhanced activity of sulphatase enzymes may increase hydrolysis of ester sulphates, and fourthly, alkaline hydrolysis of ester sulphates may occur if the soil pH is raised sufficiently. Displacement of $\text{SO}_4^{=}\text{-S}$ from the anion exchange complex by OH^- ions or $\text{HPO}_4^{=}$ ions at high pH may also be a reason for increased availability of S following liming (Kamprath, Nelson and Fitts 1958, Chao, Harward and Fang (1962)).

Another possibility advanced by Williams and Steinberg (1964) to explain increased $\text{SO}_4^{=}\text{-S}$ availability following liming, was that sulphates coprecipitated with the CaCO_3 became available after the material had been added to soil. Variable quantities of this coprecipitated $\text{SO}_4^{=}\text{-S}$ depending on the sources of CaCO_3 did prove to be available, but increased S availability was also observed where A.R. grade CaCO_3 was applied to some soils. It was concluded that release of co-precipitated S depended on the reaction of the CaCO_3 with exchangeable hydrogen and that sulphate in unreacted CaCO_3 remained unavailable. Additions of A.R. CaCO_3 caused increased S availability only when applied to acidic soils.

It is unlikely that CaCO_3 , irrespective of the content of co-precipitated $\text{SO}_4^{=}\text{-S}$, will cause increased S availability when added to alkaline soils.

5. Chemistry of Soil S Transformations

Although the influences of many environmental factors on S-mineralization have been studied the chemistry of the transformations involved is not well understood. Oxidation and reduction of inorganic S compounds and biochemical transformations are known to occur. Starkey (1966), Trudinger (1967) and Burns (1967), have reviewed the oxidation and reduction of S in soils and Freney and Stevenson (1966) and Freney (1967) have reviewed the biochemistry of organic soil S compounds. Environmental factors affecting oxidation and reduction have already been reviewed; in this section mention will be made of the organisms responsible for these transformations.

(a) Oxidation of Soil S

Most changes in the oxidation states of inorganic S compounds are caused by microbial action involving sulphur bacteria. S has long been applied to soils to acidify them with the intention of rendering other nutrients more readily available to plants (Lipman, McLean and Lint 1916). Studies of this process led to the isolation of the bacterium Thiobacillus thiooxidans (Waksman and Joffe 1922), which is a S oxidizer tolerant of low pH levels.

Other organisms responsible for S oxidation in soils include Thiobacillus thioparus, T. denitrificans, T. ferrooxidans, T. novellus,

(Starkey 1966). Most of the S bacteria are autotrophic organisms although T. novellus is a facultative bacteria which can also utilize organic media. The survival of autotrophic S bacteria in soils depends on the availability of reduced S substrates, and under normal conditions the numbers of these organisms present in soil are likely to be low (Swaby and Vitoline 1968, Vitoline and Swaby 1969).

Heterotrophic bacteria have also been considered to play a major role in the oxidation of S compounds in soils (Vishniac and Santer 1957). Starkey (1935) examined 29 soils and found evidence of autotrophic organisms in only 2. In spite of this all soils contained organisms capable of oxidising thiosulphate. Perfusion experiments (Gleen and Quastel 1953) revealed that tetrathionate was a product of thiosulphate oxidation in soils. It was shown that the presence of excess simple organic carbon substrates such as sucrose or glucose inhibited oxidation and stimulated reduction. Similar results were obtained by Trudinger (1967) with one of two heterotrophic bacterial cultures which he studied. In this case reduction of tetrathionate occurred in the presence of lactate under anaerobic conditions. Starkey (1965) stated that S is oxidised to thiosulphate and tetrathionates by heterotrophic micro-organisms and that in soils these substances are oxidised to sulphate. Recently Vitoline and Swaby (1969) have shown that heterotrophic micro-organisms are present in many Australian soils, but are generally poor oxidisers of S and thiosulphates.

(b) Reduction of Soil S Compounds

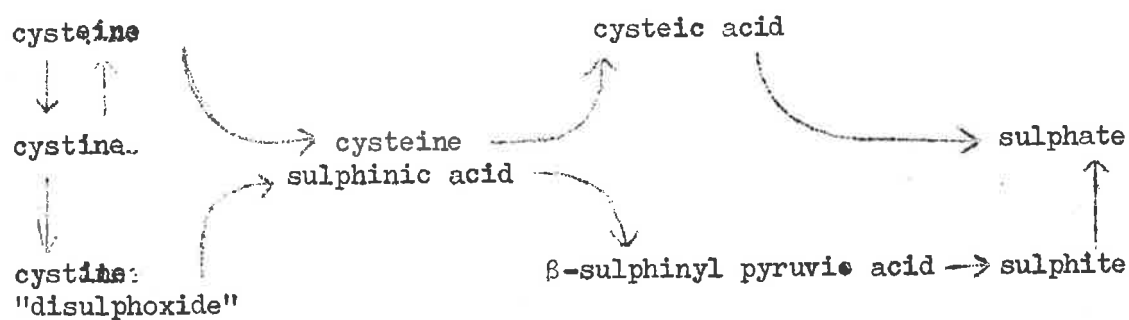
As previously explained in section II 2(b), under anaerobic conditions, reduction of oxidized or partially oxidized forms of S occurs in soil. Organisms responsible for these reactions include members of two dissimilatory bacterial genera, non-sporing Desulfo^vribrio[^] and sporing Desulfotomaculum. The optimum soil pH for their development is between 5.5 and 9.0. These bacteria are capable of reducing sulphate to sulphide and in doing so use a variety of simple organic substances such as hydrogen donors (Starkey 1966). Where metallic elements such as Fe are present they may be converted to insoluble metallic sulphides (Janitsky and Whittig 1964).

Most soil organisms can utilize $\text{SO}_4^{=}\text{-S}$ by assimilatory processes during which sulphides are formed but not released. $\text{SO}_4^{=}\text{-S}$ can be absorbed by micro-organisms and converted into cysteine (Young and Maw 1958). Possible pathways for this process have been summarized by Freney and Stevenson (1966). Cysteine readily undergoes oxidation to the disulphide cystine but in the presence of suitable reducing agents this reaction can be reversed. Production of methionine from cysteine is known to occur in Neurospora crassa and Escherichia coli (Freney and Stevenson 1966, Young and Maw 1958) and is likely in other micro-organisms.

(c) Biochemical Soil S Transformations

Bacterial oxidation and reduction and assimilatory reduction of S mainly involve inorganic S compounds as substrates. Before such conversions can influence the organic S compounds in soils they must be converted to simpler forms, and, although the conversions are accepted as being caused by micro-organisms, they are more complex than those brought about by the sulphur oxidizers and reducers. The biochemistry of the transformations involved is still largely speculative.

The decomposition of the amino acid cysteine in soils under aerobic conditions has been studied using perfusion techniques (Frederick, Starkey and Segal 1957, Freney 1958 and 1960). The end product obtained in all cases was sulphate. A possible pathway for this transformation based on the isolation of intermediates from perfusion experiments (Freney 1967) is:



On the other hand, the end products of the aerobic decomposition of methionine in soils appear to be methyl mercaptan and dimethyl disulphide (Frederick, Starkey and Segal 1957). There is, however, some doubt about the pathways and end products of methionine decomposition. Barjae (1952) found that under aerobic conditions the products of methionine decomposition in soils included sulphate and sulphite as well as mercaptans. Hesse (1957) found that methionine decomposition in forest soils of East Africa produced sulphate only.

It appears that various organisms are responsible for the decomposition of some compounds and the breakdown of a particular compound may therefore lead to the formation of different products. Under certain conditions methionine may be demethylated and converted into cysteine which is broken down to release sulphate.

Although there is some information available on the breakdown of S-containing amino acids in soils, the chemistry involved in the decomposition of the more complex organic S compounds is still obscure. The number of complex organic S compounds identified from soils is small. Before the chemical processes involved in S mineralization can be studied more information is needed about the chemical nature of the S-containing organic matter that provides the substrate for microbial breakdown. Biochemical pathways are frequently common to a number of organisms and with more basic knowledge of primary substrates inferences from pure culture studies can be made more reliably.

6. Plant S Requirements

The recent discoveries of many S deficient areas in the world (for list see review ^{by} Martin and Walker 1966) have highlighted the need to examine plant S requirements. Visible symptoms occur only when the deficiency is acute, and growth rate is depressed in most crops before this stage is reached.

The problem of immediate agricultural importance in a S-deficient area is to assess the requirement for profitable crop or pasture production. The extent of such requirements may not be governed solely by the achievement of maximum yield of dry matter, since the S content of the crop or pasture may influence its value for subsequent use. This is important where fodder crops are concerned, as S-availability is likely to affect the amino acid content, and hence the nutritional value of the material produced.

(a) Field Responses and Plant S Content Measurements

The simplest method of assessing S-requirement is the field trial. Plant tops and leaves have been analysed by various workers as a guide to S requirements; tests such as these need to be calibrated for each species, and time and method of sampling must be standardized. When correlated with yield response data, plant analysis is likely to give the most reliable estimate of S requirement. Jones (1962)

found that subterranean clover (T. subterraneum, L.) tops harvested at the early flowering stage with a $\text{SO}_4^{=}\text{-S}$ content of less than 0.17% were deficient. Later he showed (Jones 1963) that the S content in tops of pasture plants was affected by competition between species, and as growth proceeded there was a fall in the S content of the plant tops. Results of New Zealand trials (Walker and Adams 1958b) showed that grasses absorbed a higher proportion of available S than clovers in the same sward. Recently Whitehead and Jones (1969) recorded a decline in the S content of the tops of three pasture species as growth proceeded, but in the same experiment lucerne (Medicago sativa, L.) behaved differently and S content in the tops showed an irregular trend, reaching a peak late in the growth phase. This finding was inconsistent with the results of Pumphrey and Moore (1965a) who observed a decline in the S content of lucerne as growth proceeded. The four species examined by Whitehead and Jones were grown in separate swards on the same type of soil in the field and differed markedly in S uptake. From the results discussed above it is clear that the species, competition between species, and time of sampling as well as soil $\text{SO}_4^{=}\text{-S}$ content influence plant S content.

Jones and Martin (1964) claimed that the best time for sampling clover tops for diagnostic tests was at flowering and before wilting. Tests conducted so late in the growing period of annuals are of no use in deciding whether or not to add fertilizer during the current

growing season, but they do have a place in research to examine the efficiency of fertilizer practices.

Most results obtained from diagnostic tests suggest a critical value of between 0.2% and 0.25% total S in tops (expressed on a dry matter basis) at flowering is applicable to both lucerne and clovers. Critical values of S content for individual plant parts of various pasture species sampled under specified conditions have been summarized by Martin and Walker (1966).

(b) Use of N/S Ratios.

The use of N/S ratios has for diagnostic tests been investigated (Walker, Adams and Orchiston 1955, Dijkshoorn, Lampe and Van Burg 1960, Pumphrey and Moore 1965 . Using data of Ergle and Eaton (1951) Dijkshoorn et al. showed that plant S requirements depended mainly on the utilization of N in protein formation; since the results indicated an accumulation of NO_3^- -N where plants were S deficient it would appear that the converse also applies.

Dijkshoorn et al. found that protein S and N within perennial ryegrass (Lolium perenne, L.) were closely correlated with a ratio of S/N of 0.027 and since all SO_4^{2-} -S present in plants on deficient soil was converted into organic S, where N supply was adequate a S/N ratio lower than 0.027 (N/S = 37) was indicative of S deficiency.

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The N determinations were carried out by the Kjeldahl method and deemed sufficiently accurate for diagnostic purposes since NO_3^- -N was usually relatively low compared with total N. The method tested depended on all S being converted into organic (protein) form in deficient plants, and in view of this useful results might be obtained merely by testing for the presence of inorganic SO_4^{2-} -S which would indicate S sufficiency.

Pumphrey and Moore (1965a and b) investigated methods of diagnosing S-deficiency in lucerne (Medicago sativa, L.) using both total S content and N/S ratios as criteria. They found that S deficient lucerne contained less than 0.22% S at the early bloom stage and had a N/S ratio greater than 11. By comparing this value of N/S with the corresponding value obtained by Dijkshoorn et al. it can be seen that different critical values apply to different species. The main advantage of using N/S or S/N ratios is to overcome the changes in percentage S content of plant material that occur as growth proceeds. Pumphrey and Moore found that both N and S contents declined as plants neared maturity, and for a given level of S supply the N/S ratio remained relatively constant during the growing period.

Bouma, Spencer and Dowling (1969) used total plant S and reducible plant S in an attempt to provide criteria for S deficiency. Reducible

S would consist mainly of inorganic $\text{SO}_4^{=}\text{-S}$ accumulated in plant tissue in excess of plant protein requirement, together with limited quantities of ester sulphates. Curvilinear regressions of yield responses on total and reducible S content produced correlation coefficients as high as 0.947 with none lower than 0.606. These workers also reported average linear correlations from -0.760 to -0.800 between N/S ratios and yield responses. Variations in regression lines from year to year suggest that although the methods described can be used to indicate S-deficiency or sufficiency predictions of yield responses based on plant analysis would be far from exact.

(c) Estimates of S Requirement

From the work reviewed it seems that the S content of healthy leguminous pasture plants should be at least 0.2% to ensure maximum dry matter yield. This implies that a good pasture of subterranean clover producing 9,000 kg per ha of dry matter utilizes at least 18 kg per ha of S per year. Furthermore, since plants accumulate excess $\text{SO}_4^{=}\text{-S}$ over that necessary for protein synthesis (Spencer 1959, Dijkshoorn et al. 1960), the S uptake and subsequent removal under conditions of excess supply may be higher than critical values indicate.

(d) S-requirements Relative to Animal Nutrition

Although critical levels of S in plant tops may be used as indices of deficiency, levels which are adequate for optimum plant

growth may not be adequate for animal nutrition. Recently it has been shown (Reis 1965) that the growth rates and S content of wool are affected by the level of nutrition of sheep, and that S-containing amino acids fed to the abomasum of a sheep stimulated wool production.

Evidence of variations in the content of S-containing amino acids in plants, due to fertilizer application, was noted by Tisdale, Davis, Kingsley and Mertz (1950). They found that different clonal lines of lucerne differed in S-amino acid contents when grown at given levels of applied $\text{SO}_4^{=}\text{-S}$. Higher levels of fertilizer application reduced the differences, but even so the methionine and cystine contents of the lucerne were too low for it to form a balanced protein ration for animal production. The actual S-amino acid content of the diet of ruminants may not be critical if the level of S entering the rumen is sufficient for microbial synthesis of necessary compounds. Even so there is great scope for work relating fertilizer practices to the S-nutrition of livestock.

(e) Soil tests for S Availability

Attempts to correlate soil S tests with plant yield responses have met with variable success. Most S is present in organic forms and unavailable so that total S determinations are not reliable

indicators of total S availability. Soil $\text{SO}_4^{=}\text{-S}$, although available to plants, is influenced by mineralization, immobilization, leaching and reduction. Estimates of $\text{SO}_4^{=}\text{-S}$ content only indicate S status at the time of sampling. Unless soil samples are handled carefully, factors such as drying at raised temperatures (Freney 1958) may lead to misleading estimates of $\text{SO}_4^{=}\text{-S}$ content.

Williams and Steinbergs (1959) examined a number of Australian soils and found that although S extracted by 0.1N NaOH, oxidation, or reduction, was well correlated with total S, the results were poorly correlated with yield responses. Better correlations were obtained between yield and $\text{SO}_4^{=}\text{-S}$ extracted by 0.15% CaCl_2 , total water soluble S, and "heat soluble" S.

In soils where $\text{SO}_4^{=}\text{-S}$ adsorption occurs extracting solutions containing phosphate provide a better indication of S status (Ensminger 1954; Freney and Spencer 1960). Many other $\text{SO}_4^{=}\text{-S}$ extractants have been used, and these are summarized in a review by Beaton, Burns and Platou (1968). Different methods of extraction yield different results and the value of any chosen procedure needs to be assessed for a given soil type and crop.

Another method of assessing S status of soils is microbiological assay. The use of Aspergillus demonstrated by Spencer and Freney

(1960), and Naik and Das (1964) provides a reliable measure of soil S availability. Any method used must be calibrated for a particular situation and no method could be classed as generally applicable in all situations.

7. S-Fertilizers and Usage

Tisdale and Nelson (1966) have discussed the various S-containing fertilizers and their uses. Gypsum, either alone or in superphosphate, elemental S, and ammonium sulphate are the main forms of fertilizer used in Australia today. Recently the use of elemental S dissolved in liquid anhydrous ammonia has been introduced, and investigations (Parr and Papendick 1966) have shown it to be effective.

Recognition of S-deficiencies has led to investigation of methods of application and efficiency of various S-fertilizers. The fact that $\text{SO}_4^{=}\text{-S}$ is readily available to plants but is easily lost by leaching has already been discussed. S applied in superphosphate, gypsum and ammonium sulphate is susceptible to leaching and the residual value of such fertilizers depends on rainfall incidence and soil-type.

(a) Superphosphate

Donald and Williams (1954) found that most of the S from superphosphate added to soils in the Crookwell district of New South Wales (Aus.) had been taken up by plants and/or incorporated in soil organic matter. Regular superphosphate applications had led to an accumulation of available P, but the organic S which

had accumulated was not readily available to plants. It was suggested that gypsum would be a useful S-fertilizer when residual phosphorus was available.

(b) Gypsum

In other areas the accumulation of the S component of superphosphate has not been as marked. Losses of gypsum from light soils due to leaching under high rainfall conditions (Walker, Adams and Orchiston 1956, McKell and Williams 1960, Barrow 1966) have, in some cases, been so rapid that $\text{SO}_4^{=}\text{-S}$ supplies were removed from the soil before appreciable plant uptake could occur. In order to utilize the available residual P on many soils it is necessary to provide regular dressings of S-fertilizers in forms which, while maintaining supplies, are not easily leached.

Crushed rock gypsum of coarse particle size (Powrie 1967) had a much greater residual effect than either superphosphate or fine agricultural gypsum, when used to supply S on sandy soils in high rainfall areas of South Australia. The factors controlling the rates of leaching of such material were examined (Millington and Powrie 1968), and it was concluded that diffusion from the fertilizer particles in the soil solution limited the rate of dissolution and hence leaching. With materials like gypsum which are sparingly soluble it is possible to choose particle sizes which will

maintain a supply of nutrient in solution for a substantial period of time.

Contrasting results to those of Powrie were obtained by McLachlan and De Marco (1968) who found that on a clay loam soil very fine gypsum (<1mm) was a more efficient supplier of S than coarser material (up to 5 mm). It is clear that optimum particle size depends on the solubility of the material, soil type and rainfall intensity.

(c) Elemental S

To become available to plants elemental S must first be oxidized to SO_4^{2-} -S in the soil. The rate of oxidation is affected by the presence of sulphur-oxidizing organisms, soil moisture, temperature, pH and the particle size of the elemental S (Burns 1967).

Many soils contain S-oxidizing organisms which multiply in response to increases in available substrate when reduced S compounds are added to soils (Rudolf's 1922, Starkey 1950, Vitolins and Swaby 1969). Where the existence of S-oxidizing bacteria is doubtful, inoculation with desirable organisms can be effective in increasing S oxidation (Li and Caldwell 1966, Vitolins and Swaby 1969).

Optimum soil-water content for oxidation of elemental S depends on the type of soil but appears to be close to field capacity (Moser

and Olson 1953). Optimum soil temperature for S-oxidation by micro-organisms occurs between 27 and 40°C (Burns 1967), but the temperatures of most surface soils of temperate regions only reach these levels in mid-summer when the soil is likely to be too dry. Soil pH also influences the oxidation of elemental S in soils, but within the pH range normally encountered is less critical than water content or temperature. Oxidation is fairly rapid in soils ranging in pH from 4.0 to 9.6 (Burns 1967) although most thiobacilli develop better under slightly acid conditions. Oxidation of S lowers soil pH, and on lighter soils of low buffering capacity it may be desirable or necessary to counteract this effect by liming when elemental S is used as a fertilizer.

Weir, Barkus and Atkinson (1963) showed that elemental S had a useful residual effect, and that selection of appropriate particle size was important in determining availability. Fine particles have a larger surface areas exposed per unit mass than coarse particles, thus presenting a larger surface for chemical and microbial attack. Coarse particles, although remaining in the soil for long periods, may not be oxidized fast enough to sustain supplies of $\text{SO}_4^{=}\text{-S}$ for plant growth. Particle sizes of less than 100 μ gave responses in white clover (Trifolium repens, L.) for three years, by which time two thirds of the applied materials had been recovered in plant tops (Weir et al. 1963).

The main problem associated with the use of elemental S as a fertilizer is the difficulty of handling and spreading the fine material. Mixing with ordinary superphosphate or triple superphosphate to form S-enriched superphosphate is one method of overcoming the problem. Bloomfield (1966) has shown that phosphate can increase the rate of S oxidation in mixed fertilizers. Mention has already been made of investigations into the use of anhydrous ammonia-sulphur mixtures, but these require special equipment. "Biosuper" consisting of a mixture of fine rock phosphate, elemental S and containing an inoculum of sulphur bacteria (Swaby, Sperber and Vitols, 1964. Aust. Plant Nut. Conf. - personal communication), provides another method of altering P/S ratios for particular soil types.

(d) Prolonging Residual Effects

At the present time the most promising methods of correcting S deficiency with fertilizer having residual effects are the use of elemental S or gypsum of suitable particle size. The choice between the two largely depends on economics. Where transport costs are high and distances from gypsum deposits are large, elemental S would be cheaper. On the other hand the ease of handling crushed rock gypsum and the lack of acidification would favour its use where local sources of supply exist or on poorly buffered acid soils.

S-fertilization is becoming increasingly important as production levels are raised in many parts of the world. To reduce the degree of empiricism it is necessary to examine and understand the factors which influence S-availability both from soil organic matter and fertilizer materials.

III Experimental

A. Seasonal Variations and Sulphur Deficiency on Laffer Sand -

Keith, South Australia

1. Introduction

Variations in yield responses of subterranean clover (Trifolium subterraneum L.) pastures during different seasons of the same year at given sites had been noted by Powrie (unpublished) during a series of fertilizer experiments conducted between 1960 and 1963. After some years of regular applications of superphosphate at rates ranging from 108 to 300 kg per ha (1-3 cwt per acre) per annum, attempts to utilize accumulated reserves of soil P led to experiments which revealed the existence of widespread S deficiency (Powrie unpublished). Powrie's experimental sites were on sandy soils in the Upper South East of South Australia.

The aim of the present study was to find those environmental factors causing the observed variations in response to S.

During the field trial, a study was made of soil $\text{SO}_4^{=}\text{-S}$ levels at intervals over a year. Rainfall was collected and gauged at weekly intervals, and a continuous record was made of soil temperatures at 5 and 10 cm (2 and 4 inches) depth. Response of subterranean clover (cv. Bacchus Marsh) to early and late applications of fine agricultural gypsum were examined. The results of this study were used to formulate designs for subsequent experiments.

Figure 2

Map of south eastern South Australia
showing the location of Keith where the
field experiment was conducted. Mean
annual isohyets are also shown (cm).

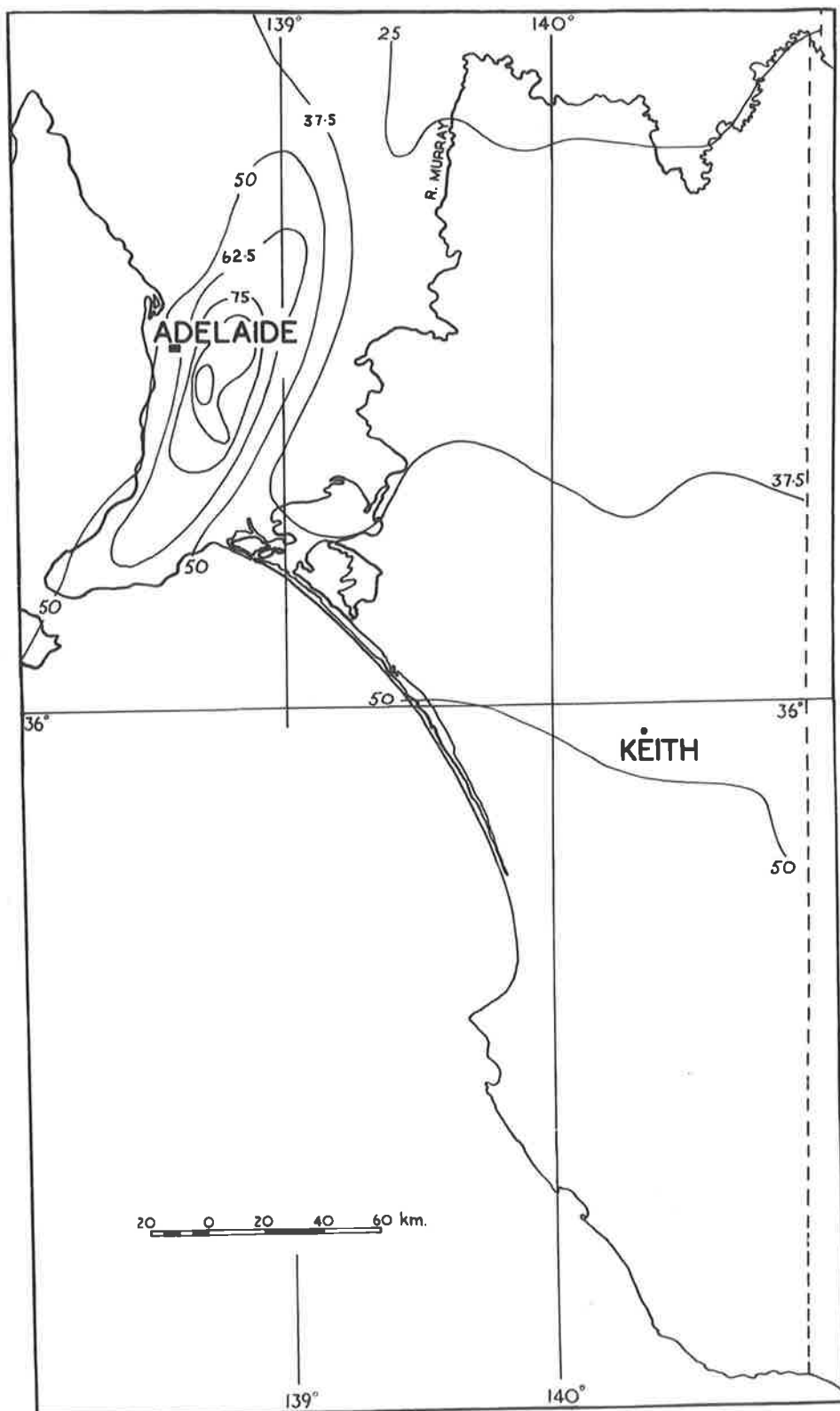
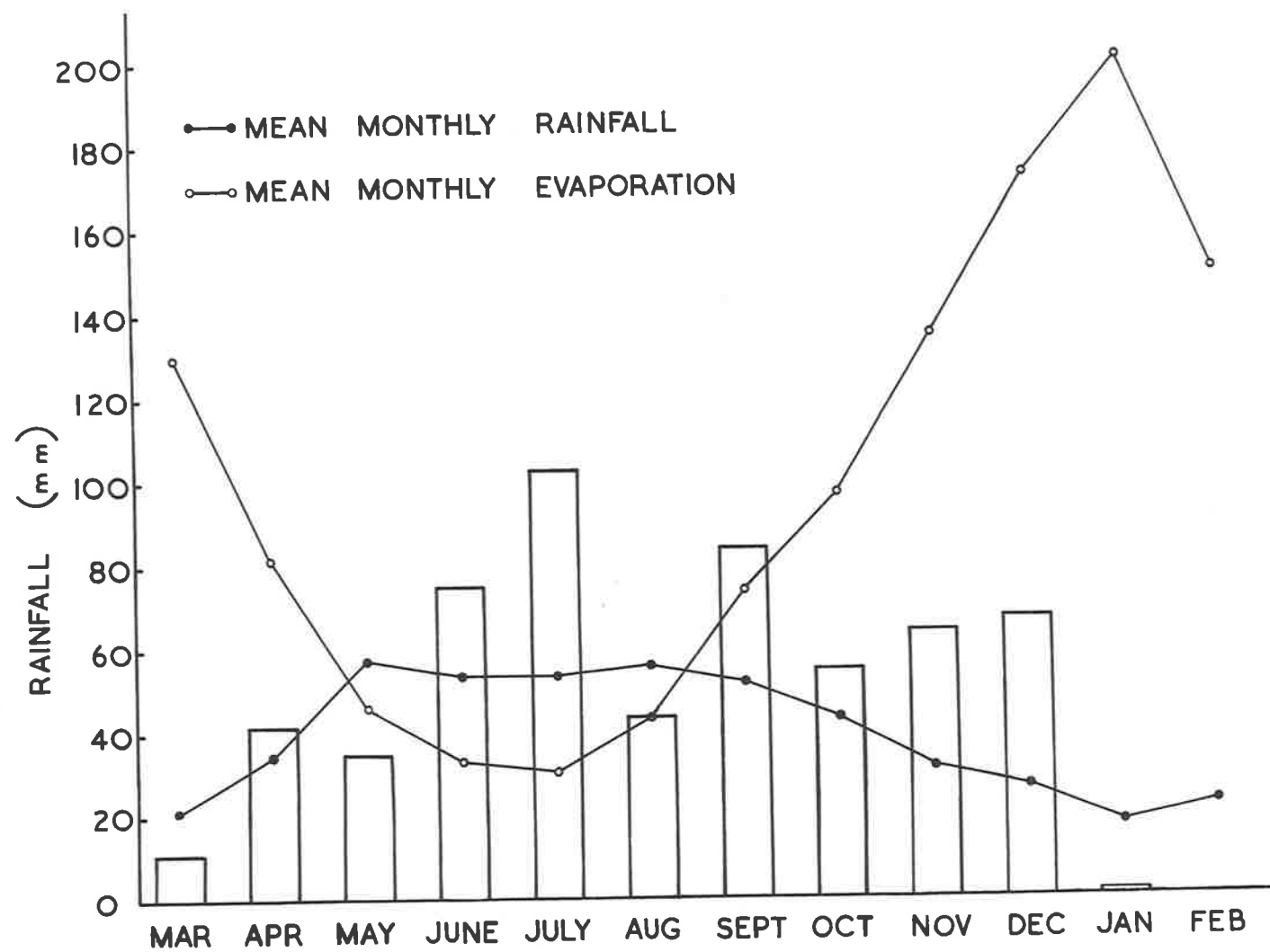


Figure 3

Mean monthly rainfall and evaporation recorded at Keith,
S.A., superimposed on a bar chart of monthly rainfall
recordings at the site of the field experiment from
March 1964 to March 1965.



The experimental site was located 16 km (10 miles) west of Keith (see map - Fig. 2). Pasture development on the sandy soils of the area has been made possible by the use of the trace elements copper and zinc together with regular applications of superphosphate.

(a) Climate

The area experiences hot dry summers and cool wet winters, with a mean annual rainfall (20 year average), recorded at Keith, (the recording station nearest to the plot site) of 467 mm (18.4 inches), two-thirds of this falling from May to October (Fig. 3). The mean estimated monthly evaporation (Commonwealth Bureau of Meteorology maps 1963) from a 36 inch open tank evaporimeter rises to a maximum of 200 mm (7.9 inches) in January (Fig. 3) and the calculated average growing season for shallow rooted annual plants is 6.9 months (Trumble 1948). Trumble calculated a drought frequency¹ of 22% for Keith. Also shown in Figure 3 is the rainfall recorded during the field experiment.

Air temperatures are lowest in July when the monthly mean is 10°C and ground frosts occur occasionally during the colder months. During the summer months extreme air temperatures sometimes reach 38°C.

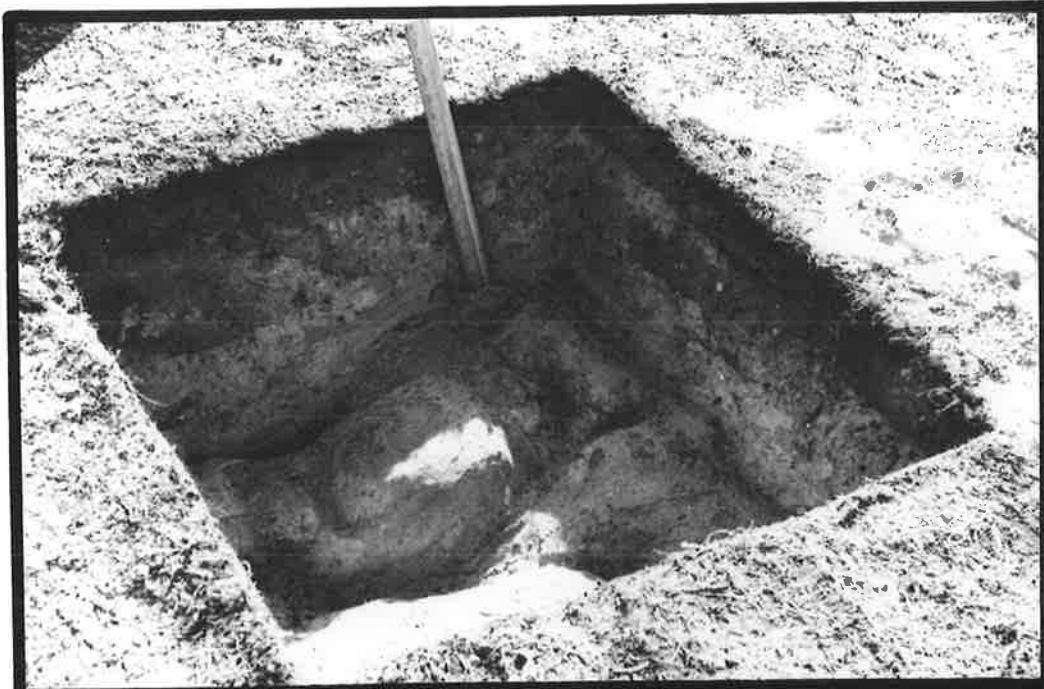
¹The number of years in 100 in which the season of continuously effective rainfall is less than 5 months.

Figure 4

Profile of Laffer Sand at the plot site showing the sandy clay domes in the bottom of the pit (30 cm).

Figure 5

Laffer sand at the plot site showing development in the A horizon due to the presence of decomposing organic matter. Note the variation in depth of dark staining and the relatively thick (up to 5 cm - 2 inches) layer of fresh organic matter at the surface.



(b) Soil

60.

The soil at the plot site is a weakly solodized solonetz (Figure 5) classified as Laffer Sand normal phase (Taylor 1933). The A₀ horizon is formed by a layer of undecomposed organic matter varying in thickness up to 2 cm depending on the time elapsed since the last cultivation. Below this is the A₁ horizon of grey sand 10 cm deep containing appreciable quantities of organic matter near the surface and becoming lighter in colour with increasing depth. The A₂ horizon is formed by a layer of white sand varying in depth from 20 to 35 cm overlaying a thin layer of sandy clay showing weakly developed columnar structure with domed tops (Fig. 4). Beneath the sandy clay layer is a deep limestone marl containing lumps of solid limestone. The limestone prevented satisfactory routine sampling of this layer.

The top soil (0-10 cm) has a pH of 6.0 (4:1 paste) and the pH rises to 8.5 at the surface of the sandy clay layer containing free calcium carbonate. The principle nutrients which limit plant growth are N, P, S, Cu and Zn. Details of the chemical characteristics of the soil from the plot site are listed in Table 1, the mechanical analysis of the 0-10 cm and 10-20 cm layers of the profile is shown in Table 2.

TABLE 1Chemical Properties of Laffer Sand from the Plot Site

Depth (cm)	0-10	10-20	20-30 (sand)	20-30 (clay)
Organic C (%)	1.30	0.27	0.10	0.50
Total N (%)	0.090	0.031	0.010	0.062
Total S (%)	0.013	0.003	0.002	0.005
C:N:S	144:10:1.4	87:10:1.0	100:10:2.0	81:10:0.8
pH	6.0	6.2	7.4	8.5

TABLE 2Mechanical Analysis of Laffer Sand from the Plot Site

Depth (cm)	Percentage Composition			
	0-10	10-20	20-30 (sand)	20-30 (clay)
Clay	3	2	2	19
Silt	1	0	1	1
Fine Sand	76	82	81	63
Coarse Sand	20	15	16	17

2. Keith Field Trial, 1964

The site chosen for the trial had previously been used by J.K. Powrie for a P x S factorial fertilizer response experiment of randomized block design, replicated six times. During this experiment the plots had been sown to Bacchus Marsh subterranean clover pasture and had received applications of fertilizer treatments in both 1962 and 1963 (Table 3).

TABLE 3Fertilizer Rates 1962 - 1963

Treatment	$\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$	$\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$	CaCO_3
	(kg/ha)	(kg/ha)	(kg/ha)
Nil	0	0	120
P	125	0	50
S	0	125	70
P + S	125	125	0

No harvests were taken from the site in 1963 and the dry matter produced was left on the plots. The site was selected to provide a set of plots of known recent fertilizer history where measurements could be made of residual S effects. Before being used by Powrie the site had been under subterranean-clover pasture for approximately eight years, during which it received biennial dressings of 225 kg per ha of superphosphate containing the trace elements Cu and Zn.

(a) Site Preparation

On April 14th, 1964, in mid autumn, the plots were cleared of plant debris by mowing and raking. Three weeks later, on May 5th, the area was rotary hoed to a depth of 7.5 cm and raked smooth. A basal application of KH_2PO_4 at a rate equivalent to 31.3 kg per ha of P was spread by hand.

(b) Treatments

At the commencement of the trial only two treatments, nil and fine agricultural gypsum ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$) were used. Figure 6 shows the plot layout and also shows the positions of treatments used in the 1962-63 P x S trial. The original plots measured 4.02 x 8.05 m (20 x 40 lk) and each was divided into two plots measuring 4.02 x 4.02 m (20 x 20 lk) for the experiment now being described. $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ was applied at a rate equivalent to 41.7 kg per ha of S to one randomly selected half of each original plot. Subsequently when it was found that leaching losses had removed most of the applied S from the root zone by mid winter, a late application of $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ was applied at the same rate as the first. This was spread by hand on August 19th, 1964, after the first harvest had been taken.

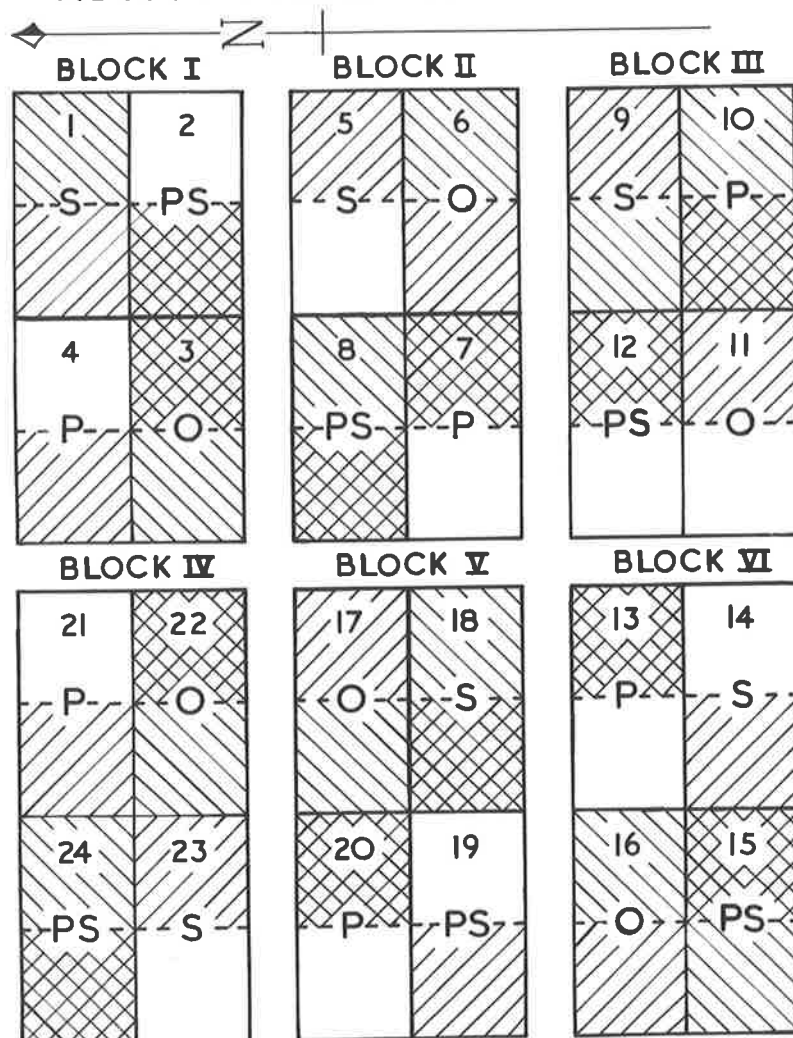
The effects of P treatments from the previous experiment were eliminated by the heavy basal application of P made at the commencement of the current study, and the only treatments of the previous experiments regarded as being effective were the S applications.

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Figure 6

Plot layout showing arrangement of treatments in 1962-63
and in the current (1964) experiment.

KEITH FIELD TRIAL 1964



1962-63 TREATMENTS

- O — NIL
- P — PHOSPHORUS ($\text{CaHPO}_4 \cdot \text{H}_2\text{O} \equiv 25.2 \text{ kg. P per ha.}$)
- S — SULPHUR ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O} \equiv 23.3 \text{ kg. S per ha.}$)
- PS — PHOSPHORUS + SULPHUR (at above rates)

1964 TREATMENTS

- NIL
- EARLY APPLICATION S ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O} \equiv 41.7 \text{ kg. S per ha.}$)
- LATE APPLICATION S
- EARLY + LATE APPLICATION S

On this assumption the late S treatment was made to randomly selected half-plots within each replicate to give the following factorial combinations:

Prior S	Early S	Late S
	x	x
Nil S	Nil S	Nil S

The locations of the current S treatments are shown in Figure 4 by shading while those of the previous fertilizer treatments are shown by letters.

(c) Fertilizer Application and Seeding

The plots were sown on May 6th, 1964. Application of fertilizer at seeding was made by mixing the material with sand from the relevant plot and spreading the mixture by hand. Immediately before spreading a measured quantity of subterranean clover seed (cv. Bacchus Marsh) equivalent to a seeding rate of 250 kg per ha was added to the fertilizer sand mixture. Inoculation was considered unnecessary as the area had previously been under pasture containing the same strain of subterranean clover which was well nodulated.

After the seed and fertilizer had been spread each plot was raked lightly to cover the seed, and the whole site was then rolled twice with a grooved roller (Fig. 7), the second rolling being at right angles to the first. Fences were erected to enclose the area.

Figure 7

Plots after seeding during the first rolling. A second rolling was made at right angles to the first.



(d) Emergence

Few seedlings had emerged before May 25th when the first rainfall after sowing was recorded. Good emergence of subterranean clover had occurred by June 5th but much brome grass (Bromus mollis L.) and silver grass (Danthonia spp.) had also germinated and this persisted throughout the experiment. At this stage the plots were sprayed with D.D.T. to prevent damage by red legged earth mites.

(e) Environmental Measurements

Commencing on May 26th, 1964, when a standard 8 inch (20.3cm) rain gauge and a twin probe mercury-in-steel thermograph were installed at the site, rainfall and soil temperature were recorded. Rainfall measurements were made weekly by an employee from a nearby farm and were compared with the sum of daily recordings made at the farm-house 1.2km (3/4 mile) from the experimental site. Soil temperatures at 5 and 10 cm depth were recorded continuously on weekly charts.

(f) Soil Sampling

Soil samplings were made on nine occasions during a one year period. Samples were collected using a twin concentric tube sampler designed by J.K. Powrie (Fig. 8). The sampler was fitted with a brass protective cap and was driven to the desired depth in the soil, with a rubber-headed mallet. The inner tube containing the soil was withdrawn and emptied, while the outer tube which bore the depth

Figure 8

Soil sampling equipment, showing from left to right, brass protective cap, inner tube for removing cores, outer tube with depth markings and rubber-headed mallet. The samplers are 44 cm long with an internal diameter of 3.2 cm.



graduations remained in the hole to prevent surface soil falling in. After reinserting the inner tube the sampler was driven down to the next sampling depth.

At each sampling at least eighteen cores were taken from each plot and the cores from each layer were bulked, placed in a plastic bag and packed in dry ice to prevent microbiological transformations occurring. The first sampling was carried out on March 4th, 1964, before the site was prepared for seeding, and samples were taken from 0-10 and 10-20 cm layers of each plot of J.K. Powrie's original P x S trial. Subsequent soil samplings were confined to the plots of the current experiment in blocks I, III and V as the amount of analytical work involved in a six block sampling would have been too great to handle. Samples were taken from the 0-10 and 10-20 cm depths at all samplings while at seeding and final harvest samples were also taken from the 20-30 cm layer.

(g) Soil Measurements

The frozen soils were thawed and the total weight of each sample was recorded for bulk density determinations. Sub-samples were taken for moisture content determinations and part of each original sample was air dried at 60°C and stored in a sealed tin to await total S determination. The remainder of each sample was stored moist in a plastic bag under refrigeration at 1-2°C until extracts for SO_4^{2-} -S determination were prepared. The maximum time of storage was 6 months.

Extracts for soil $\text{SO}_4^{=}\text{-S}$ determinations were made by shaking 50 g of moist soil with 100 ml of 0.15% CaCl_2 solution for 30 minutes. Where clay was present (samples from 20-30 cm) $\text{SO}_4^{=}\text{-S}$ extracts were prepared by shaking 20 g of moist soil with 100 ml of KH_2PO_4 solution containing 500 ppm of P (Ensminger 1954). After shaking, the soil extracts were filtered through Whatman 42 filter papers and the filtrates used for the $\text{SO}_4^{=}\text{-S}$ determinations. For total S determinations the extracts were prepared by fusing 2.5 g of oven dry soil with 1.25 g of NaHCO_3 - Ag_2O mixture (Steinbergs et al, 1962) in a muffle furnace at 550°C for 3 hours. A solution of 0.1N $\text{NaH}_2\text{PO}_4\text{H}_2\text{O}$ in 2N acetic acid (Bardsley and Lancaster 1960) was then used to prepare a 1:10 soil : solution extract from the fused material. After shaking for 30 minutes the solid matter was allowed to settle and aliquots of the supernatant were taken for S determinations.

(h) Methods of S Determination

The two methods used for the determination of S in the soil extracts were the turbidimetric method of Massoumi and Cornfield (1963) and the methylene blue method of Johnson and Ulrich (1959). Samples from the first two samplings were analysed by the turbidimetric methods, which, in spite of the use of a flask inverter to ensure uniform handling of batches of 24 determinations, gave inconsistent results. Many repeat determinations were made and an excessive number of standards was necessary to ensure satisfactory

results. A comparison of the two methods revealed that the methylene blue method gave comparable results with fewer repeats.

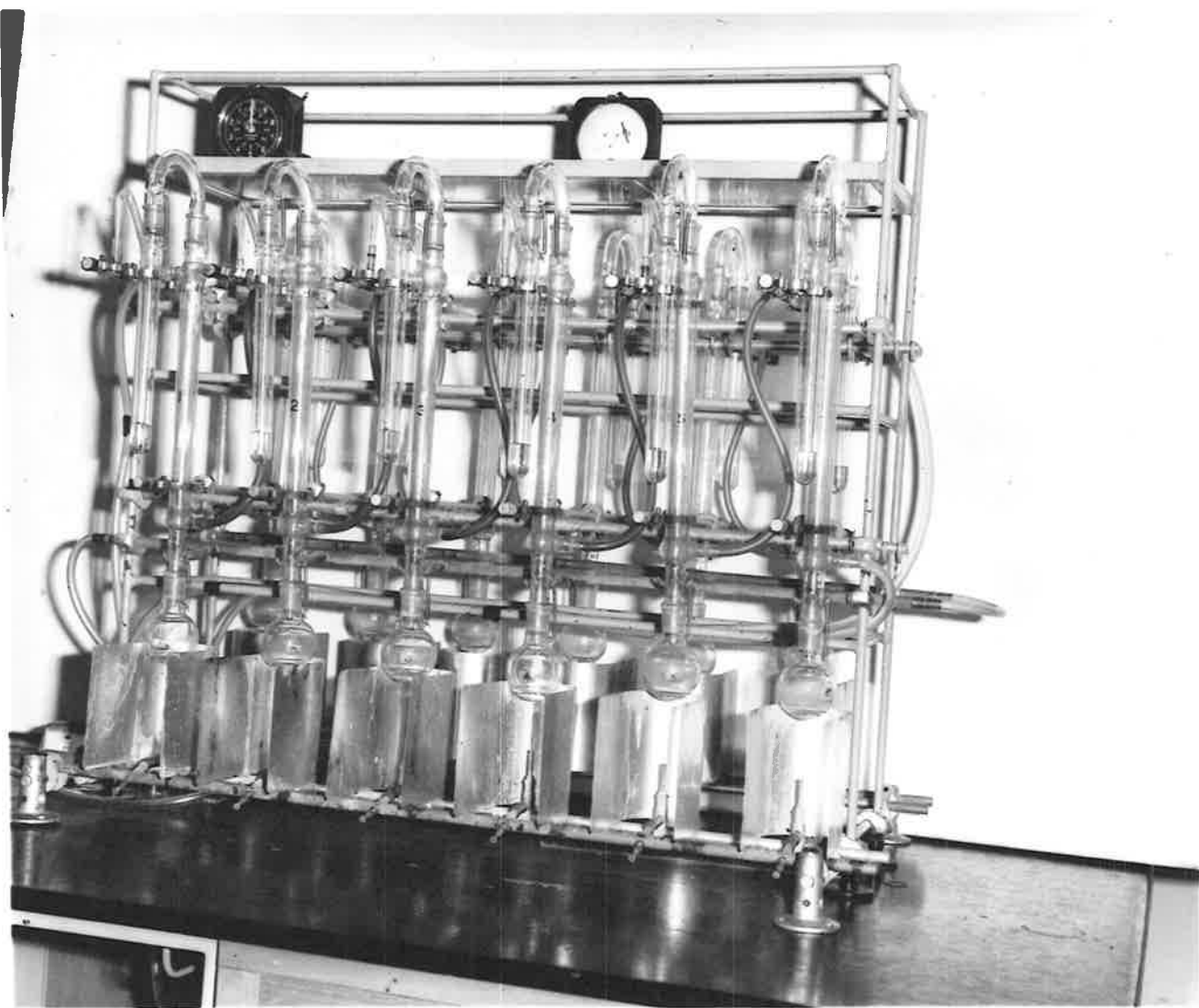
A bank of 12 Johnson and Nishita (1952) stills (Fig. 9) of modified design (Fig. 10) was constructed. The modifications were prepared after examining apparatus constructed by C.S.I.R.O. at Nedlands, W.A. Further modifications were made to enable the stills to be assembled side by side with the nitrogen supply being fed from the rear through an inlet tube which was permanently mounted below the condenser water jacket. This arrangement allowed the sample flasks to be changed without the need to disconnect the nitrogen lines, thereby minimizing the risk of breakage and reducing the time taken to change a batch of samples. Cones and sockets replaced the original spherical joints and the design of the wash columns permitted dismantling for cleaning. The nitrogen supply to each still was controlled by a needle valve mounted in a manifold at the rear of each bank of stills. Using this apparatus an experienced operator could perform 72 determinations per day including standards.

The reducing agent used for the methylene blue method of S determinations was the same as that described by Johnson and Ulrich (1959), but after use it was regenerated by evaporating the excess water from the distilled extracts over a bunsen flame in a fume hood. Nitrogen was bubbled through the reducing agent while regeneration was

Figure 9

Bank of 12 Johnson Nishita stills for S determinations.

The stills were erected in sets of 6 arranged back to back
on a scaffold mounted on an island bench.

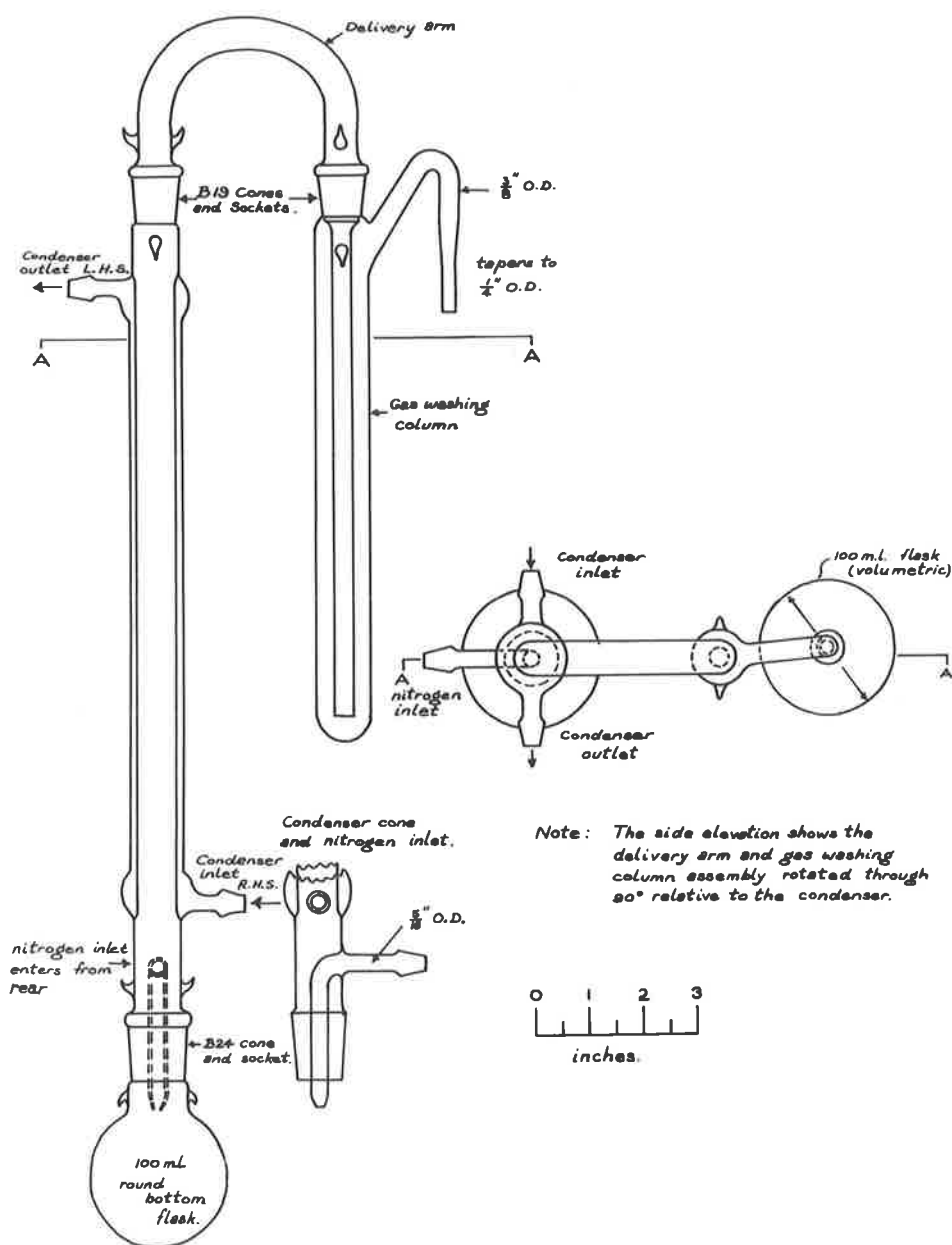


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Figure 10

Diagram of modified Johnson Nishita apparatus.

MODIFIED JOHNSON NISHITA APPARATUS



being carried out and a careful watch was kept to ensure that the temperature did not exceed 115°C . Tests (Jennings 1962) revealed that each batch of reducing agent was still usable, without further additions of hypo-phosphor^o_us acid, after being used and regenerated twice.

It was found that by increasing the amount of reducing agent from 4 to 6 ml per sample better recoveries of S were obtained. After being used 3 times the reducing agent was discarded. Where determinations were carried out on nitric/perchloric acid extracts of plant material during later experiments of the study, the reducing agent was discarded after being used only once.

(i) Harvests

Four harvests of plant tops were made on the following dates:

Harvest 1 August 19th

" 2 September 16th

" 3 October 14th

2 4 October 27th.

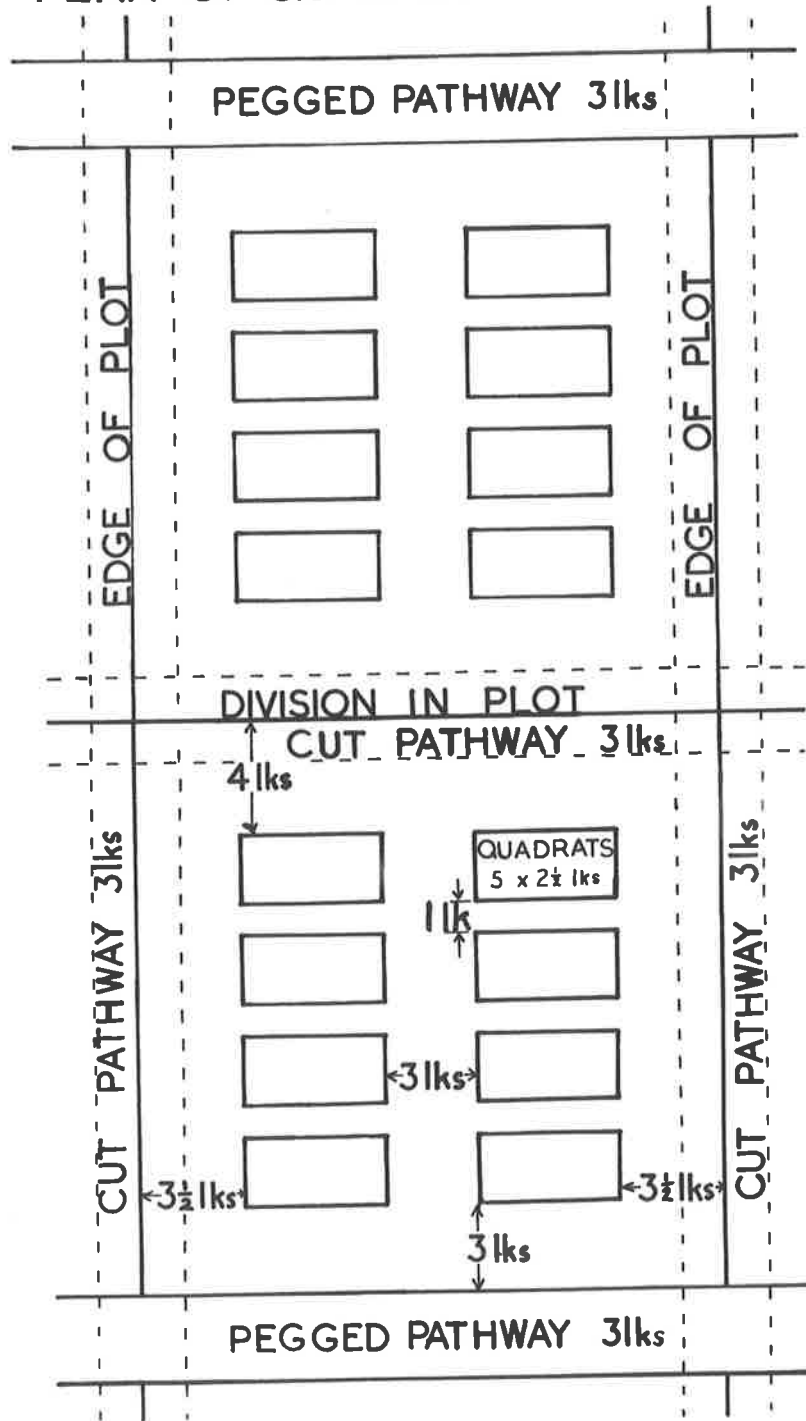
At each harvest quadrats measuring 100 x 50 cm (5 x 2½ lk) were cut from 2 randomly selected positions in each plot. The plan showing the location of the quadrat positions and the arrangement of the buffer pathways for one plot is shown in Figure 11.

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Figure 11.

Plan of quadrat cuts. Prior to harvest 1, two quadrat positions from each half-plot were randomly assigned to each harvest. Measurements are quoted in links since the plots were laid out and harvested using British system measuring equipment. (1 lk = 20 cm).

PLAN OF QUADRAT CUTS 1964



Harvesting was carried out with a portable shearing machine using a hand-piece fitted with a wide-toothed comb. The moist plant material was weighed and subsampled for botanical separation. The main samples and the subsamples were oven dried and total dry matter yields were measured. For harvests 2 to 4 inclusive where there was a substantial quantity of plant material to handle, dry matter yields were calculated by drying sub samples.

Some particles from the hand-~~spread~~^{spread} late application of gypsum had lodged on the foliage and were still visible at the time of the second harvest. The plant material from this and the subsequent two harvests, to be used for chemical determinations, was lightly rinsed with deionized water to remove contamination before it was dried.

(j) Plant Analysis

Dried samples of clover and grass tops from each harvest were ground in a laboratory hammer mill and extracts were prepared by nitric/perchloric acid digestion (Johnson and Ulrich 1959). The digests were filtered and made up to volume with distilled water (50 or 100 ml where appropriate) and aliquots were then analysed for SO_4^{2-} -S content by the turbidimetric method of Butters and Chenery (1959). Similar aliquots of blank nitric/perchloric acid extracts were added to the SO_4^{2-} -S standards to ensure that the comparisons were valid.

(k) Results

(i) Yield response and plant S uptake - Response in total dry matter yield to added $\text{SO}_4^{=}\text{-S}$ is shown in Figure 12. Statistical tests were performed by analysis of variance on log transformed data. The effects of the previous (1962-63) S treatments were not significant at any harvest and no residual effect from those applications was evident. In compiling the data from which Figure 12 was drawn the 1962-63 treatments were omitted and only the current (1964) treatments were considered.

At harvest 1 the yield differences due to the early applications of gypsum were not significant, and the corresponding difference in S uptake were small. The small differences in yield were not surprising, as the total dry matter production at that stage was small and had probably been limited by low soil temperatures (see pot experiment I) between germination and the harvest data (see soil temperature range Fig. 15).

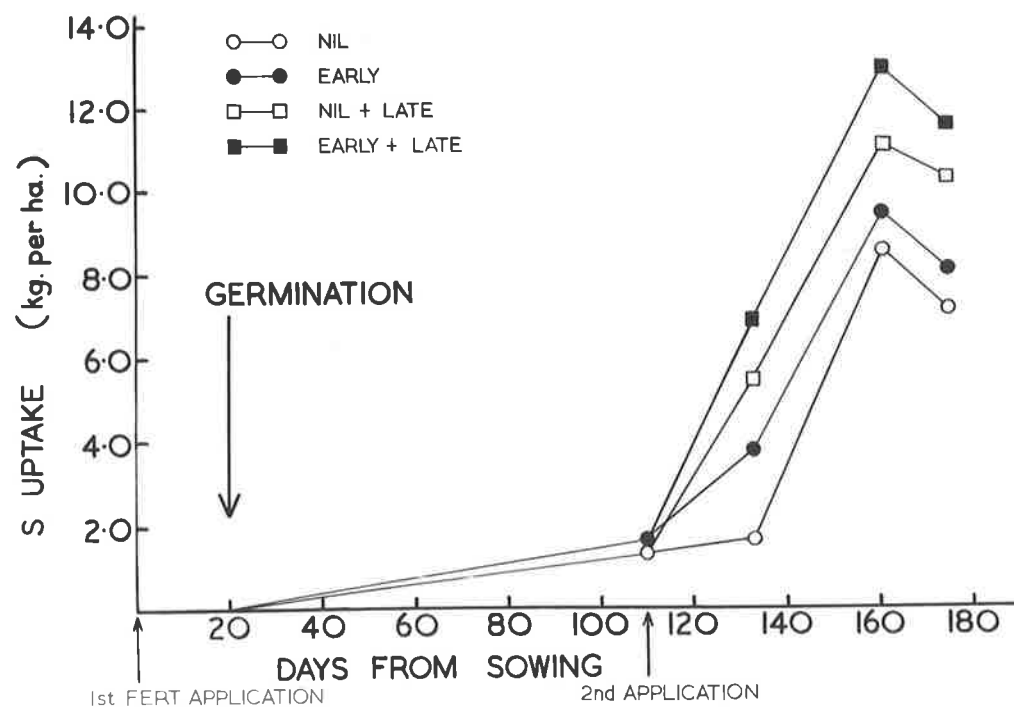
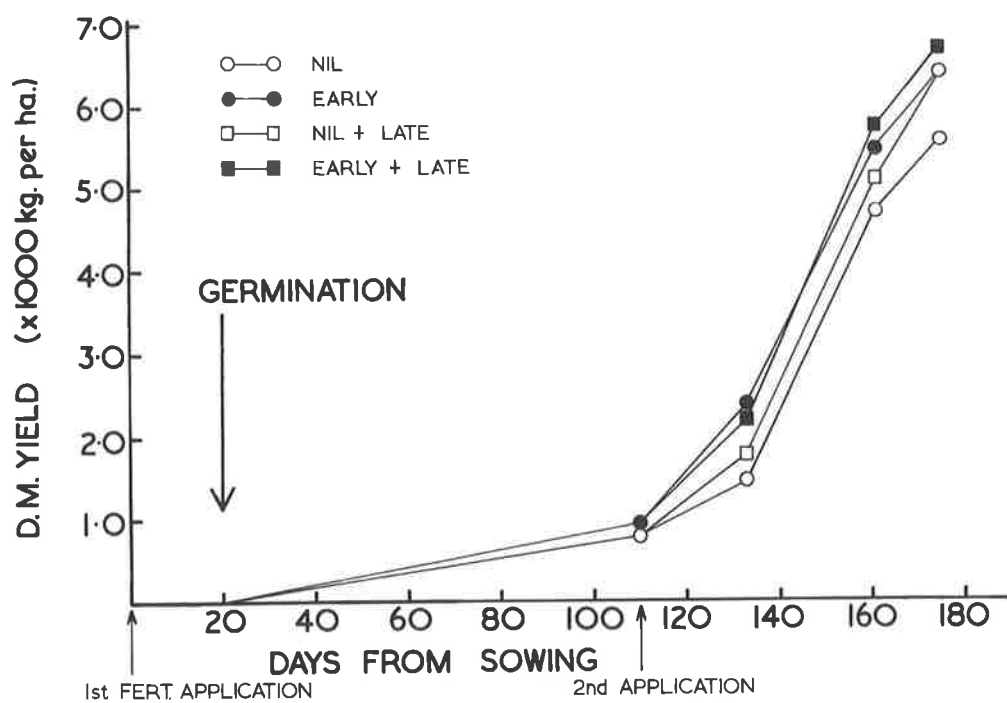
The effects of the late application of gypsum for harvests 2-4 inclusive are shown in Figure 12. At harvest 2 it was evident that the growth rates on all treatments had increased with the onset of warmer weather (reflected by rising mean weekly soil temperatures - Fig. 10), and there was a highly significant ($P \leq 0.01$) response in

Figure 12

Total dry matter yield responses to S treatments
(1964 field experiment).

Figure 13

Total S uptake by plant tops (1964 field experiment).



yield to the early application of gypsum. However, significant ($P \leq 0.5$) interaction between the previous S treatments (1962-63) and the early application treatments of 1964 was recorded. This was the only occasion when such an interaction proved significant.

Although the S uptake and the percentage S contents of both grass and clover were higher where plots had received a late application of gypsum, there was no significant yield response due to this treatment.

At harvest 3 yield differences due to the late application of fertilizer were still not significant but those due to the early application were ($P \leq 0.01$). In spite of increased dry matter yields the actual differences were of the same order of magnitude as those recorded at harvest 2, and responses to the early application appear to be due to variations in $\text{SO}_4^{=}$ -S uptake earlier in the season. The pattern and magnitude of S uptake (Fig. 13) between harvest 2 and 3 was similar for all treatments, suggesting that availability was similar.

Harvest 4 was taken at the onset of senescence and no significant yield differences due to fertilizer treatments were recorded in spite of a further increase in total dry matter yield from all plots. The decrease in total S uptake between harvests 3 and 4 may have been due

to leaching of $\text{SO}_4^{=}\text{-S}$ from senescent leaves which had taken up excess S at an earlier stage of growth. Shedding of older leaves would also have had a similar effect. There was a significant decline in the percentage S content of both the subterranean clover and grass components of the pasture during this growth phase.

Table 4 shows the mean % S contents of the grass and clover tops harvested from each treatment. The values presented in this table are with few exceptions below the 0.2% that many workers regard as the critical level for indication of S deficiency (Pumphrey and Moore 1965a; McNaught and Chrisstoffels 1961; Harward, Chao and Fang (1962). Those values greater than or equal to 0.2% (underlined in Table 4) occurred only in material from plots that had received the late application of gypsum and the higher values persisted only for harvests 2 and 3. Some of the increase may have been caused by gypsum powder adhering to the leaves in spite of careful washing prior to extraction.

(ii) Soil $\text{SO}_4^{=}\text{-S}$ changes - The soil $\text{SO}_4^{=}\text{-S}$ levels recorded in the 0-10 cm and 10-20 cm layers of the soil at each sampling date during the trial are shown in Figure 14. The values shown for treated plots on the days of application were calculated from the amount present on the controls (estimated by interpolation) and the fertilizer added.

After both early and late applications of gypsum there was a rapid decline in the soil $\text{SO}_4^{=}\text{-S}$ levels of the treated plots. At the first sampling after each application there was a significant

TABLE 4

S Content of Tops*

S Treatment	Species	S Content %			
		Harvest 1	Harvest 2	Harvest 3	Harvest 4
Nil.	Grass	0.16	0.14	<u>0.20</u>	0.10
	Clover	0.15	0.17	0.16	0.15
Early	Grass	0.18	0.15	<u>0.20</u>	0.10
	Clover	0.17	0.16	0.17	0.14
Late	Grass	-	<u>0.28</u>	<u>0.28</u>	0.13
	Clover	-	<u>0.36</u>	<u>0.22</u>	0.19
Early + Late	Grass	-	<u>0.26</u>	<u>0.27</u>	0.13
	Clover	-	<u>0.36</u>	<u>0.20</u>	<u>0.20</u>

* Each value is the mean of 6 replicate samples.

rise in the $\text{SO}_4^{=}\text{-S}$ levels in the 10-20 cm layer of the treated plots, indicating that loss from the surface had been partly due to leaching. By August 5th the $\text{SO}_4^{=}\text{-S}$ levels of the top 20 cm of soil of both untreated and early application plots were lower than the levels recorded on March 5th at the end of the summer. Since no samples were taken between May 26th and August 5th, the rates of decline in soil $\text{SO}_4^{=}\text{-S}$ could have been much faster than indicated. By the date of the first harvest (August 19th), the applied $\text{SO}_4^{=}\text{-S}$ had disappeared from the surface 20 cm of soil; as mentioned previously very little growth had occurred at that stage.

Following the late application, made on August 5th, there was an initial rapid decline in the soil $\text{SO}_4^{=}\text{-S}$ levels, but this slowed down between harvests 2 and 4. The weather during this period was warmer, and at no stage did the mean weekly minimum soil temperature (Fig. 15) fall below 10°C ; although the rainfall (Fig. 3) was still well above average, evapotranspiration would have been higher than that experienced immediately after the early application of gypsum. At this stage of growth there was complete canopy development and ground cover.

Another period of high rainfall occurred in late November and early December, and the sampling taken immediately afterwards, on December 7th, revealed that a further reduction of $\text{SO}_4^{=}\text{-S}$ had occurred

Figure 14

Fluctuating soil $\text{SO}_4^{=}\text{-S}$ contents in the 0-10 cm and 10-20 cm layers of Laffer Sand measured on 9 sampling dates over a period of 1 year from March 1964 to March 1965. Note the rapid fall of soil $\text{SO}_4^{=}\text{-S}$ after each application of fine gypsum.

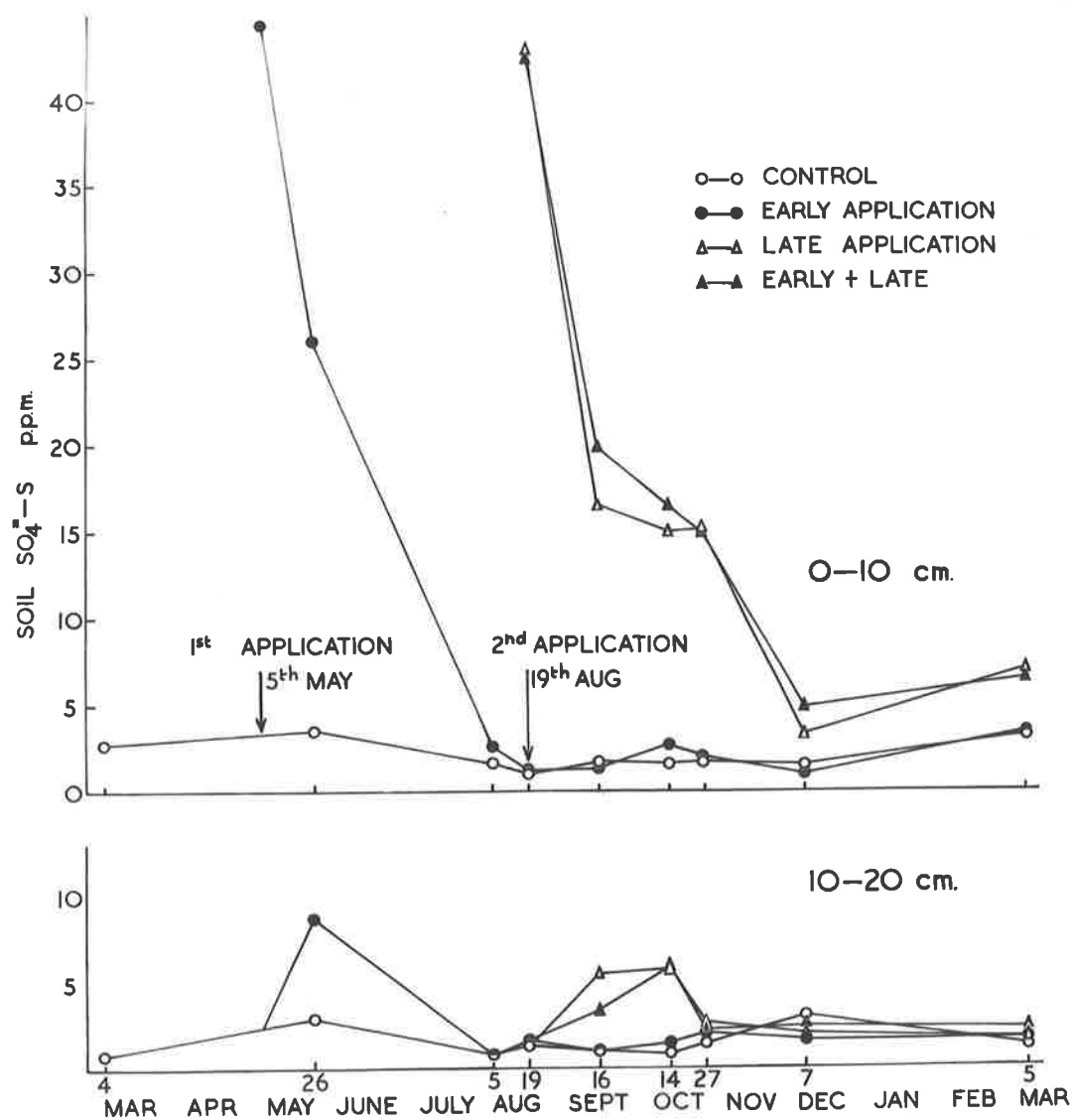
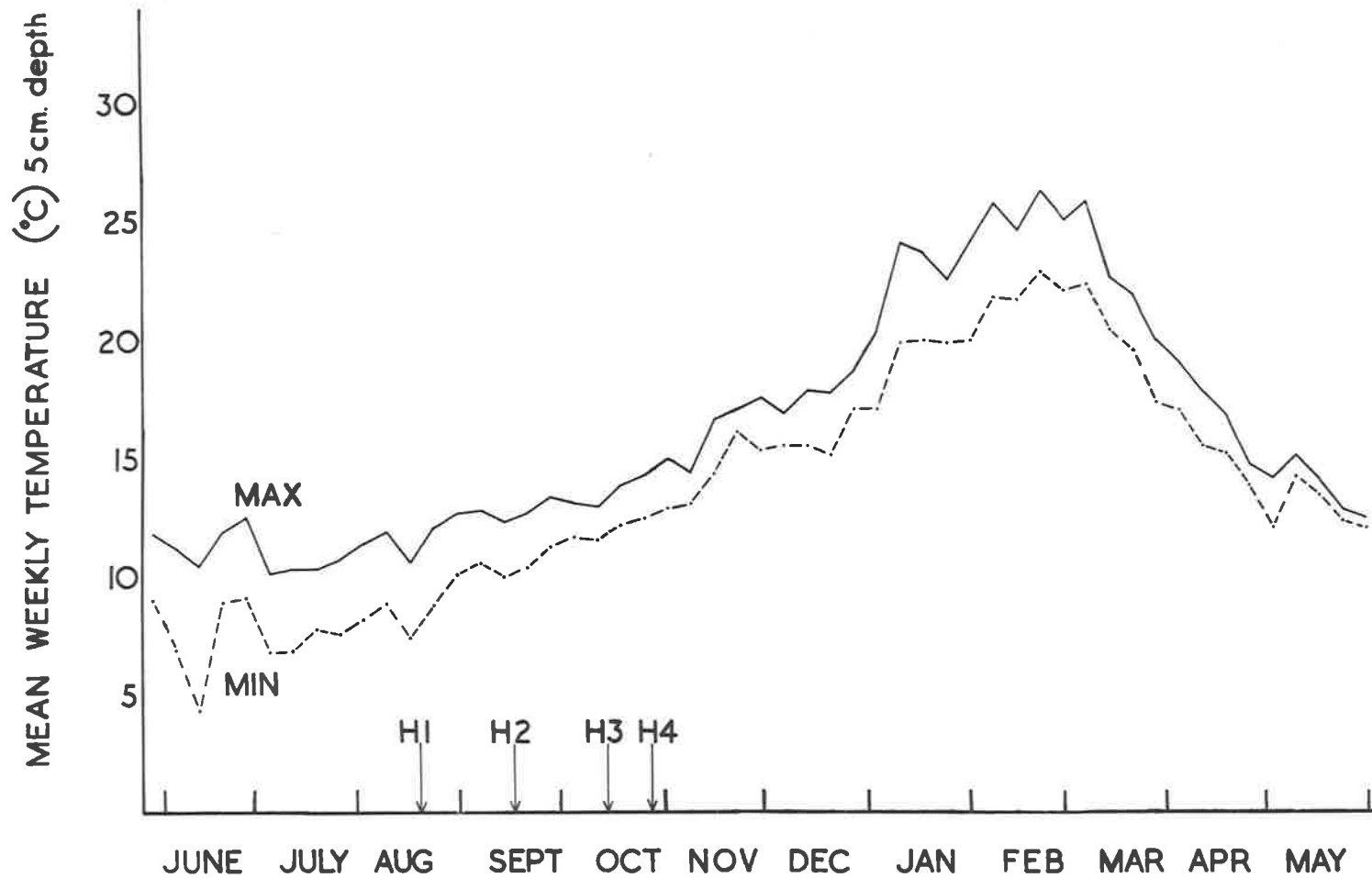


Figure 15

Variations in mean weekly soil temperature at 5 cm depth recorded during 1964 and 1965 at the site of the field experiment. Harvest dates are indicated by H1, H2, H3 and H4.



from the plots that received the late applications of gypsum. The subsequent summer months of January and February 1965 were extremely dry, and the last sampling of the trial taken on March 5th revealed that a slight rise in soil $\text{SO}_4^{=}\text{-S}$ had occurred in the 0-10 cm layers of all treatments. At this stage there was still a residual effect from the application of gypsum, but the rise of $\text{SO}_4^{=}\text{-S}$ on all treatments was similar. During the period between samplings 8 and 9 the highest mean weekly maximum and minimum soil temperatures for the year were recorded (Fig. 15).

(iii) $\text{SO}_4^{=}\text{-S}$ in clay - Samples from the sandy clay layer, normally encountered between 20 and 30 cm deep, were collected at samplings 2 and 7 made on May 25th and October 27th respectively. Variations in depth to the surface of the clay caused variable amounts of clay to be obtained from each sampling hole and plot and may have led to some variability in results.

TABLE 5
 $\text{SO}_4^{=}\text{-S}$ in Clay

S Treatment	$\text{SO}_4^{=}\text{-S}$ (ppm)		LSD		
	Sampling 2	Sampling 7	5%	1%	0.1%
Nil	7.6	11.8	-	-	-
Early	15.6	11.4	3.0	-	-
Late	-	16.6			
Early + Late	-	18.1			
L.S.D. 5%	-	4.36			
1%	-	-			
0.1%	2.2	-			

Table 5 summarizes the measurements obtained for extractable $\text{SO}_4^{=}\text{-S}$ at the two samplings. Values for sampling 2 are the means of 12 duplicate determinations and those for sampling 7 are the means of six duplicate determinations. A highly significant ($P \leq 0.001$) increase in $\text{SO}_4^{=}\text{-S}$ due to addition of gypsum was recorded at sampling 2 made only three weeks after the first application. This rise indicated that some of the gypsum had already been leached at least as far as the sandy clay layer in a relatively short time and after only 20 mm of rain had fallen. Most of the rain fell in showers lasting for several hours on the day before sampling. By sampling 7 differences due to the early application of gypsum had disappeared, but there was a rise in the $\text{SO}_4^{=}\text{-S}$ content of the nil treatments and a fall in the corresponding +S treatment.

The late application of gypsum caused an increase in $\text{SO}_4^{=}\text{-S}$ in the clay layer of plots that received this treatment but the effect was not as large as that observed in sampling 2. A longer time (10 weeks) had elapsed between the second application and sampling 7 than was the case with the early application and sampling 2.

(iv) Total soil S - Total S determinations were made on soil samples collected on five sampling dates during the trial. The intention was to subtract values obtained for $\text{SO}_4^{=}\text{-S}$ and thereby obtain estimates of organic S and organic S changes. However, it was found that detection of small changes (of the order of 5 ppm) in the relatively large quantity of total S (up to 150 ppm) was

impracticable. Results obtained by the methylene blue method of S determination were multiplied 10 times to give the total S content of the soil samples. The 2.5 g sub-sample of oven dry soil used to prepare each extract was small relative to samples of 1 kg or more collected in the field, and subsampling was complicated because organic matter tended to separate from the dry sand. Both of these factors contributed to unreliable results, and the tolerance of ± 5 ppm for the chemical determination was of the same order as likely changes in total soil S.

A summary of total S results is presented in Table 6 with LSDs applicable to significant differences. Only when additions of readily mobile $\text{SO}_4^{=}\text{-S}$ had been made prior to the sampling (e.g. samplings 2 and 4) were differences in total S between treatments significant. By comparing the results in Table 6 with those presented in Figure 14, it can be seen that rises and falls in total soil S were mainly due to variations in $\text{SO}_4^{=}\text{-S}$ content, but the method of total S determination limited the accuracy with which these changes could be followed.

An important feature of Table 6 is the fall in total S content of the top 20 cm of the profile observed at sampling 9, made after the dry summer period of early 1965. This fall was significant ($P < 0.001$) compared with S content of the top 20 cm measured at

TABLE 6
Total Soil S

Sampling No.	Date	Depth (cm)	S content (ppm)				LSD		
			Nil S	Early S	Late S	E + L	5%	1%	0.1%
1	4.3.64	0-10	124	-	-	-	-	-	-
		10-20	27	-	-	-	-	-	-
2	25.5.64	0-10	113	153	-	-	-	-	27.8
		10-20	30	41	-	-	-	-	10.4
4	19.8.64	0-10	113	125	-	-	-	10.0	-
		10-20	35	42	-	-	-	-	-
7	27.10.64	0-10	124	120	132	130	-	-	-
		10-20	36	36	33	38	-	-	-
		Total	160	156	165	168			
9	5.3.65	0-10	108	110	117	112	-	-	-
		10-20	31	33	31	30			
		Total	139	143	146	142			

sampling 7. Since there was a rise in $\text{SO}_4^{=}\text{-S}$ content of the top 20 cm of soil over the same period (Fig. 14) the decline was accompanied by net mineralization involving a fall in organic S content. The fall in total S may have been due to leaching of some mineralized $\text{SO}_4^{=}\text{-S}$ or volatilization. During the period between samplings 7 and 9 there were two factors that could have contributed to the fall. Firstly heavy rainfall in November and December 1964 would have caused increased leaching at a time of the year when leaching was normally low; and secondly removal of dry plant debris by mowing and raking prior to sampling 9 would have reduced the amount of fresh organic S normally added to soil reserves in this period. Since $\text{SO}_4^{=}\text{-S}$ levels in the top 20 cm of the soil of the nil treatment remained close to 3.0 ppm (approx. 3.0 kg per ha) for most of the growing period, a net uptake of 7.5 kg per ha of S by plants suggests that some might have been supplied by mineralization of organic reserves.

(v) Estimation of S mineralization - Since $\text{SO}_4^{=}\text{-S}$ levels in the top 20 cm of the soil of the nil treatment remained close to 3.0 ppm (approx. 3.0 kg per ha) for most of the growing period, the net uptake of 7.5 kg per ha of S by plants in this treatment suggests that some might have been supplied by mineralization of soil organic reserves. Using values of soil $\text{SO}_4^{=}\text{-S}$ (corrected for bulk densities measured on the dates of samplings) the mean net change in soil $\text{SO}_4^{=}\text{-S}$ between sowing and harvest calculated for

the nil S treatment was 0.2 kg per ha. The calculation is shown below:

Soil SO_4^{--}S (top 20 cm) at sowing	= 3.6 kg/ha
" " " " at harvest 4	= 3.4 " "
Net difference in SO_4^{--}S	= 0.2 " "

Assuming that the fall of 0.2 kg per ha was due to plant uptake a further 7.3 kg per ha of S was supplied to plants either by mineralization, accession from the atmosphere or from depths greater than 20 cm. If all of the 7.3 kg per ha of S was supplied from the soil organic S, initially calculated at 150 kg per ha in the top 20 cm, the effective mineralization during the experiment was 4.9%.

Measurement of S accession in rainfall made at a nearby site in 1965 (Powrie, personal communication) suggests that less than 2 kg per ha per year of S is added by this method. Much of this fell in the winter when leaching rates were high and this would minimize any beneficial effect. Soil sampling showed that few plant roots were deeper than 20 cm at harvest 4, but nevertheless uptake from the surface of the sandy clay could have provided S for plant growth and caused an overestimation of mineralization. Continual leaching losses on the other hand would lead to the underestimation of mineralization.

In view of the possible errors in the estimate it is presented here only as an indication of the value that could be ascribed to

mineralization; it will be seen in Sections IIIB, C and D the value was similar to those obtained in pots.

(j) Discussion

Firstly there were no signs of residual effects from previous S applications; secondly the rapid losses of applied gypsum from the soil rendered this fertilizer ineffective; and thirdly there was a rise in the $\text{SO}_4^{--}\text{-S}$ content of the surface soil during the summer.

Neither plant yields nor surface $\text{SO}_4^{--}\text{-S}$ determinations gave any indication of residual effects from previous applications of S fertilizers. Even so the total S content of 124 ppm in the top 10 cm of soil at the start of the experiment was much higher than the 30 ppm found in the top 10 cm of a similar virgin soil used in pot experiment II (Section IIIC). It appeared that the only residual effect of S due to the superphosphate and gypsum previously used on the site was a gross increase in organic S. No residual effects were evident from the previous trial.

The lack of residual effects is not surprising in view of the losses of $\text{SO}_4^{--}\text{-S}$ applied as fine gypsum in the current experiment. Similar material was used to supply S in the previous trial and therefore would have been lost, presumably by leaching, without leaving any significant residual effect. In the current experiment

risers in the $\text{SO}_4^{=}\text{-S}$ content of the 10-20 cm and 20-30 cm layers following losses from the 0-10 cm layer where gypsum had been applied suggest that much of the S was being leached below the root zone. The first sign of the downward movement occurred after only 20 mm of rain, further suggesting that on these soils $\text{SO}_4^{=}\text{-S}$ is readily leached.

Yield responses to gypsum application were small. Values of plant S content in Table 4 confirm that in nearly all cases the plants were either S-deficient or bordering on deficiency levels below which some reduction in yield would be expected. The late sowing contributed to reduced yields but low yield is unlikely to account for the low S content; rather the reverse is likely to be true.

Rapid removal of $\text{SO}_4^{=}\text{-S}$ from the root zone in the early stages of development reduced the effectiveness of the +S treatment, and suggested that under moderate rainfall fine gypsum is an unsatisfactory method of S fertilization on the sandplain soils. Even the late application of S was rapidly lost, and no yield response was observed in spite of slightly increased uptake. To be effective a continuous supply of S appears to be required during the first half of the growing period.

A small percentage of the late application persisted in the soil until the harvest 4, but this was ineffective in increasing plant growth at this stage. There was, however, a measurable increase in the $\text{SO}_4^{=}\text{-S}$ level in the soil following the hot dry summer period. This rise in $\text{SO}_4^{=}\text{-S}$ may have been due to several factors. Firstly the soil was moist in early December when the warm period commenced, as in shown in Sections III B and C, and conditions would have been favourable for mineralization of organic S. Secondly the prolonged rainless period (Fig. 3) when soil temperatures were high (Fig. 15) dried the soil to low water content (e.g. 1.4% in the top 10 cm in December at sampling 8 and 1.5% in March at sampling 9). Williams (1967) among others has shown that drying a soil at moderately high temperatures (e.g. 25-30°C) increases the amount of extractable $\text{SO}_4^{=}\text{-S}$. The increase in $\text{SO}_4^{=}\text{-S}$ observed over the summer period is in agreement with findings of Joffe (1933; 1940). In view of the losses of $\text{SO}_4^{=}\text{-S}$ that were observed in May 1964 before pasture establishment, the value of S mineralized over the summer-autumn period depends on the amount and intensity of rains falling at the start of the growing period. Any S mineralized during summer is likely to be lost by leaching before plants are able to utilize it. The findings of this experiment and those of Barrow (1966), McKell and Williams (1960), Hogg and Cooper (1964), and Hogg (1965) show that caution should be used when interpreting the results of experiments using fine gypsum.

Since this experiment was carried out, Powrie (1967) has demonstrated that the particle size distribution of the gypsum influences its resistance to leaching and hence its value as a fertilizer. Millington and Powrie (1968) showed that particle size can be varied to control availability and resistance to leaching.

B. Factors Affecting Soil Sulphur Transformations on Laffer Sand.

1. Introduction

From measurements made during the field experiment it was apparent that plants on the nil S treatments were able to obtain significant quantities of $\text{SO}_4^{=}\text{-S}$ from the soil in spite of the small quantity present throughout the growing period. To examine the importance of mineralization on plant S supply, a series of pot and incubation experiments were carried out.

Incubation experiments by Barrow (1960a, b, c, 1961a) examined the effect of C, N, S content on the decomposition of soil organic matter. Williams (1967) studied factors affecting the mineralization of soil organic matter. Both of these workers used incubation temperatures of $27 - 30^{\circ}\text{C}$ and the measurements made during the Keith field experiment indicated that such high temperatures occur in the top 5 cm of temperate region soils only in mid to late summer. There is little doubt that the high soil temperatures experienced during this period are partly responsible for increases in $\text{SO}_4^{=}\text{-S}$ release by mineralization. However, under the influence of autumn rains S mineralized in summer is likely to be leached from the top-soil.

In order to examine the importance of S mineralization during the growing season, tests were conducted at appropriate temperatures

and the experiments to be described were designed to cover the temperatures that would be encountered during the growing period.

2. Pot Experiment I. 1965¹

This experiment was designed to examine the influence of soil temperature, plants, and added $\text{SO}_4^{2-}\text{-S}$ on S transformations in Laffer sand.

(a) Soil Preparation

A bulk sample of the top 10 cm of soil was taken at the site of the field experiment. The soil had a gravimetric water content of 5% when collected and was air dried (to less than 0.5% soil water content) and stored. Before use the soil was ^{ie} ~~se~~ved in a rotary screen (6.5 cm) to remove plant debris and to ensure a low initial $\text{SO}_4^{2-}\text{-S}$ content was leached using deionized water. This was done by placing samples of the soil in stainless steel Buchner funnels of 10 kg capacity and leaching with 30 l of water. After leaching, the samples were dried by suction to a gravimetric water content of 5 to 10% and stored under refrigeration at 1-2°C until sufficient soil had been prepared.

1

A report of this experiment, entitled "Soil Sulphur balance studies in the presence and absence of growing plants" has been accepted for publication in "Soil Science".

The soil was brought to a gravimetric water content of 10% by the addition of de-ionized water and thoroughly mixed in a cement mixer. Fifty two 1 kg samples (oven dry basis) were then taken and each was mixed with 20 ml of the required fertilizer solution using an electric mixer, bringing the soil samples to a final water content of 12%. Properties of the leached soil are summarized in Table 7.

TABLE 7

Soil Properties

Coarse sand (%)	20
Fine sand "	76
pH	6.0
C (%)	1.50
N "	0.09
S "	9.912
SO ₄ ⁼ -S (ppm)	1.2

(b) Design of Experiment

The following treatments were applied in factorial combination:

- (i) Soil Temperatures - 10, 15, and 20°C
- (ii) Sulphur - Nil and 30 ppm S (as Na₂SO₄)
- (iii) Presence and absence of plants.

Each treatment was replicated 4x. Replications of $\text{SO}_4^{2-}\text{-S}$ and plant treatments were made within individual temperature tanks.

(c) Fertilizer Application, Potting and Sowing

A basal application of potassium dihydrogen phosphate (KH_2PO_4) equivalent to 30 ppm of P was made to all pots. A solution containing sodium sulphate (Na_2SO_4) supplying 30 ppm of S was added to twenty six of the pots. Each mixed soil sample was placed in a metal pot 11 cm deep and 10.0 cm diameter, which had been coated internally with bitumen paint and lined with a polythene bag. The soil was compacted to a bulk density of approximately 1.3 g per cc by tapping each pot gently on a concrete floor while it was being filled. Immediately after filling, on 6th August, 1965, fourteen seeds of subterranean clover (Trifolium subterranean L. cv. Bacchus Marsh) were sown in twelve pots of each fertilizer treatment. Before sowing the seed had been freshly inoculated with a suitable strain of Rhizobium trifolii.

The pots were kept indoors at temperatures between 7 and 10°C while the seeds germinated prior to imposing the temperature treatments. After seven days the seedlings were thinned to eight per pot and the temperature treatments were commenced. A thin layer of washed gravel was placed on the surface of the soil to minimize evaporation losses, and to bring each pot to a constant weight. The pots were placed in randomized blocks in controlled

temperature tanks within the glasshouse (Figure 16). The pots were divided into three groups of 16, each of which was subjected to a temperature treatment.

Four pots of soil, two with, and two without added $\text{SO}_4^{2-}\text{-S}$, were stored under refrigeration throughout the experimental period. These were used to check S recoveries at the end of the experiment.

(d) Soil Temperature Control

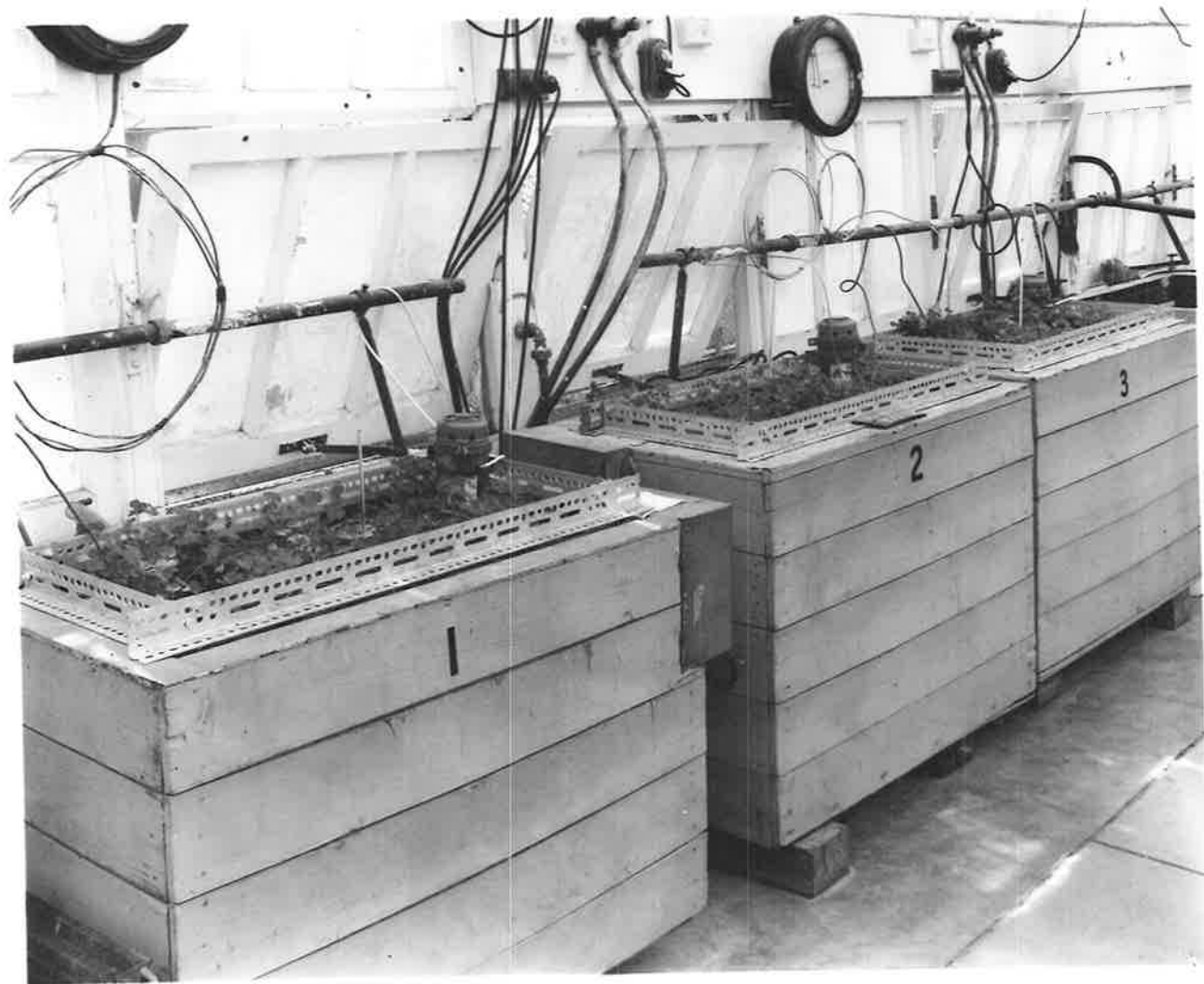
The temperature tanks were insulated, thermostatically controlled copper water baths, equipped with heating units and circulating pumps. Two of the tanks (10° and 15°C treatments) were also fitted with refrigeration coils. A steel frame inside of each tank supported the pots so that the top of the soil in each pot was level with the surface of the water in the tank. A 0.5 cm hardboard shield with holes to admit the pots covered each tank.

Tank temperatures were monitored continuously with mercury-in-steel recorders which were checked daily against mercury-in-glass thermometers placed in the pots. The temperature of the tanks was maintained within $\pm 1^\circ\text{C}$ of the desired figure except on very hot days when fluctuations were as high as $\pm 2^\circ\text{C}$. Soil temperature was within 0.5°C of tank temperature at all times. A continuous recording was kept of air temperature and humidity within the

100.

Figure 16

Pot experiment I - showing the tanks used to control
soil temperature.





glasshouse. The air temperature, measured with mercury-in-glass thermometers 3 cm above the soil within the clover canopies, was independent of tank temperature.

(e) Watering

The moisture content of the pots was maintained by the daily addition of de-ionized water to constant weight. On hot days towards the end of the experiment when transpiration losses were high the pots were watered twice daily. The pots in each replicate within each temperature tank were randomized weekly.

(f) Harvesting and Analyses

The experiment was terminated sixty days after the soil temperature treatments were imposed. The plant tops were harvested and oven dried at 60°C. The soil core from each pot was fragmented carefully after first removing the gravel from the surface, and the roots were separated from the soil by dry sieving. Soils were sealed in preserving jars under refrigeration at 1-2°C pending analysis. Roots received a light washing with de-ionized water and were oven dried at 60°C. The dry matter yields of the roots and tops were recorded and the plant samples were ground for analysis.

Digests of plant tops and roots were prepared by oxidizing 250 mg of plant material in 8 ml of a 3 : 1 mixture of nitric and

perchloric acids. The digests were filtered and made up to 100 ml. Soil extracts for total S and $\text{SO}_4^{=}\text{-S}$ determinations were prepared using the methods described for the field experiment. All determinations were performed using the methylene blue method (Johnson and Ulrich 1959). Organic S in the soil was obtained by subtracting the soil $\text{SO}_4^{=}\text{-S}$ from the total soil S. S balance calculations were made to obtain mineralization and immobilization estimates.

(g) Results

(i) S recoveries - The results presented in Table 8 show the calcium chloride extractable $\text{SO}_4^{=}\text{-S}$ content and the total S content of the soils in the four pots which were stored under refrigeration at $1-2^{\circ}\text{C}$ for the duration of the experiment. The soils were analysed before and after storage and the values for the duplicate extracts are shown.

From Table 8 it can be seen that satisfactory recovery of added $\text{SO}_4^{=}\text{-S}$ was achieved in both total S and $\text{SO}_4^{=}\text{-S}$ determinations before and after storage. No apparent transformations occurred between S fractions during storage of the samples in the refrigerator. The mean base levels of 1.2 mg/pot of $\text{SO}_4^{=}\text{-S}$ and 120.4 mg/pot of total S were used in all subsequent calculations.

(ii) Summary of significance tests - All tests for significant differences due to treatment effects were carried out by analyses

TABLE 8

Soil Sulphate-S and Total S Contents

Treatment		SO ₄ ⁼ -S (mg/pot)		Total S (mg/pot)	
Added SO ₄ ⁼ -S (ppm)	No.	Before cold storage	After cold storage	Before cold storage	After cold storage
0	1	1.1	1.2	118.8	119.6
	1	1.3	1.2	118.8	120.5
	2	1.1	1.0	121.8	121.4
	2	1.0	1.1	121.0	121.0
	Mean	1.2	1.2	120.1	120.6
30	3	31.4	32.1	148.9	150.7
	3	31.4	31.9	149.9	150.7
	4	30.7	30.5	151.7	150.4
	4	31.0	30.6	151.5	150.1
	Mean	31.2	31.2	150.2	150.4

of variance and are summarized in Table 9. Prior to analysis all data concerning soil S and S balance measurements were transformed to natural logarithms.

(iii) Plant yields and S uptake - At harvesting, plants from the nil S treatment were showing visible ^msymptoms of S deficiency. Petioles were reddish and the leaflets pale green to yellow with the severest symptoms occurring at the highest temperature. Figure 17 shows the type of growth response due to S additions at the three temperatures while Figure 18 shows the mean dry matter yields of tops and roots. Soil temperatures had a marked effect on the dry matter yields of the tops, but the effect on root growth was less marked.

Highly significant ($P \leq .001$) responses in the dry matter yield of tops due to added $\text{SO}_4^{=}\text{-S}$ were recorded at each soil temperature, but the responses in root yield although significant ($P \leq 0.5$) were small. Where $\text{SO}_4^{=}\text{-S}$ was added significant responses in the yields of tops ($P \leq .001$) were observed with each increase in soil temperature. Where no S was added there was no yield response when soil temperature was raised from 15°C to 20°C and in this range S supply appeared to be the sole limiting factor.

Table 10 shows that the percentage S contents of the tops or roots of plants were similar within each of the S treatments. The

TABLE 9

Pot Experiment I - Significance of Treatment Effects

Treatment ¹ Component	T	S	P	TxS	TxP	SxP	TxSxP
Soil SO ₄ ⁼⁼ -S	*	***	***	ns	*	ns	ns
Loss of Soil Organic S	ns	ns	***	ns	ns	*	ns
Loss of Soil/ Plant S	*	***	***	*	ns	ns	ns
Recovery of Soil/ Plant S	ns	***	***	**	ns	ns	ns
Plant S Uptake (Tops)	*	***	-	ns	-	-	-
Plant S Uptake (Roots)	ns	***	-	ns	-	-	-
Plant S Uptake (Total)	ns	***	-	ns	-	-	-
Dry Matter Yield (Tops)	***	***	-	ns	-	-	-
Dry Matter Yield (Roots)	ns	*	-	ns	-	-	-
Dry Matter Yield (Total)	**	***	-	*	-	-	-
% S Content (Tops)	ns	***	-	ns	-	-	-
% S Content (Roots)	ns	***	-	ns	-	-	-

Significance levels:

*** - P 0.001 ns - not significant

** - P 0.01

* - P 0.05

(1) - Treatment Symbols:

T - Soil temperature

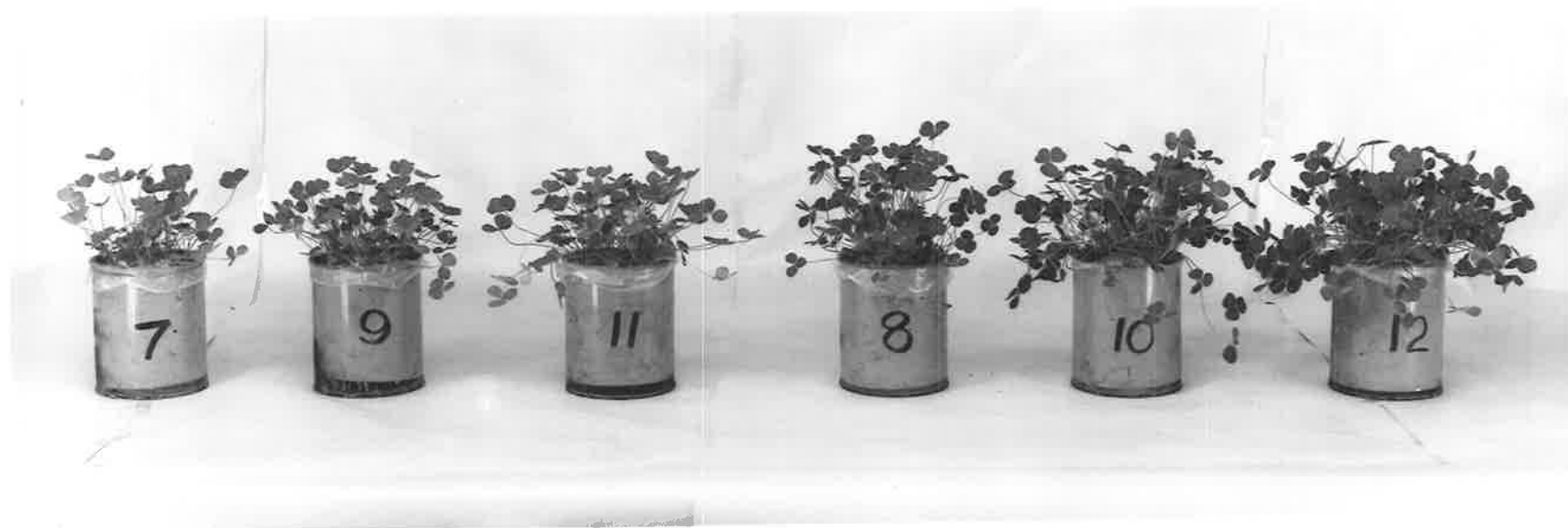
S - Sulphur

P - Plants.

Figure 17

Pot experiment I, showing pots ready for harvesting from left to right

Nil S			+S (30 ppm)		
Soil temperature					
10°C	15°C	20°C	10°C	15°C	20°C



107.

Figure 18

The influence of soil temperature and added $\text{SO}_4^{=}\text{S}$ on the dry matter yield (in g/pot) of subterranean clover tops and roots (pot experiment I).

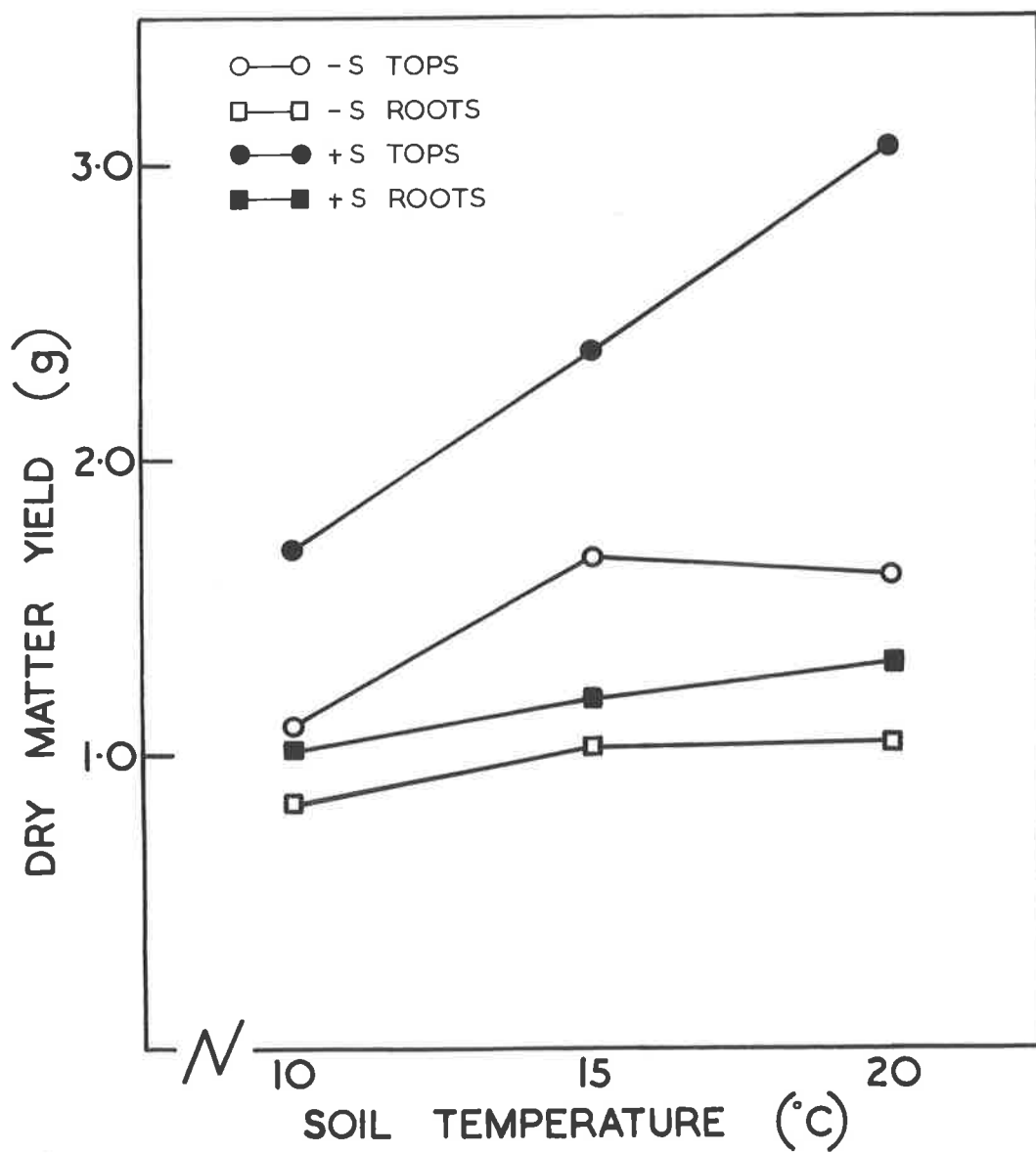


TABLE 10

Plant S Analyses*

Treatment $\text{SO}_4^{=}\text{-S}$ (ppm)	S content (%)			
	0		30	
Temp °C	Tops	Roots	Tops	Roots
10	0.08	0.10	0.42	0.64
15	0.09	0.12	0.41	0.62
20	0.06	0.11	0.39	0.55

TABLE 11

Total S Uptake*

$\text{SO}_4^{=}\text{-S}$ (ppm)	S uptake (mg per pot)					
	0			30		
Temp °C	Tops	Roots	Total	Tops	Roots	Total
10	0.82	0.86	1.68	7.16	6.31	13.47
15	1.53	1.20	2.73	9.60	7.42	17.02

* All figures are the means of four replicates.

levels in the tops from the nil S treatment are indicative of severe S deficiency at all temperatures based on standards for clover summarized by Ensminger and Freney (1966). Table 11 lists values for total S uptake by plants, and in view of the similarity of % S contents within each S treatment (Table 10) total S uptake for each S treatment was closely related to plant yield.

(iv) Soil S measurements and balance calculations - Balances were prepared for total S and $\text{SO}_4^{=}\text{-S}$ using total soil S values, soil $\text{SO}_4^{=}\text{-S}$ values and plant uptake data according to equations (1) and (2). S added in the seed was negligible and no S was detectable in the de-ionized water used throughout the experiment. Levels of total soil S and soil $\text{SO}_4^{=}\text{-S}$ at the start of the experiment that were used for balance calculations are shown in Table 8.

$$\Delta S = (S_H + S_P) - (S_I + \text{SO}_4^{=}\text{-S}_A) \dots \dots \dots (1)$$

$$\Delta \text{SO}_4^{=}\text{-S} = (\text{SO}_4^{=}\text{-S}_H + S_P) - (\text{SO}_4^{=}\text{-S}_I + \text{SO}_4^{=}\text{-S}_A) \dots \dots \dots (2)$$

Where H - content of soil at harvest

P - content of plants at harvest

I - initial content of soil

A - added.

Values for total S balance (ΔS) and $\log_e \Delta S$ are shown in Table 12 together with the mean values of soil S content (S_H) and plant S uptake (S_P) measured at harvest. Appropriate values

of LSD for the log transformed data are given in the attached sub-table. In all treatments there was a net loss of S from the soil/plant system. S losses were greater where $\text{SO}_4^{=}\text{-S}$ had been added ($P \leq 0.001$). Less S was lost from the planted than from the bare pots ($P \leq 0.001$).

For the purpose of calculating net changes in the soil $\text{SO}_4^{=}\text{-S}$ fraction, plant S uptake was regarded as occurring in the form of $\text{SO}_4^{=}\text{-S}$. The initial soil $\text{SO}_4^{=}\text{-S}$ levels are shown in Table 8 while final soil $\text{SO}_4^{=}\text{-S}$, plant S contents and net $\text{SO}_4^{=}\text{-S}$ balance values ($\Delta\text{SO}_4^{=}\text{-S}$) calculated using equation (2) are shown in Table 13.

Positive values of $\Delta\text{SO}_4^{=}\text{-S}$ show that net mineralization of $\text{SO}_4^{=}\text{-S}$ occurred and negative values show that $\text{SO}_4^{=}\text{-S}$ was either immobilized or lost from the system. Where $\text{SO}_4^{=}\text{-S}$ was not added a small net amount of S was mineralized in the planted pots. Where $\text{SO}_4^{=}\text{-S}$ was added between one sixth and one third of the added $\text{SO}_4^{=}\text{-S}$ was either immobilized or lost, the change being smaller in the planted pots.

By subtracting soil $\text{SO}_4^{=}\text{-S}$ values from the corresponding total soil S values an estimate of the soil organic S content was obtained. The initial organic S content of the soil was 119 ppm and Table 14 lists mean organic S contents and organic S balance at harvest. Neither S nor temperature treatments influenced the

TABLE 12
Total S Balance

Treatment	$\text{SO}_4^{2-}\text{-S}$ (ppm)	0			30		
	Temp ($^{\circ}\text{C}$)	10	15	20	10	15	20
No plants	Soil S (S_H) (mg/pot)	111.4	114.0	112.2	130.9	127.3	125.7
	Change (ΔS) (mg/pot)	-9.0	-6.4	-8.2	-19.5	-23.1	-24.7
	$\log_e \Delta\text{S}$	2.22	1.90	2.11	2.98	3.16	3.22
Plants	Soil S (S_H) (mg/pot)	115.3	116.4	112.9	123.5	123.8	121.5
	Plant S (S_P) (mg/pot)	1.7	2.7	2.1	13.5	17.0	19.0
	Change (ΔS) (mg/pot)	-3.4	-1.3	-5.4	-13.4	-9.6	-9.9
	$\log_e \Delta\text{S}$	1.27	0.41	1.67	2.56	2.29	2.31

	5%	1%	0.1%
L.S.D. $\log_e \Delta\text{S}$ shown above (applicable to plant or S treatments).	0.51	0.69	0.91

TABLE 13

$\text{SO}_4^{=}$ -S Balance

Treat- ments	SO ₄ ⁼ -S (ppm)	0			30		
	Temp (°C)	10	15	20	10	15	20
No Plants	Soil SO ₄ ⁼ -S at harvest (S _H) (mg/pot)	1.3	1.2	1.4	22.5	20.7	22.0
	log _e S _H	0.19	0.15	0.30	3.11	3.03	3.08
	Change in SO ₄ ⁼ -S (ΔSO ₄ ⁼ -S) (mg/pot)	0.1	0.0	0.2	-8.7	-10.5	-9.2
Plants	Soil SO ₄ ⁼ -S at harvest (S _H) (mg/pot)	0.9	0.6	0.1	11.5	7.6	6.6
	Plant S (S _P) (mg/pot)	1.7	2.7	2.1	13.5	17.0	19.0
	S _H + S _P	2.6	3.3	2.2	25.0	24.6	25.6
	log _e (S _H + S _P)	0.95	1.18	0.74	3.22	3.20	3.24
	Change in SO ₄ ⁼ -S	1.4	2.1	1.0	-6.2	-6.6	-5.6

	5%	1%	0.1%
L.S.D. $\log_e S_H$) (applicable to plant or S)	0.56	0.75	0.99
" $\log_e (S_H + S_P)$ treatment differences only).			

NOTE: L.S.D. not obtained for $\text{SO}_4^{=}\text{-S}$ as not possible to apply required log transformation to values of differing sign.

changes in organic S and the LSDs listed apply to differences due to plant treatments only.

(h) Discussion and Conclusions

(i) S losses - The most interesting result was the apparent loss of significant quantities of S from the soil and soil/plant systems. Recovery tests (Table 8) suggest strongly that the loss was real and not due to analytical errors. Volatile S compounds may be lost from plant material during drying, but recent work by Grundon and Asher (personal communication) suggests that such losses would be far too small to account for those of the size found in the current experiment; moreover losses occurred from the bare as well as from the planted pots.

Mechanisms to explain the loss are a matter of conjecture, but volatilization due to the activity of soil micro-organisms is one possible explanation. Production of H_2S in soils under strongly reducing conditions has been discussed in the literature review, but at no stage was the average air space in the sand less than 39% (corresponding to a soil water content of 12%). Nevertheless, the possible existence of local sites of reduction should not be overlooked.

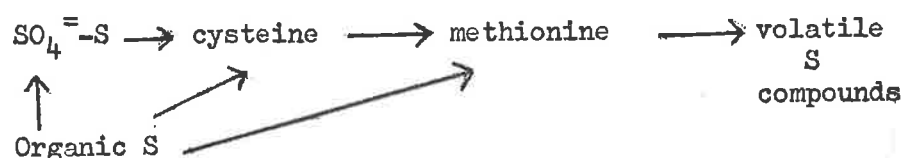
Barjac (1952) and Frederick, Starkey and Segal (1957) have

TABLE 14

Soil Organic S Balance

Treatments	SO ₄ ⁼ -S	0			30		
	Temp (°C)	10	15	20	10	15	20
No Plants	Final Organic S (mg/pot)	110.1	112.8	110.7	108.4	106.5	103.7
	Loss of Organic S (mg/pot)	+9.1	+6.4	+8.4	+10.8	+12.7	+15.5
	log _e change	2.19	1.84	2.06	2.29	2.52	2.69
Plants	Final Organic S (mg/pot)	114.4	115.8	112.8	112.0	116.2	114.9
	Loss of Organic S (mg/pot)	+4.7	+3.4	+6.5	+7.2	+3.0	+4.3
	log _e change	1.47	1.10	1.80	1.81	0.95	1.00
					5%	1%	0.1%
L.S.D. log _e change in organic S (plant treatment comparisons only).					0.77	1.04	1.36

observed the emission of volatile S compounds including methyl mercaptan and dimethyl disulphide from soils under anaerobic conditions. These volatile compounds are products of methionine decomposition. Micro-organisms capable of producing methionine from cysteine are known to exist (Young and Maw, 1958), and, since sulphate can provide S for cysteine synthesis, it is possible that volatile S losses under aerobic conditions may occur via the following pathways:



In an experiment of Freney and Spencer (1960) the disappearance of $\text{SO}_4^{=}\text{-S}$ measured in a similar balance study was attributed to immobilization, but since no measurements of total S were reported such an interpretation is open to doubt. Their results, reproduced here in Table 15, are consistent with the present study; more immobilization appeared to occur as larger quantities of $\text{SO}_4^{=}\text{-S}$ were added in soils and the presence of plants reduced the apparent S immobilization or in some instances resulted in net mineralization.

TABLE 15

Mineralization or Immobilization of $\text{SO}_4^{2-}\text{-S}$ *

(Freney and Spencer 1960)

Soil No.	$\text{SO}_4^{2-}\text{-S}$ (ppm)	Plants		No Plants	
		36	108	36	108
1	C:S = 63:1	1.9	-12.5	-17.7	-55.2
2	85:1	2.0	-10.8	-12.9	-19.6
3	84:1	6.1	-7.7	-13.7	-41.7
4	112:1	0.5	-13.4	-31.2	-70.6
5	139:1	2.5	-4.5	-10.2	-32.8

* All values in the body of the table are in ppm,

- ve values indicate "immobilization"

The C/S ratios of all soils shown in Table 15 were less than 200:1 and, therefore, according to Barrow (1960c) would have favoured mineralization of S. In view of the current results Freney and Spencer may have confounded immobilization and volatilization of S.

The results of both experiments show that plants reduce S losses, and this may occur in two ways. Firstly, by competing with micro-organisms for available S, plants may limit the amount of S which is converted into microbial products. Secondly,

frequent replacement of water lost by transpiration improves the oxygen supply in soil and minimizes the occurrence of reducing conditions favourable for the production of H_2S .

(ii.) Soil temperature and mineralization - An unexpected result of the experiment was the lack of influence of soil temperature on net S mineralization. By contrast, Williams (1967), in incubation experiments observed increases in S mineralization with increasing soil temperature. He incubated three different soils at 10, 20 and 30°C for 64 days and recorded little or no S mineralization at 10°C. However, at 20°C and 30°C variable quantities of S were mineralized from each soil. Even though different quantities of S were mineralized in the different soils it can be shown that the same percentage (1.3% at 20°C) of the total S present was mineralized from each of the 3 soils.

In the current experiment very little net S mineralization occurred in the bare pots, but the mean net S mineralized in the planted pots of the nil S treatment was 1.25% of the total S originally present. The C/S ratios of the soils of Williams' experiments and the Laffer Sand of the current study were all between 105 and 125. This ratio would in general favour S mineralization, Barrow (1960c).

The amount of S mineralized at each temperature was too small to correct the S deficiency of the subterranean clover plants. In winter and spring, when temperatures in the top 10 cm of Laffer Sand under pasture are close to 10°C and always less than 20°C, similar low rates of mineralization may be expected. However, as plant growth rates in the field are lower than in the glasshouse the contribution of S supplied by mineralization is likely to be higher relative to plant requirements. Even so, mineralization of S on these soils will still be insufficient to permit maximum economic yields, and field experiments (Powrie, personal communication) have shown a continuing need for S fertilizers on these soils.

3. Incubation Experiment I.

The Influence of Soil Water and $\text{SO}_4^{=}\text{-S}$ on Net S Mineralization.

(a) Introduction

The gravimetric water content of the top 10 cm of soil measured at each sampling date during the field experiment varied from as low as 1.4% after a dry summer period to as high as 15.7% in winter. Low soil water contents may prevent soil microbial activity while high soil water contents cause poor aeration and favour the development of anaerobic organisms. Moser and Olson (1953) demonstrated that optimum conditions for oxidation of S occurred at soil water contents slightly above field capacity. Recently, Williams (1967) has shown that soil water contents affect microbial transformations responsible for S mineralization. The experiment to be described was designed to examine the effect of soil water content on S mineralization in a Laffer Sand from an area of developed pasture. The object was to assess the optimum conditions of soil moisture for S mineralization likely to be encountered during the growing period.

Throughout the incubation experiments the terms 'net mineralization' and 'net immobilization' have been used to discuss increases and decreases respectively in the concentration of inorganic $\text{SO}_4^{=}\text{-S}$

and $(\text{NO}_3^- + \text{NH}_4^+)\text{-N}$ in the soil. Evidence of S losses from soil plant systems by volatilization shows that a measured gain or loss in $\text{SO}_4^{2-}\text{-S}$ is not necessarily an indication of actual mineralization or immobilization. In the sections which follow the terms "net mineralization" or net immobilization denote net positive or negative balance rather than implying that total mineralization or immobilization has been measured. Other workers have used the terms in a similar manner without qualification.

(b) Soil and Soil Preparation

The soil chosen was from the same sample collected for pot experiment I. Soil properties are shown in Table 7, the only difference in this experiment being that $\text{SO}_4^{2-}\text{-S}$ (at 1.0 ppm) was slightly lower initially. A soil water content/suction curve for low suctions between 0 and 136 cm of water is shown in Figure 19. This curve was prepared by draining a sample of the soil on a sintered glass funnel using a variable height manometer. Four samples drained on a tension plate at one third of an atmosphere (approx. 340 cm of water) retained a mean gravimetric water content of 5.8%.

Preparation of the soil was carried out by air drying, sieving, and leaching with de-ionized water as for pot experiment I. If a soil is air dried there is an increase in extractable sulphate, and such treatment may be likened to the effects of the dry summer

and autumn periods on soil sulphur availability (Williams 1967). In order to assess potential mineralization during the winter-spring growing period, the soil was leached after being air dried to remove any $\text{SO}_4^{=}\text{-S}$ released during drying.

After leaching the soil was again air dried to 1.5% water content. The initial $\text{SO}_4^{=}\text{-S}$ analysis was made after this drying and showed a $\text{SO}_4^{=}\text{-S}$ content of 1.0 ppm indicating that any further release of $\text{SO}_4^{=}\text{-S}$ due to the second drying was minimal.

Two hundred and forty samples of leached soil each weighing 250 g were individually prepared for incubation by hand mixing with appropriate aliquots of fertilizer solution and de-ionized water to bring them to the required moisture content. A basal addition of KH_2PO_4 equivalent to 30 ppm of P was mixed with each soil sample. No trace elements were added, since the field from which the sample was taken had received regular dressings of copper and zinc sulphates.

(c) Design of Experiment

(1) Treatments:

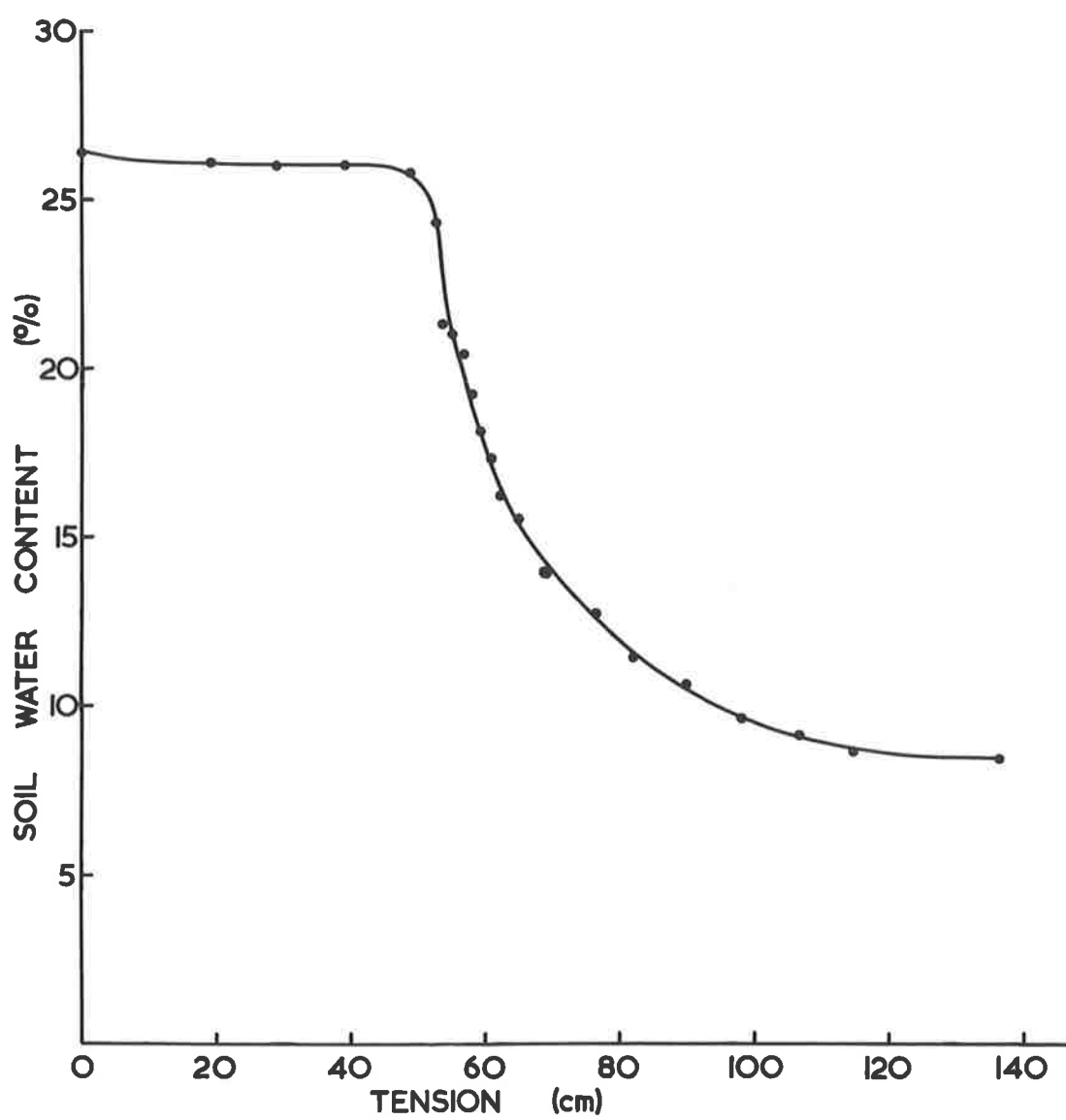
- (i) Gravimetric soil water content: 3, 6, 9, 12, 18, and 24%
- (ii) Sulphur: nil and 30 ppm S applied as Na_2SO_4 in solution.

(2) Replication and Sampling:

Four replicates were prepared and each replicate contained five similar samples for destructive sampling at 12 day intervals.

Figure 19

Soil water content (gravimetric) suction curve for
Laffer Sand used in the incubation experiments.
The curve was prepared by draining the soil on a
sintered plate at the tensions shown.



(d) Potting

After mixing, each sample was placed in a container (Fig. 20) made by removing the neck from a 225 ml polythene bottle. All containers were trimmed to a constant tare weight to simplify the task of checking water contents by weighing. The soil in each container was packed to a bulk density of 1.3 g/cc by gently compressing successive layers with a pestle. Sets of five similar samples were placed in large (4.5 l capacity) high density polythene jars fitted with screw top lids. In each jar was a polythene bottle containing 50 ml of 5% NaOH solution to absorb CO_2 evolved from the soil.

The bottom of each jar contained 5 ml of 0.1N HCl to maintain humidity without absorbing CO_2 . To obtain blank values for CO_2 determinations four incubation jars containing no soil samples were prepared, and the absorbers from all incubation vessels were changed at appropriate intervals.

(e) Conduct of Experiment

The incubation jars were placed in a constant temperature room which was maintained at 20°C for the duration of the experiment. Each day the lids were removed for five minutes to allow replenishment of the air within the jars. The CO_2 absorber bottles were

124_c

Figure 20

Incubation apparatus - from left to right -
soil container, incubation vessel with lid and
"O" ring seal, and CO₂ absorber bottle.



initially changed at 6 day intervals but changes were reduced to coincide with the 12 day intervals between samplings from day 36 to day 60.

One soil sample was removed from each incubation jar at intervals of 12 days after the commencement of the experiment. Samples were placed in plastic bags and stored under refrigeration at $1-2^{\circ}\text{C}$ until extracts were prepared for analysis.

During the experiment it was intended to maintain a constant soil water content for each treatment by the addition of water to constant weight, but it was found that water losses were low and additions were only necessary at 6 day intervals.

(f) Chemical Analyses

The $\text{SO}_4^{=}\text{-S}$ content of all soil samples were determined after the incubation period and net mineralization or immobilization calculated. Since the experiment was only intended to examine the effect of soil water content on $\text{SO}_4^{=}\text{-S}$ no measurements were made of total S changes.

$\text{SO}_4^{=}\text{-S}$ extracts were prepared by extraction with 0.15% CaCl_2 and determinations were carried out by the methylene blue method described in sections III A 2 (g) and (h).

The CO_2 content of the absorbers was determined by taking 10 ml aliquots of each absorber solution and adding a slight excess of 3N BaCl_2 solution to each aliquot to precipitate the carbonate as BaCO_3 . Several drops of phenolphthalein were then added to the samples and they were slowly titrated to a colourless end point using 1N HCl. Aliquots from the blank absorbers were treated in a similar manner.

The amount of CO_2 evolved from each incubation vessel was calculated from the difference in titre between the blank and the soil sample absorbers (Stotzky 1965).

Soils from samplings 1, 3, and 5 were analysed for $\text{NO}_3^- + \text{NH}_4^+ - \text{N}$. The determinations were made using aliquots of CaCl_2 extracts prepared for the sulphate determinations. Steam distillation with MgO and $\text{Ti}_2(\text{SO}_4)_3$ (Bremner 1965) followed by collection of the distillate in boric acid indicator, and subsequent titration with standard acid was used for $\text{NO}_3^- + \text{NH}_4^+ - \text{N}$ determinations. Distillations were carried out in a Hoskin's still as described by Bremner (1965).

(g) Results

(i) Net mineralization of S - All results are expressed as ppm of S in oven dry soil. There was a net increase of $\text{SO}_4^{2-} - \text{S}$ in soils of all treatments after 60 days incubation with one exception (Fig. 21). The exception was the soil of the +S treatment at 24% water

content where a consistent pattern of decreasing $\text{SO}_4^{=}\text{-S}$ content was observed.

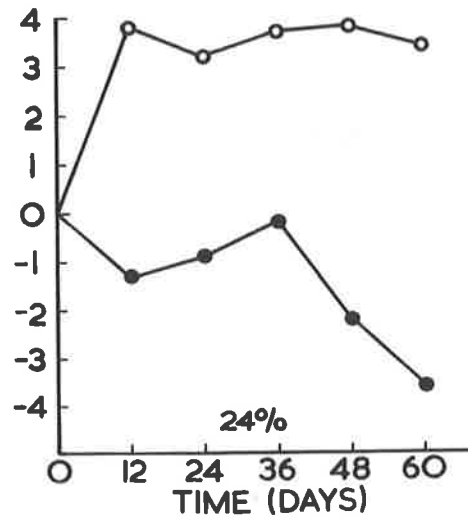
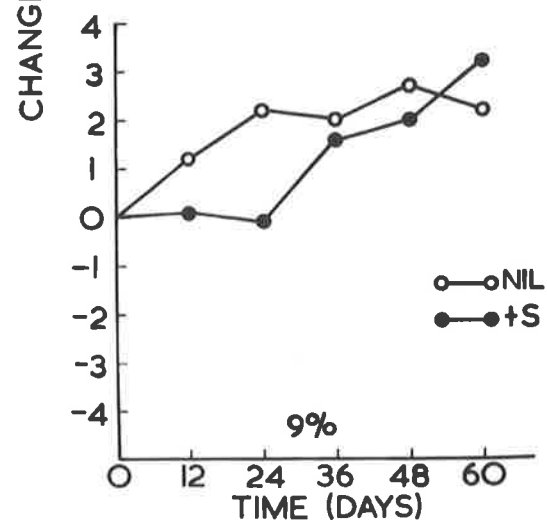
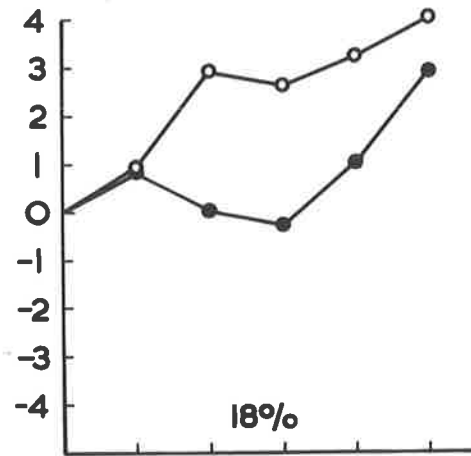
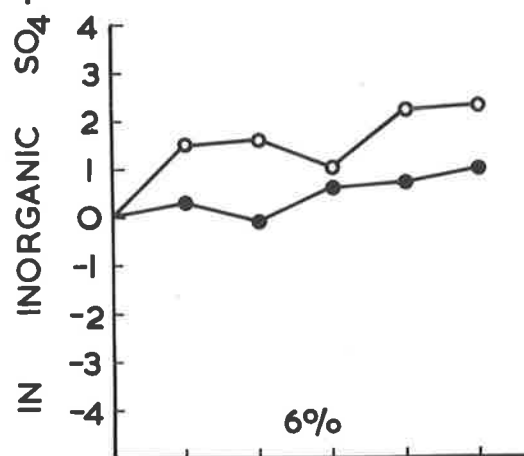
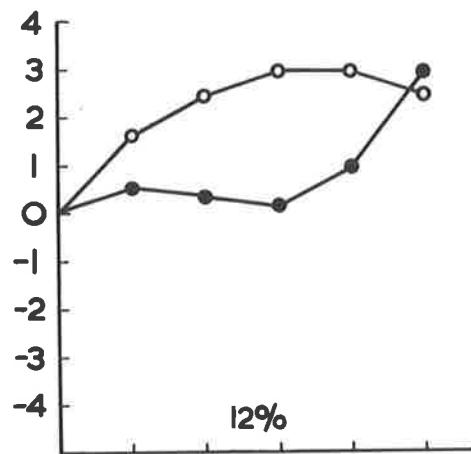
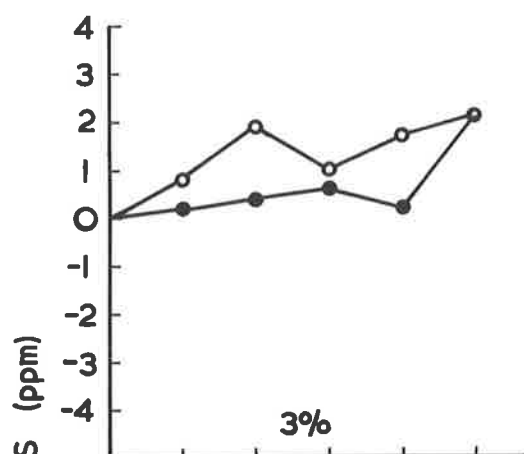
All soil samples of the nil S treatment regardless of soil water content showed a net gain in $\text{SO}_4^{=}\text{-S}$. Although the amounts mineralized between sampling intervals were rather erratic there was a slow increase in mineralization over the 60 day period. The maximum net mineralization observed occurred in soil of the nil S treatment at 18% water content resulting in an increase of 4 ppm in $\text{SO}_4^{=}\text{-S}$. This represented a net mineralization of 3.3% of the original organic S (119 ppm) present in the sample.

The results from the +S treatments were more variable and the net mineralization lagged behind that of the nil treatments. Added $\text{SO}_4^{=}\text{-S}$ appeared to suppress net mineralization until after the second sampling at 24 days.

Although the net mineralization of S in soil of the nil treatment at 24% water content was relatively high (3.4 ppm), the pattern of release at this moisture level differed from other treatments in that nearly all of the S was mineralized within the first 12 day sampling period. For the remaining 48 days the soil $\text{SO}_4^{=}\text{-S}$ level remained constant.

Figure 21

Changes in the concentration of soil $\text{SO}_4^{=}\text{-S}$ in Laffer Sand at six different soil water contents incubated at 20°C , in the presence and absence of added $\text{SO}_4^{=}\text{-S}$. The +S treatment received 30 ppm of applied S and all values shown have been corrected to show changes and not absolute $\text{SO}_4^{=}\text{-S}$ content. All measurements are expressed on an oven dry soil basis.



Since the prime aim of the experiment was to examine the effect of soil water content the results obtained for S mineralization together with corresponding results for N mineralization from samplings 1, 3 and 5 are presented plotted against soil water content in Figure 22.

As far as the nil treatments were concerned the results show that the highest soil water content used (24%) gave consistently high net S mineralization values. It has already been explained that after the initial release very little further mineralization occurred at this soil water content. The fall in soil $\text{SO}_4^{=}\text{-S}$ levels at this water content when $\text{SO}_4^{=}\text{-S}$ was added to the soil suggests that reducing conditions favouring the production of volatile products such as H_2S arose in soils of this treatment. Support for this theory is provided by the corresponding reduction in N mineralization which accompanied the rapid lowering of $\text{SO}_4^{=}\text{-S}$ levels between days 48 and 60. A similar effect was not evident in the nil treatment but this may have been due to shortage of $\text{SO}_4^{=}\text{-S}$ substrate for reduction.

(ii) Nitrogen mineralization - The pattern of N mineralization shown in Figure 23 was more consistent than that for S mineralization. Low soil water content (3%) retarded N mineralization quite markedly during the first 12 day incubation period. There was a slight

Figure 22

Changes in the concentrations of $(\text{NO}_3^- + \text{NH}_4^+)$ -N and $\text{SO}_4^{=}$ -S in Laffer Sand incubated at 20°C at various soil water contents in the presence or absence of added $\text{SO}_4^{=}$ -S. All measurements are expressed on an oven dry soil basis.

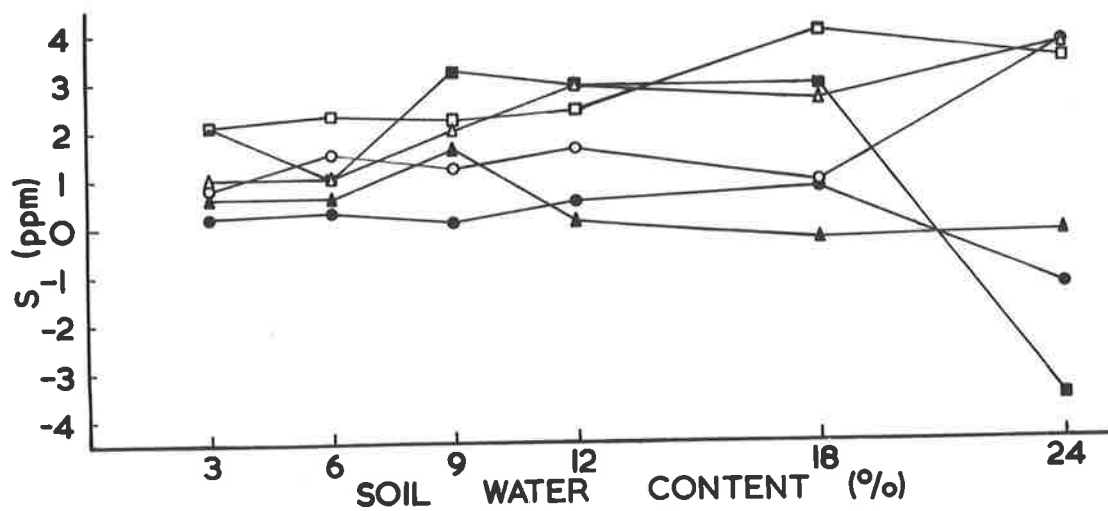
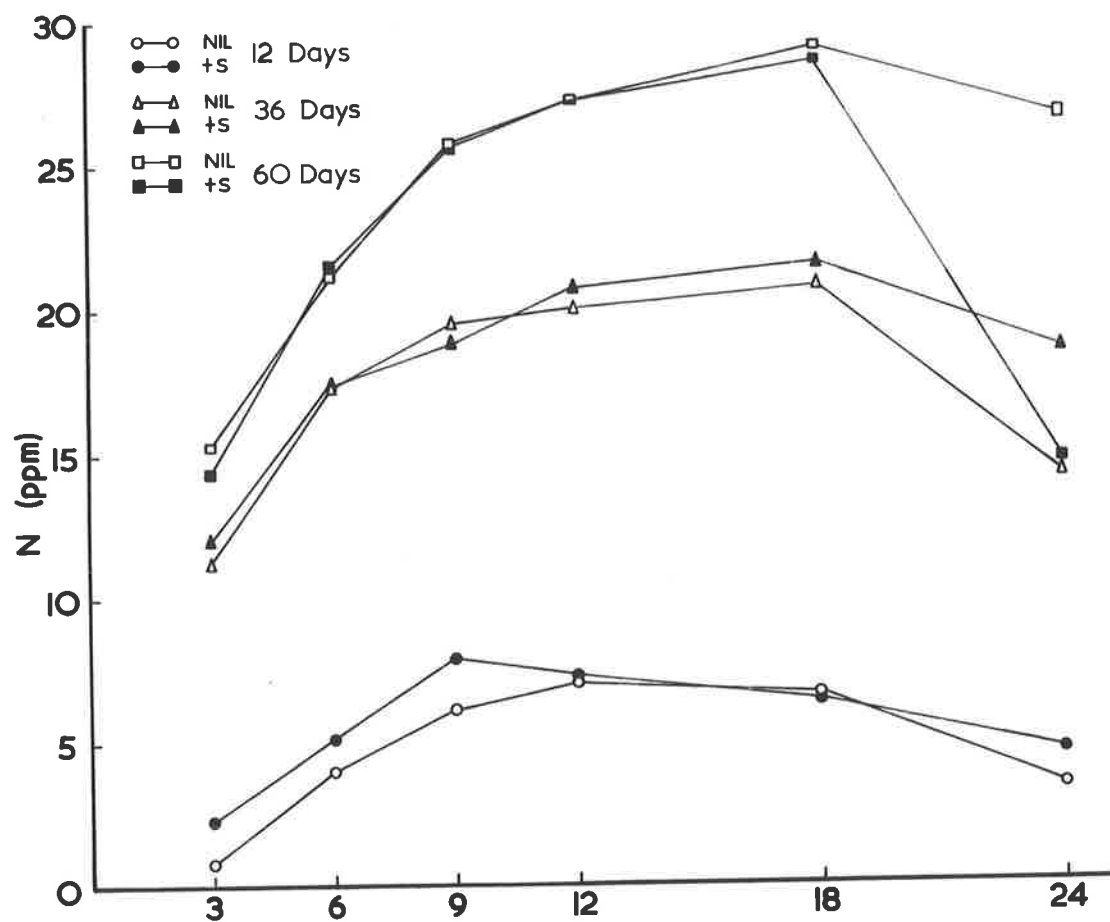
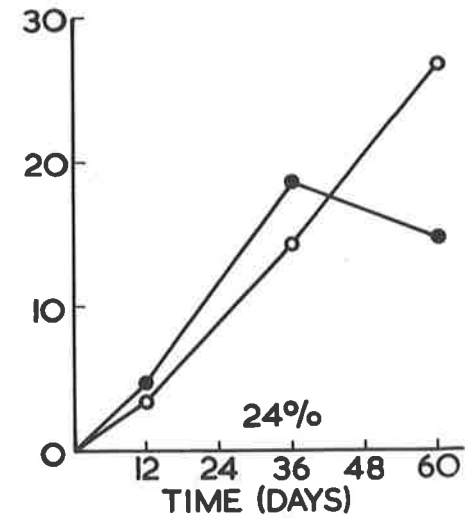
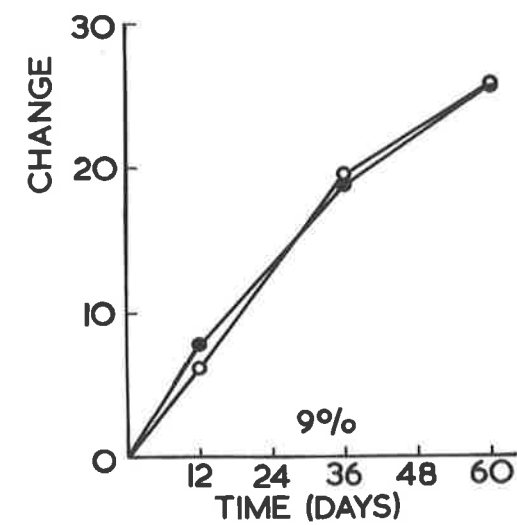
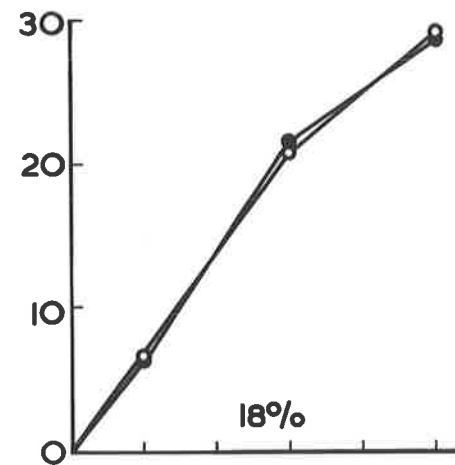
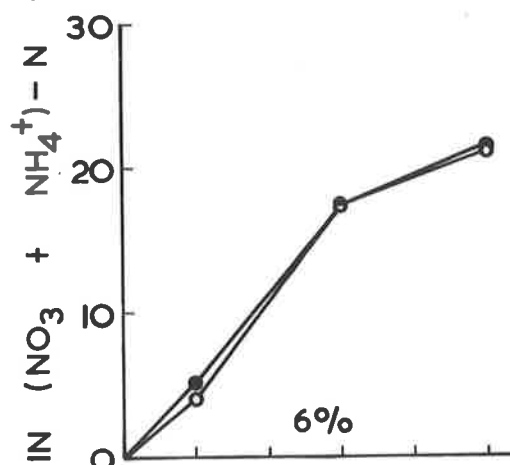
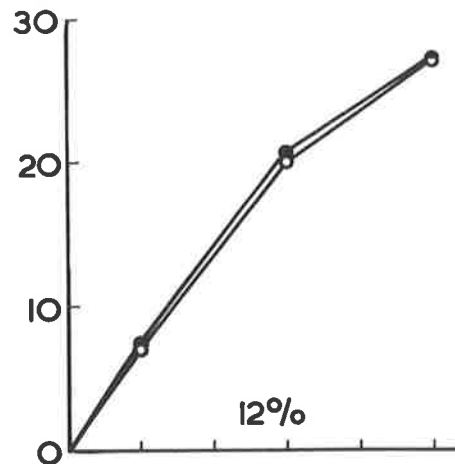
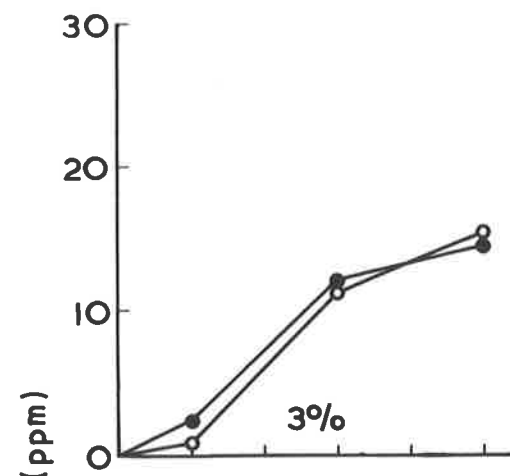


Figure 23

The influence of soil water content and added $\text{SO}_4^{=}\text{-S}$ on net mineralization of N from Laffer Sand incubated at 20°C . The +S treatment received 30 ppm of added $\text{SO}_4^{=}\text{-S}$. All measurements are expressed on an oven dry soil basis.



decrease in mean N mineralization rates between 36 and 60 days. Additions of $\text{SO}_4^{--}\text{-S}$ had no influence on N mineralization at soil water contents up to 18%. At 24% soil water content there was a sharp decrease in N mineralized after 36 days, and during the last 24 day period there was a decline in the mean mineral content of the soil of the +S treatment.

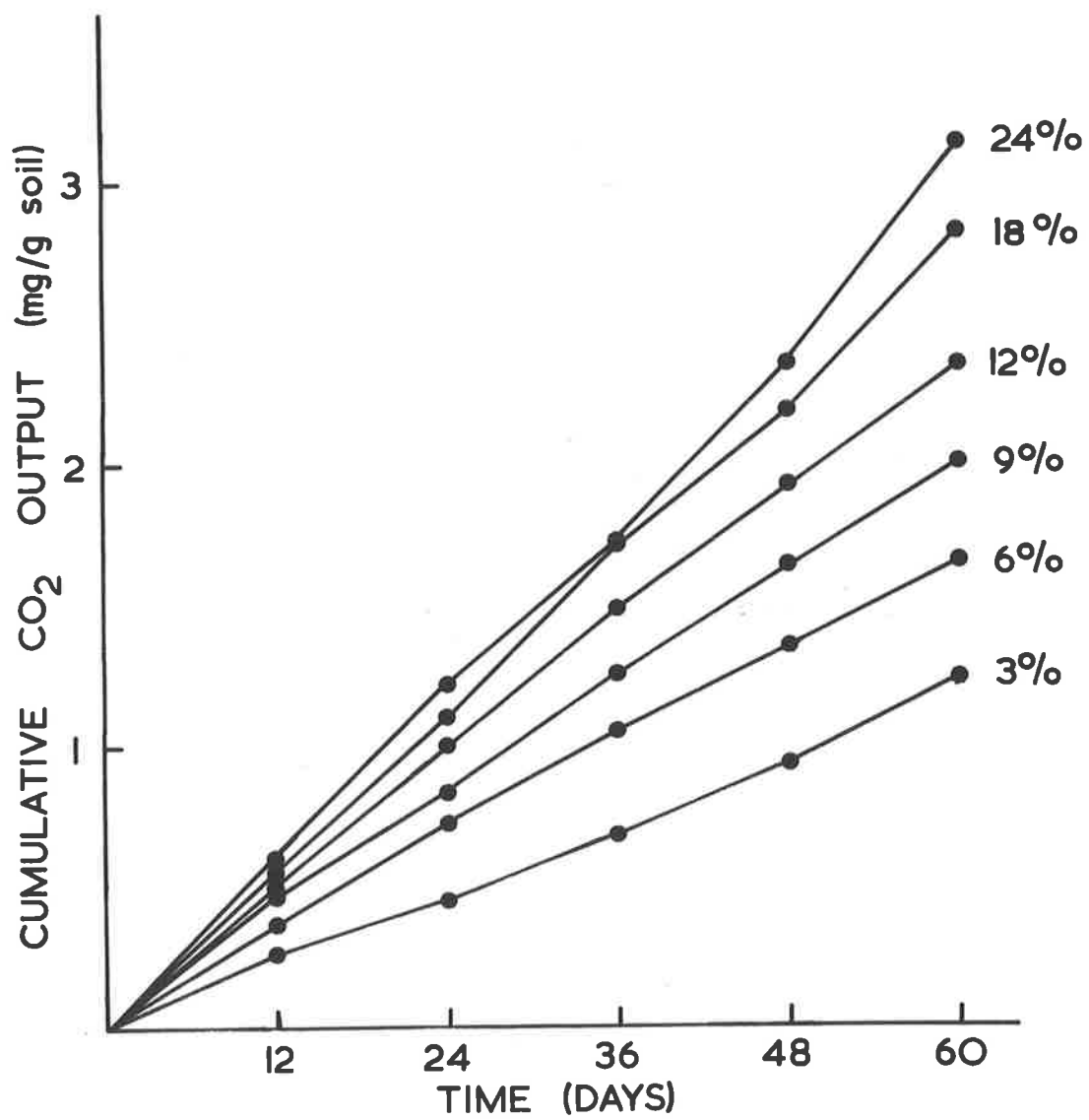
Figure 22 shows that optimum soil water contents for N mineralization occurred at the comparatively high soil water content of 18%. There was, however, very little increase in N mineralization between 12 and 18% soil water content.

(iii) Carbon dioxide release - The amount of CO_2 released depends on the respiration rate of soil micro-organisms, and as such gives a crude index of soil microbial activity. The graph of cumulative CO_2 release in mg per g of soil (Fig. 24) shows that CO_2 output increased with increasing soil water content. Separate values for nil and +S treatments are not shown since there was no significant difference between them.

It can also be seen from Fig. 24 that the rate of CO_2 production at each soil water content remained approximately constant for the duration of the experiment, indicating that microbial activity had reached a steady state.

Figure 24

The evolution of CO_2 from Laffer Sand at different soil water contents during incubation at 20°C . All quantities are expressed on an oven dry basis.



(h) Discussion

The constant rate of CO_2 evolution observed during the experiment differs markedly from the effects observed when fresh organic residues with large C:N or C:S ratios are added to soils. Constant rates of CO_2 emission show that the soil microbial population is in a state of equilibrium and that relatively stable ratios of C:N:S exist in the soil organic matter, as one would expect to find in a field soil which has been under pasture for a long period of time. Under permanent leguminous pasture small additions of organic matter occur continuously with shedding of leaves and the decay of senescent roots. Such a gradual accumulation of organic matter of relatively high N content would presumably give rise to a steady state of microbial activity.

Increased additions of organic matter following senescence and desiccation in summer would occur, resulting in a flush of mineralization following the autumn rains. During the winter growing period, when leaching intensities are high, (as shown by the field experiment) organic matter decomposition would be likely to occur at steady rates similar to those measured in this experiment. However, rates of mineralization in the field will differ due to lower soil temperatures and the effect of plant stimulation. Leaching, by continually removing soluble breakdown products, may also influence the rate

of mineralization and if excessive may remove newly released $\text{SO}_4^{2-}\text{-S}$ before uptake by roots can occur.

The soil moisture determinations made during the field experiment showed that for most of the normal growing period soil water contents remain between 9% and 15%, and as such would not impose drastic limitations on soil microbial activity. From this experiment, it seems that the rate of S-mineralization likely to be encountered under developed pastures would be of the order of 3% of total S in 60 days. Since the growing period lasts a little more than six months, 9% or 10 ppm of total S from a soil containing 120 ppm of S might be released.

4. Incubation Experiment II

The Influence of Soil Temperature and Leaching on Net S Mineralization

(a) Introduction

Conditions simulated in the previous incubation experiment were representative of a soil held at a constant water content without the influence of leaching normally experienced in the field. It was thought that removal of soluble end products of microbial activity by leaching might enhance the rate of net S mineralization. A further incubation experiment was carried out to test this theory.

(b) Soil and Soil Preparation

A sample from the same batch of soil used for pot experiment I and the previous incubation experiment was passed through a 2 mm sieve, and 75 g sub-samples of the sieved material were placed in plastic incubation vessels. Unlike the previous experiments the soil was not leached and air dried before being placed in the incubation vessels.

(c) Leaching Apparatus

The incubation vessels were made by drilling a number of 1.0 mm diameter holes in the bottom of polystyrene vials. "Porvic" filter discs were cut to fit the inside of the vials and were placed over

the holes in the bottom. The vials were fitted with polyethylene screw caps.

The vessels were designed to permit leaching of the soil to be carried out without disturbing the samples. Initial leaching was carried out by free drainage followed by removal of excess water by suction. To accomplish this process, a series of vacuum trap vessels were made using glass preserving jars of approximately 1 kg capacity.

A hole was cut in the tinfoil inner seal lid of each jar, and a ring, made from copper tubing of suitable size to hold a vial fitted with a Gooch grommet was soldered to the seal to surround the hole. A piece of 5 mm copper tube to enable evacuation was also passed through the seal and soldered in place. To collect the leachate, a 100 ml polyethylene bottle fitted with a small funnel was placed in the preserving jar below the vial. Figure 25 shows a soil sample on the assembly, ready for evacuation following initial free drainage.

(d) Design of Experiment

The experiment was to compare S-mineralization from unleached and intermittently leached samples of the same soil. At the same time, the effect of temperature of incubation was also studied using

138.

Figure 25

Apparatus for leaching soil samples without disturbing
them during incubation experiments.



a multi range incubator.

The following treatments were imposed:

- (i) Leaching - Unleached and leached every 10 days
- (ii) Temperatures - 8.5, 12.5, 16.5, 21.0, 24.5, 29.0 and 32.5°C.

Duplicate samples of leached and unleached treatments were incubated at each temperature in a multi-range incubator. The incubator was housed in a heated room where the temperature remained about 20°C during the experiment. Variations in room temperature above 20°C caused increases of up to 1.5°C above the desired incubator temperatures. The values quoted for temperature treatments are therefore nominal, but there was sufficient interval between each temperature to prevent overlapping of treatments even on the hottest of days. For most of the time the temperatures were maintained to within 0.5°C of the desired value.

(e) Conduct of Experiment

A preliminary test revealed, that after three successive leachings the $\text{SO}_4^{=}$ content of the leachate fell to barely detectable levels. The test was conducted using four 75 g (O.D. basis) samples of soil similar to those used for the main experiment. Each sample was leached with four 75 ml aliquots of de-ionized

water and the results of the test are shown in Table 16.

TABLE 16

Sample No.	$\text{SO}_4^{=}\text{-S}$ Removed (ppm)			
	1st Leaching	2nd Leaching	3rd Leaching	4th Leaching
1	6.7	1.1	1.3	0
2	6.0	0.9	0.7	0
3	7.6	1.1	0.4	0.2
4	6.1	0.5	0.2	0

To ensure that all samples used for the main experiment contained a uniformly low base level of $\text{SO}_4^{=}\text{-S}$, they were leached with three consecutive 75 ml leachings. The soil in each vial was then brought to a gravimetric water content of 12% by addition of de-ionized water and all vials were placed in the incubator.

The incubation period commenced on 14th October 1967 and the experiment was terminated on 23rd December 1967 after eight 10-day sampling intervals.

At each sampling, the vials of the leached treatment were leached with 75 ml of de-ionized water. After leaching, the soils were restored to the correct gravimetric water content and replaced in the incubator. The process took about two hours to complete. The

vials were weighed before and after leaching and the volume of leachate calculated by difference.

Daily checks were made on soil water content and additions were made as necessary. To reduce evaporation losses the lids were loosely fitted to the vials, and were removed once daily to permit aeration.

When the experiment was terminated, the soils of the leached and unleached treatments were all leached with 3 x 75 ml aliquots of de-ionized water to ensure an almost complete removal of $\text{SO}_4^{=}\text{-S}$.

(f) Chemical Analyses.

The $\text{SO}_4^{=}\text{-S}$ contents of the leachates were determined by the methylene blue method (described in Section III A2(h)) and results were expressed as $\text{SO}_4^{=}\text{-S}$ leached in ppm of oven dry soil.

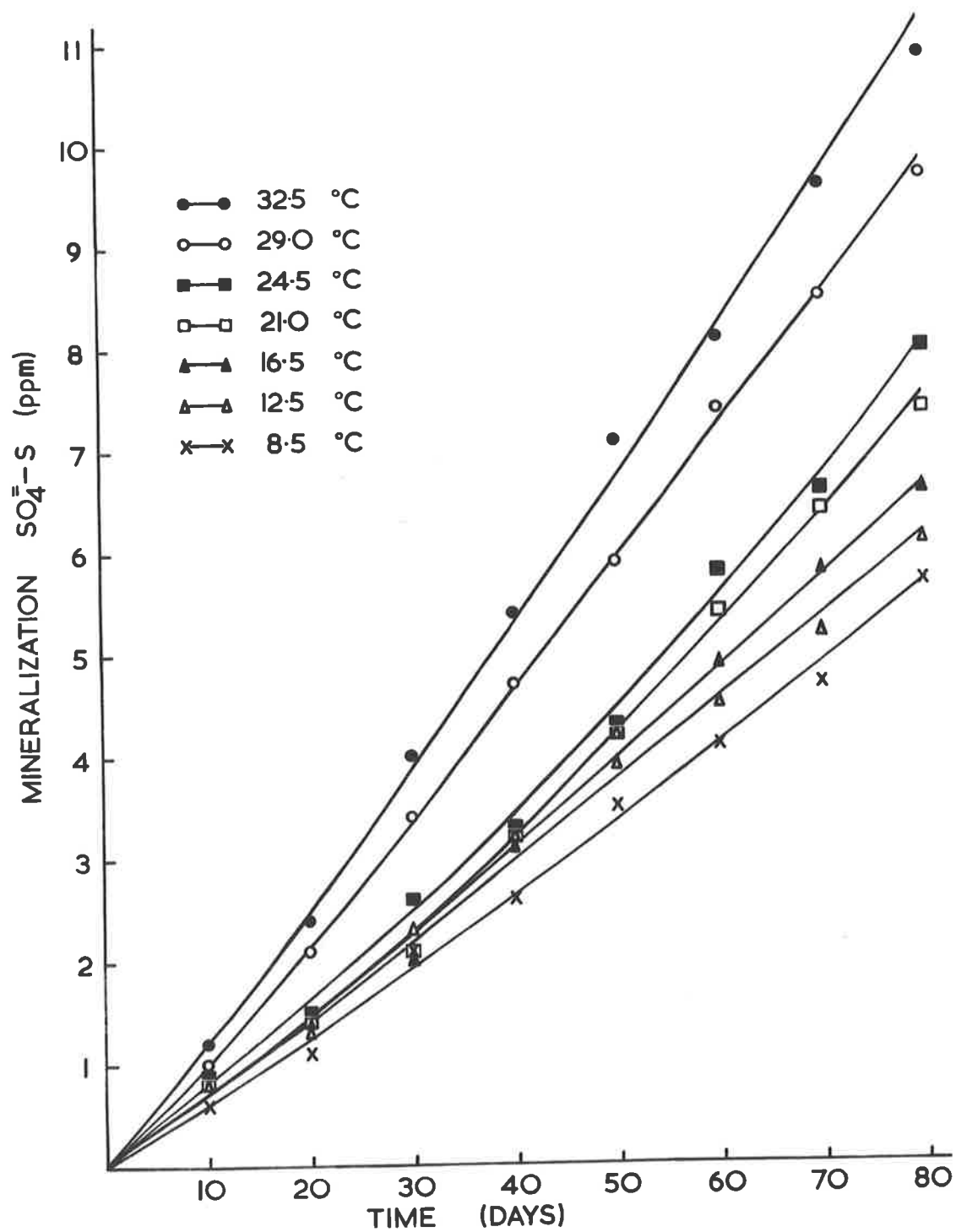
(g) Results

(i) Release of $\text{SO}_4^{=}\text{-S}$ from leached soil - Apart from the variable values obtained for release of $\text{SO}_4^{=}\text{-S}$ at the early samplings, a clear-cut picture of cumulative S-mineralization was obtained (Fig. 26). The graph indicates that a slight lag phase occurred during the first sampling interval after which a uniform release of $\text{SO}_4^{=}\text{-S}$

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Figure 26

The influence of soil temperature on $\text{SO}_4^{=}\text{-S}$ extracted
from Laffer Sand by intermittent leaching.



took place. An increase in temperature from 8.5°C to 32.5°C approximately doubled the $\text{SO}_4^{=}\text{-S}$ output in 80 days.

(ii) Release of $\text{SO}_4^{=}\text{-S}$ from unleached soil - Total $\text{SO}_4^{=}\text{-S}$, leached from soils of the unleached treatment by three successive leachings at the end of the incubation period, was lower than that obtained from soil by regular leaching throughout the incubation period (Fig. 27). A difference in total $\text{SO}_4^{=}\text{-S}$ removed between leached and unleached soils was present at all temperatures used. Values for total $\text{SO}_4^{=}\text{-S}$ removed from the leached soil, shown in Figure 27, include the additional quantity removed in the second and third successive leachings made at the final sampling.

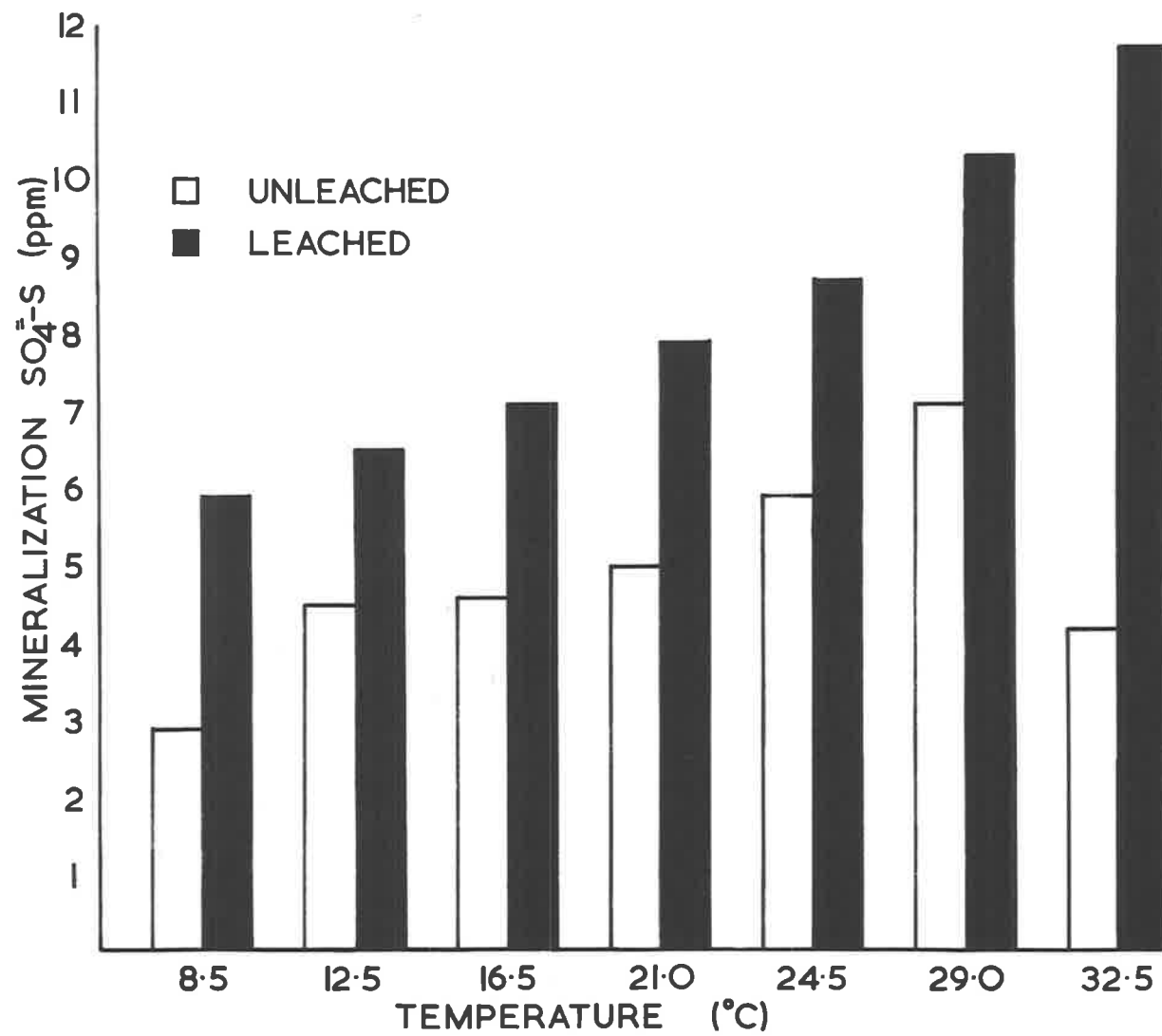
The fall in $\text{SO}_4^{=}\text{-S}$ removed from the unleached samples at 32.5°C is an unexplained feature of the pattern of S mineralization in the experiment. Although the results obtained from the unleached soils were more variable than those from the leached soils, the increase in S-mineralization with temperature was roughly parallel up to the 29°C temperature treatment.

(h) Discussion

(i) Leaching effects - It is clear from this experiment that periodic leaching enhanced the release of $\text{SO}_4^{=}\text{-S}$ from soil organic matter. Many reasons could be advanced for this enhancement.

Figure 27

A comparison of $\text{SO}_4^{=}\text{-S}$ removed from intermittently leached and unleached samples of Laffer Sand incubated at various temperatures for 80 days.



Removal of toxic end products may have helped to maintain soil microbial activity at a higher level in the leached soil. Periodical leaching and draining would have replaced soil air favouring the development of aerobic organisms; and the population of anaerobic organisms capable of reducing S compounds may have been higher in the unleached soils.

The depth of soil in the vials was 5 cm with a surface area of 12.5 cm. In the field an equivalent leaching intensity on the same depth of soil would have required the application of 60 mm of water to an already moist soil, no allowance being made for evapotranspiration or retention of water in the leaching zone. Even in the wettest years it is doubtful whether such a leaching intensity would occur in areas of Laffer Sand on as many as eight occasions during the growing period. However, evidence from the field experiment indicated that at certain times substantial leaching of $\text{SO}_4^{=}\text{-S}$ did occur, and although the effect may not have been as drastic as that in the present study, leaching could increase the amount of S mineralization. Such stimulation of mineralization would be of limited value to surface rooted annuals, including subterranean clover, due to leaching of $\text{SO}_4^{=}\text{-S}$ so released below the root zone.

(ii) Soil temperature - The most clear cut influence of temperature on net S mineralization so far observed in the project was seen in

this experiment. There was an increase in $\text{SO}_4^{=}\text{-S}$ release with each increase in temperature, but an unusual feature was that the increases became larger at higher temperatures. Williams (1967) showed that the extractable $\text{SO}_4^{=}\text{-S}$ removed by either KH_2PO_4 or CaCl_2 was increased by approximately 50% by extracting a soil after vacuum drying at 35°C compared with extraction after air drying at 20°C . Increasing temperatures of incubation may increase the release of more labile forms of organic S besides increasing microbial activity, and this could be one explanation for the larger increases in $\text{SO}_4^{=}\text{-S}$ output at higher temperatures.

Variations in soil temperatures within the range recorded in the field during the growing period of subterranean clover (i.e. $10\text{--}15^\circ\text{C}$) had a small effect on $\text{SO}_4^{=}\text{-S}$ released from both the leached and unleached soils. In summer soil temperatures as high as 25 to 30°C are recorded, and Williams (1967) has shown that such conditions cause increased S-mineralization which may enhance S supply for autumn sown pastures.

The low recovery of $\text{SO}_4^{=}\text{-S}$ from the unleached samples at 32.5°C may have been caused by two factors. Firstly, increased release of $\text{SO}_4^{=}\text{-S}$ at the high soil temperature may have provided a high concentration of substrate for S-reducing micro-organisms.

Secondly, because leaching was not carried out, poor aeration may have quickly resulted due to increased microbial activity at high temperature thus providing ideal conditions for sulphate reduction and volatilization. An objection to the theory, is that this was the only soil of the series to break the trend of increasing SO_4^{2-} -S release and a more gradual effect might be expected.

C. The Influence of Pasture Development on Soil/Plant Sulphur
Balance

Pot Experiment II

1. Materials and Methods

(a) Introduction

The experiments previously described were conducted using soil from the site of the field trial and of similar organic matter content. Barrow (1960c) has shown that the quantity and chemical composition of the organic matter present in soil affects the mineralization of S. Fertilizer and pasture history affect the total accumulation and quality of soil organic matter (Williams and Donald 1957); and so the final major experiment of this project was designed to examine the influence of topdressing history and resultant organic matter accumulation on potential S-mineralization.

Subterranean clover was again used as the test plant. Use of a nodulated legume avoided the need for N applications which would have interfered with stable C/N ratios in the soil organic matter. A wider range of soil temperatures (10-30°C) was used to cover the extremes used previously by various workers in incubation experiments. Incubation experiment II showed that $\text{SO}_4^{=}\text{-S}$ accumulation doubled over this range.

(b) Soils and Soil Preparation

Samples of the top 10 cm of six soils of known pasture history were collected from profiles of Laffer Sand (Taylor 1933) near Keith, South Australia. All the soils, with the exception of the virgin samples, had been under subterranean clover pasture from the time of development and had received regular annual or biennial applications of superphosphate. The developmental history and approximate total superphosphate applications, together with relevant chemical details of each soil are shown in Table 17.

All soils, except No. 5, carried subterranean clover dominant pastures. The pasture on soil 5 was sparse and contained much capeweed (Cryptostemma calendula, L.). This soil had been under pasture for longer than any other in the series and showed the characteristic symptoms of water repellent sands (Bond 1968) frequently encountered beneath older degenerate pastures.

The soil samples were collected in September 1967. The water content of all samples was low (between 3% and 7%). The general appearance of the pasture on all soils was poor, following the driest winter ever recorded in the area. Pastures had been heavily grazed due to shortage of feed caused by the drought.

TABLE 17

History and Properties of Soils - Pot Experiment II

Soil No.	1	2	3	4	5	6
Years of Pasture	0	1	2	3	27	10
Total Superphosphate (kg/ha)	0	270	405	540	4,000	1,450
Organic C (%)	0.54	1.24	0.90	1.46	1.02	1.50
Kjeldahl N (%)	0.022	0.035	0.052	0.098	0.072	0.094
Total S (ppm)	30	38	55	116	95	139
C:N	24.5	35.4	17.3	14.9	14.2	16.0
C:S	180	323	164	126	107	108
N:S	7.3	9.2	9.5	8.4	7.6	6.8

TABLE 18

Basal Fertilizer Solution and Equivalent Application Rates(50 ml added per 1 kg of soil)

Substance	Concentration (Solution)	Concentration of element in Soil (ppm)	$\text{SO}_4^{2-}\text{-S}$ (ppm)
KH_2PO_4	3.514 g/l	P = 40	-
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	49.9 mg/l	Cu = 0.64	0.32
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	100.0 mg/l	Zn = 1.14	0.56
$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$	2.53 mg/l	Mo = 0.05	-
$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	0.26 mg/l	Co = 0.013	-

Samples were air dried and sieved through a 1.0 cm screen to remove coarse organic matter such as woody roots. All samples were leached with de-ionized water. Leaching was carried out by placing 10 kg samples of soil in 10 l plastic buckets. Gauze-covered holes in the bottoms of the buckets allowed the water to drain through without losing the soil. Each 10 kg sample was leached with 30 l of water. The wet soil was then spread on plastic sheets on a cement floor and air dried to approximately 6-7% water content.

(c) Design of Experiment

- (i) Soils:- 6 soils of differing pasture history (Table 17).
- (ii) Fertilizer Treatments:- Nil and 40 ppm of S applied as sodium sulphate (NaSO_4).
- (iii) Soil Temperature Treatments:- 10°C , 17°C , 24°C and 30°C .

Four replicates were provided within each temperature treatment making a total of 192 pots for the experiment.

(d) Potting and Fertilizer Application

The water content of each soil was determined by oven drying subsamples. As each pot was prepared the soil water content was brought to 12% by the addition of fertilizer solution plus de-ionized water. Appropriate S applications were included in

fertilizer solutions which contained P and a basal mixture of trace elements (Table 18). Trace elements were added in this experiment because the virgin soil had not received any previous fertilizer application. The trace element mixture was made using sulphates of copper and zinc, so a small basal application of S (0.9 ppm) was made to each soil. All base values of soil S were corrected for this addition.

The pots used in the experiment were the same as those used for pot experiment I and were described in Section IIIB 2(c). Samples of soil (1 kg O.D. basis) were hand mixed with 50 ml of appropriate fertilizer solution and addition de-ionized water. The soil in the pots was compacted by gently tapping them on the concrete floor until the bulk density was approximately 1.3 g/cc.

1

When all soils were potted ten seedlings of subterranean clover (Trifolium subterraneum, L. cv. Bacchus Marsh) were sown in each pot. The seedlings had been germinated on a wire mesh screen in a bowl of de-ionized water. A suspension of an appropriate strain of Rhizobium trifolii had been added to the water to ensure satisfactory inoculation. The sown pots were then placed in controlled temperature tanks.

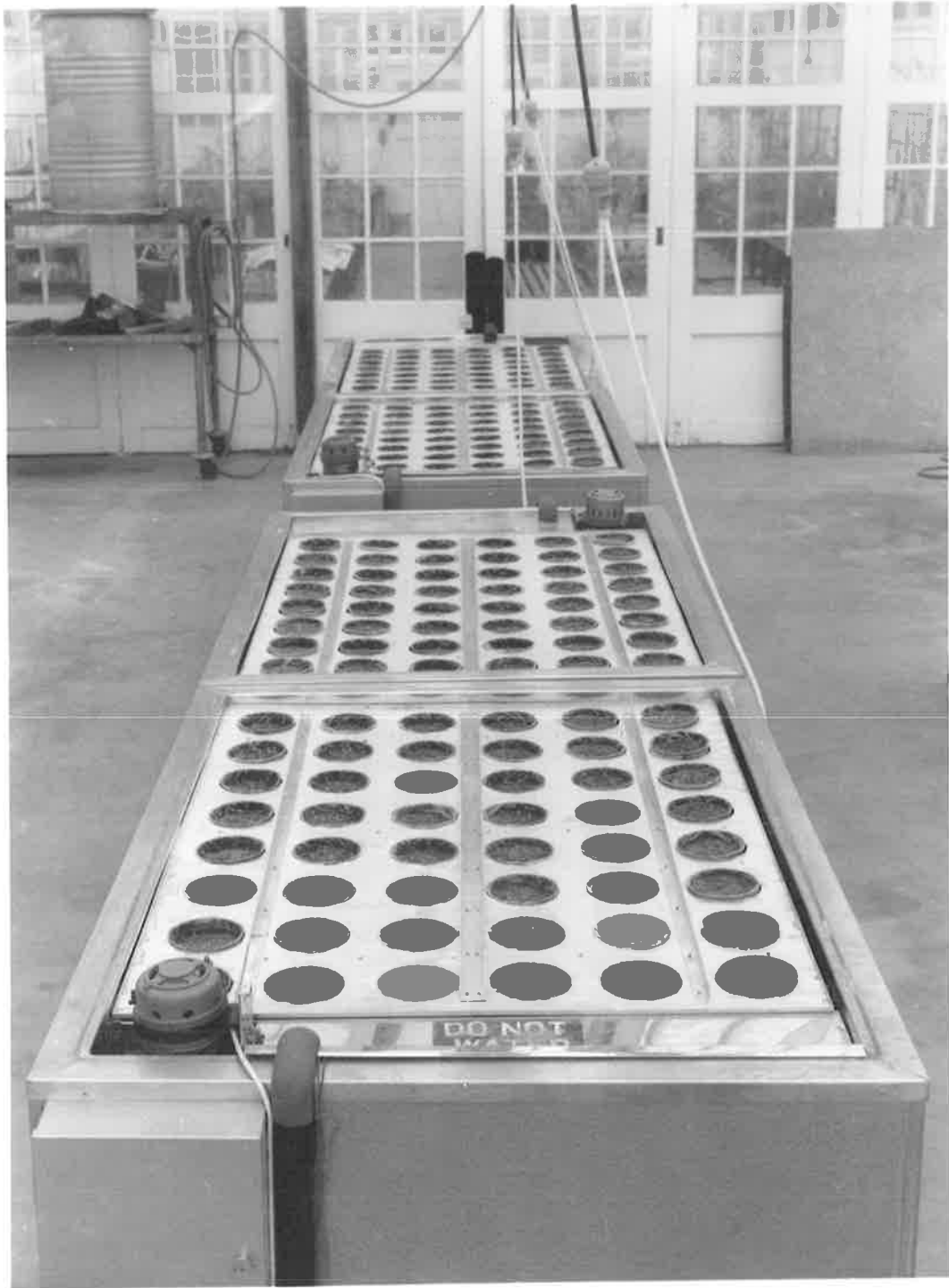
(e) Temperature Tanks

Tanks of a new and improved design were used for this experiment (Figure 28). The internal lining was of stainless steel surrounded by insulating material in an external steel cabinet. Each tank was self contained and was equipped with a $\frac{1}{2}$ h.p. refrigeration unit located underneath. Heating was provided by reverse cycle refrigeration thereby eliminating the need for separate heating elements. A narrow compartment at one end of each tank housed the refrigeration coil and water was circulated through this compartment and the main tank by an electric pump.

Each tank was fitted with a steel frame to support the pots. The frame was adjusted so that the tops of the pots were level with the rim of the tank. The surface of the water in the tank was at the same height as the soil surface in the pots. Thin aluminium covers, with holes to admit the pots, covered the water in each tank to limit evaporation and heat transfer. The tanks were located along the centre-line of an open-sided glasshouse. Tank temperatures were maintained within $\pm 1^{\circ}\text{C}$ of the desired value. Pot temperatures were within 1°C of the tank temperatures.

Figure 28

Pot experiment II - The arrangement of the temperature tanks within the glasshouse, showing the pots immediately after sowing. The circulating pump, control box, and refrigeration coil compartment, are seen at the end of the tank in the foreground nearest to the camera.



(f) Management

The pots were sown and placed in their respective temperature treatments on 18th Nov. 1967. A few plants did not survive transplanting and were replaced from a fresh supply of germinated seedlings, maintained for several days after the pots were placed in the tanks. By the end of the first week all pots contained 10 successfully established seedlings. The pots were watered daily by adding de-ionized water to bring them to a constant weight corresponding to a gravimetric soil water content of 12%.

To minimize positional effects between and within tanks, rerandomization was carried out at ten day intervals. Each temperature was allocated to another tank and the pots were removed from the tanks while the temperatures were changed. Changeover took about five hours to complete. Positions of replicates were also changed.

During the experiment two infestations of aphids occurred and all pots were taken outside the glasshouse and sprayed with 'Shell' 'Phosdrin', a S-free systemic insecticide of short residual effect (2-3 days). On each occasion a complete kill of aphids was achieved.

(g) Harvests

To ensure depletion of the $\text{SO}_4^{=}\text{-S}$ supply of the soils it was decided to make two harvests. The first harvest was made by removing the clover tops at the soil surface after which regrowth was allowed to occur. The second was made at the conclusion of the experiment when roots were also harvested.

Harvest I was made on the 26th December 1967 after 39 days of growth. At this stage the canopies of the plants at 24°C were beginning to overlap causing mutual shading (Figure 29). After cutting, the tops were placed in "manilla" envelopes and dried at 60°C . The dried material was ground in a laboratory hammer mill.

Harvest II, the final harvest, was made on 31st January 1968 after a further 35 days of regrowth. Mutual shading had again become a problem. Cutting of tops took a day to complete and root harvests a further seven days.

To minimize damage to roots and to prevent regrowth of tops while harvesting was in progress, the pots awaiting root separation were covered with aluminium discs and kept in the tanks at a temperature of 4°C . The soil from each pot was carefully dismantled by hand and the major parts of the root system removed intact.

Figure 29

Pot experiment II - showing plants immediately before harvesting. Note that while canopies of subterranean clover plants at high soil temperatures (24°C in foreground) were mutually shading each other, those from plants grown at low soil temperatures (10°C in second tank from rear) were still completely separated.



Remaining roots were separated by dry sieving, and the roots were rinsed by placing them on a sieve and dipping them gently in clean de-ionized water. Washed root material was allowed to dry on sheets of blotting paper before being placed in "manilla" envelopes and oven dried at 60°C. After oven drying, tops and roots were weighed to determine dry matter yield and were ground for analysis in a laboratory hammer mill.

The soil samples from which the roots had been separated were placed in plastic bags and stored moist at 1-2°C in a refrigerator.

(h) Chemical Analyses

Plant S uptake and soil S determinations were made using the methods described for pot experiment I. Due to the large number of samples duplicate extracts were not prepared, and single determinations only were carried out, except where the results were erratic, when repeat determinations were performed. As expected, total S determinations were variable and not as reliable for balance calculations as those of the previous pot experiment.

2. Results

(a) Statistical Analyses

Basic measurements from this experiment were punched on cards and all calculations for derived data and statistical analyses were performed by computer. Statistical tests were carried out by analysis of variance on untransformed data because the logarithmic transformation could not be applied to S balance values of differing sign.

Analyses of variance were carried out on complete sets of data incorporating the treatments of sulphur, soils, and soil temperature. These tests frequently showed significant first order, and sometimes second order interactions. Table 19 lists the quantities analysed and significance levels of factors affecting them. Interpretation of the results was difficult due to the large number of significant interactions.

The F ratios for S treatments were large relative to those obtained for other treatments. By separating the data into sets for nil and +S treatments many interactions were removed, providing a clearer picture of soil and soil temperature effects. A summary of significance tests on the two sets of data is shown in Table 20.

TABLE 19

Pot Experiment II - Significance of Treatments
Effects from Analyses of Variance Including all Treatments

Quantity Measured	Treatments and Interactions						
	Sulphur (S)	Soils (So)	Temp (T)	SxSo	SxT	SoxT	SxSoxT
Total Dry Matter Yield	***	***	***	NS	***	***	NS
Dry Matter Yield Tops HI	***	***	***	*	***	***	*
Dry Matter Yield Tops HII	***	***	***	NS	***	***	NS
Dry Matter Yield Roots	***	***	***	NS	***	*	NS
% S Content, Tops HI	***	NS	***	**	**	*	NS
% S Content, Tops HII	***	**	***	NS	**	NS	*
% S Content, Roots	***	***	***	***	***	NS	NS
S Uptake, Tops HI	***	***	***	***	***	**	**
S Uptake, Tops HII	***	**	***	NS	***	NS	NS
S Uptake, Roots	***	***	***	***	***	NS	NS
Total S Uptake	***	***	***	***	***	NS	NS
Total $\text{SO}_4^{=}\text{-S}$ Recovery	***	***	***	*	NS	*	NS
Net S Balance	***	NS	***	**	NS	**	NS

*** $P \leq 0.001$ ** $P \leq 0.01$ * $P \leq 0.05$

NS Not significant.

TABLE 20

Pot Experiment II - Significance of TreatmentEffects from Analyses of Variance Excluding Sulphur Treatments

Sulphur Treatment	Nil			+ 40 ppm		
Quantity Measured	Soil	Temp	SoxT	Soil	Temp	SoxT
Total Dry Matter Yield	**	***	**	***	***	**
Dry Matter Yield, Tops HI	***	***	*	***	***	***
Dry Matter Yield, Tops HII	**	*	**	**	***	NS
Dry Matter Yield, Roots	*	NS	NS	**	***	NS
% S Content, Tops HI	**	***	*	*	***	NS
% S Content, Tops HII	***	***	NS	NS	***	*
% S Content, Roots	***	***	NS	***	***	NS
S Uptake, Tops HI	***	***	*	***	***	**
S Uptake, Tops HII	***	***	*	**	***	NS
S Uptake, Roots	***	***	NS	***	***	NS
Total S Uptake	***	***	**	***	***	NS
Total SO ₄ ⁼ -S Recovery	***	***	*	***	***	NS
Change in SO ₄ ⁼ -S	**	***	*	***	***	NS
Total S Recovery	***	*	NS	***	*	NS
Net S Balance	NS	*	NS	NS	*	NS
Change in Soil Organic S	*	**	NS	NS	NS	NS

*** $P \leq 0.001$ ** $P \leq 0.01$ * $P \leq 0.05$

NS Not Significant.

(b) Plant Dry Matter Yields

(i) Total dry matter yield - Total dry matter yields (Fig. 30)

were obtained by summing yields of the three components:

Harvest I tops + Harvest II tops + Roots

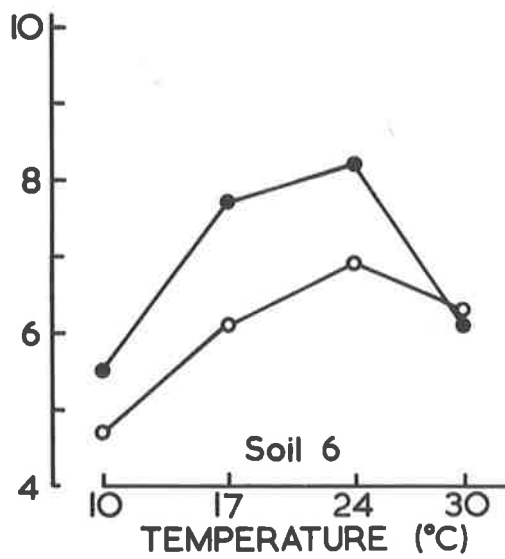
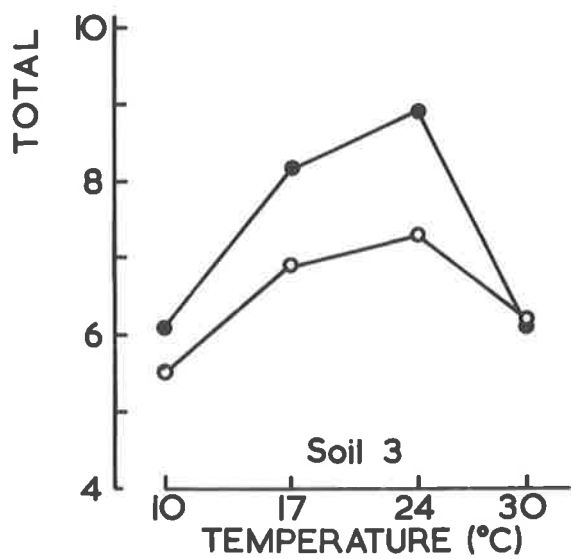
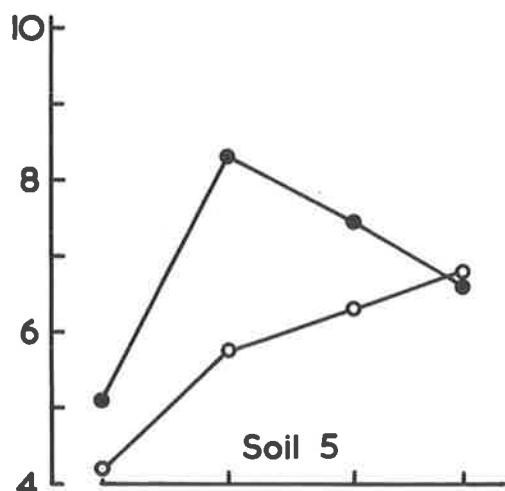
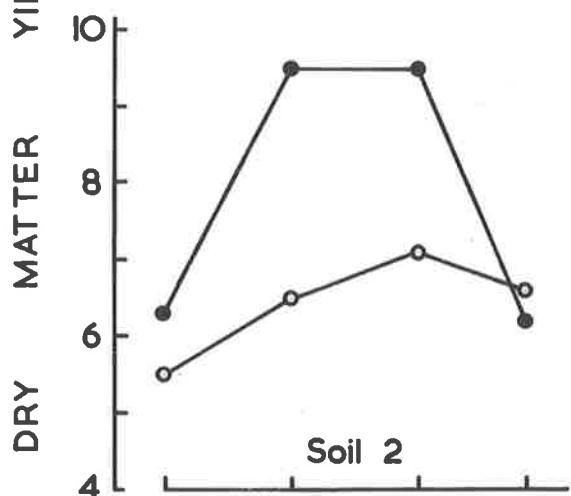
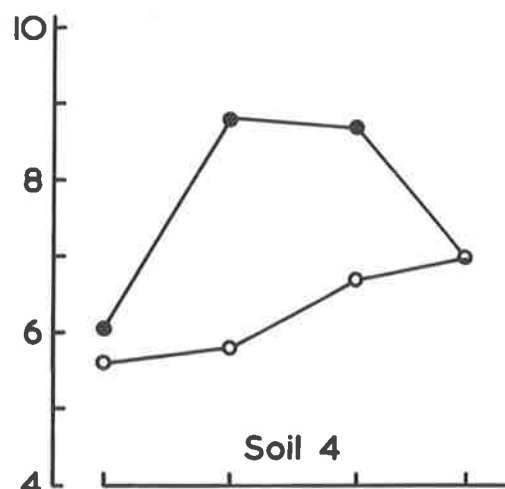
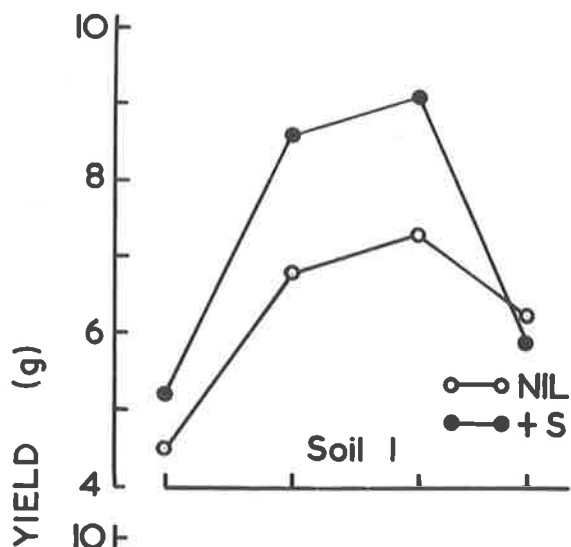
Effects of various factors on each of these components are discussed in parts (ii) to (iv).

Additions of $\text{SO}_4^{=}\text{S}$ significantly ($P \leq 0.001$) influenced yields of total dry matter from all soils, but the strongest effects were observed at soil temperatures of 17 and 24°C. At a soil temperature of 30°C there were no significant differences in total dry matter yield due to added S on any soil. There was a decline in total dry matter yield at a soil temperature of 30°C compared with yield at 24°C and the decline was highly significant ($P \leq 0.001$) on all soils for the +S treatment. A similar but smaller and less significant ($P \leq 0.05$) decline in dry matter yield was observed on soils 1, 2, 3 and 6 of the nil S treatment, but on soils 4 and 5 yields at 24°C and 30°C soil temperatures were similar. At 30°C soil temperature only small differences in dry matter yields were observed between different soils.

(ii) Dry matter yields of tops (harvest I) - At harvest I the differences in dry matter yields due to soil or S treatments were

Figure 30

Pot experiment II - The influence of soil temperature, soil, and sulphate sulphur on total dry matter yields of subterranean clover from harvests I and II (including roots) measured in g/pot.



164.

Figure 31

Pot experiment II - The influence of soil temperature,
soil, and sulphate sulphur on the dry matter yield of
subterranean clover tops at harvest I measured in g/pot.

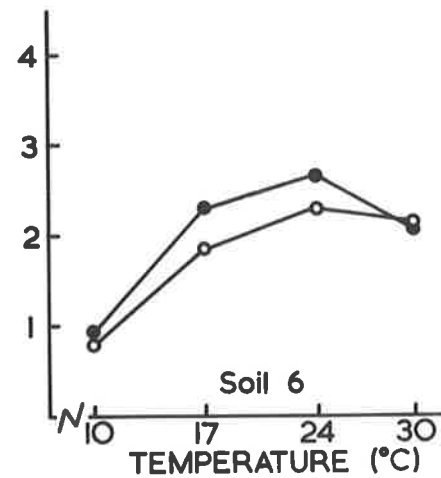
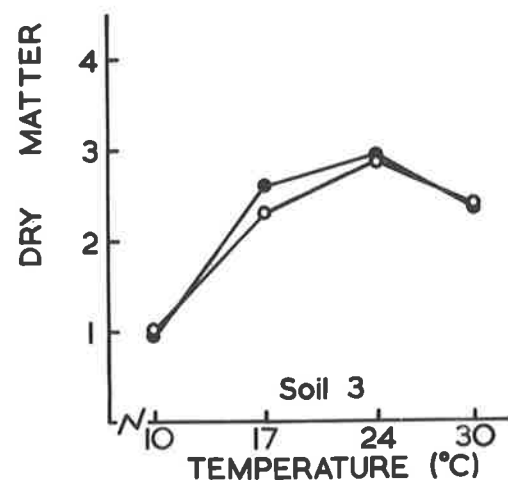
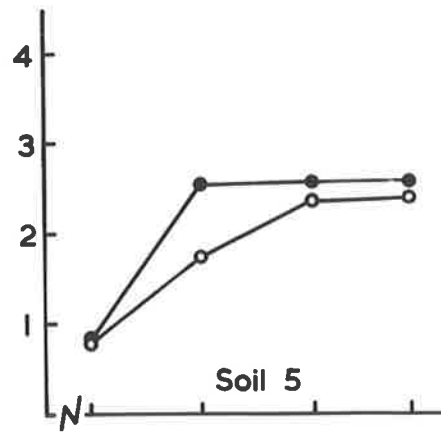
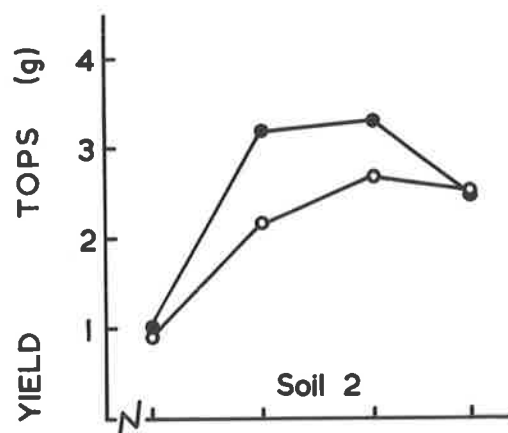
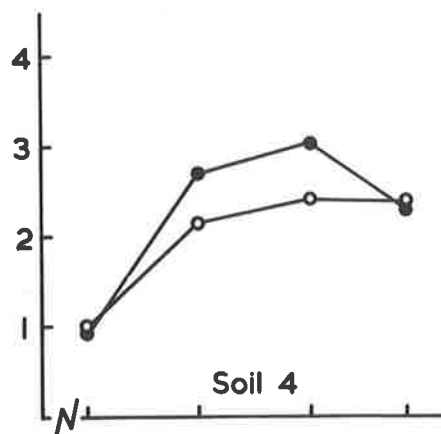
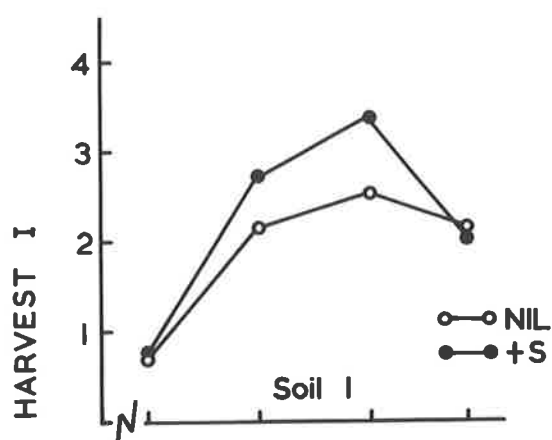


Figure 32

Pot experiment II - The influence of soil temperature,
soil, and sulphate sulphur on the dry matter yield of
subterranean clover tops at harvest II measured in g/pot.

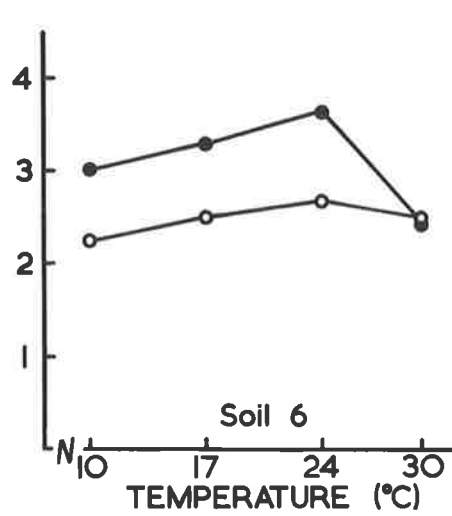
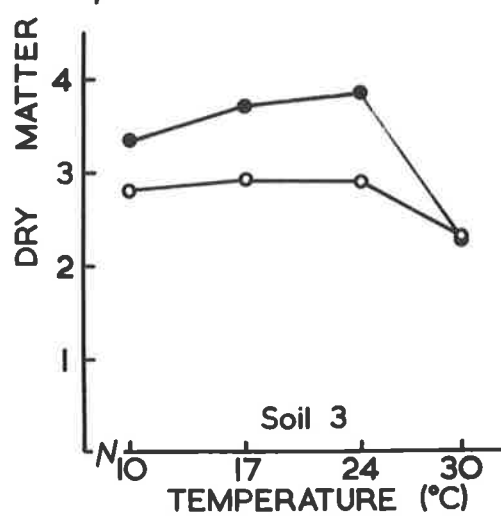
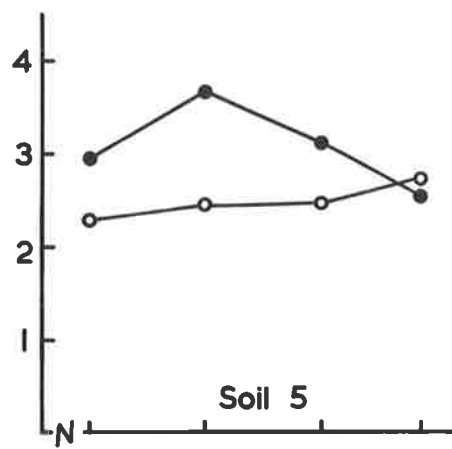
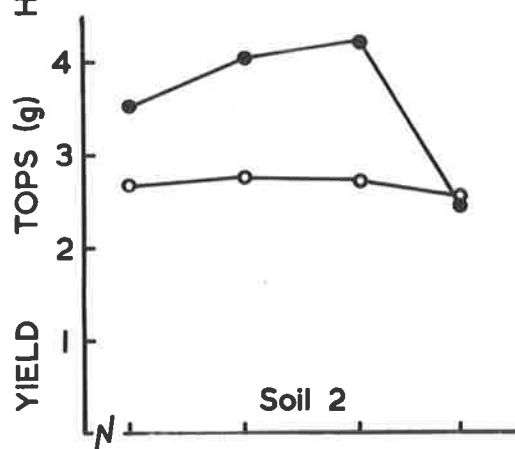
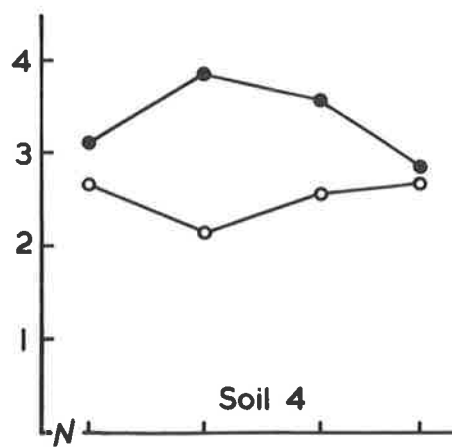
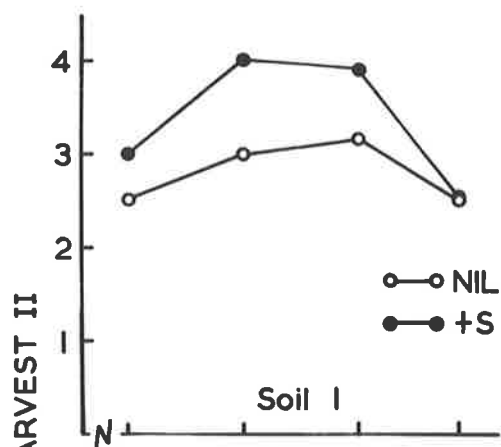
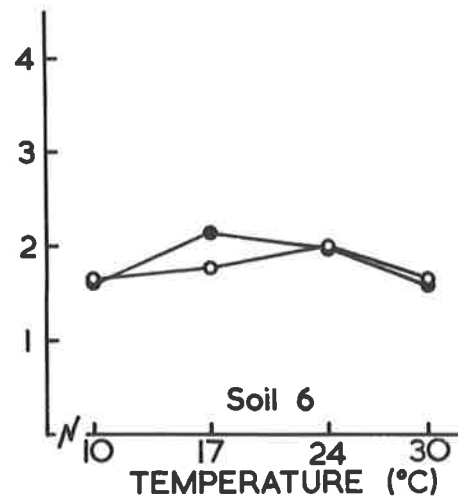
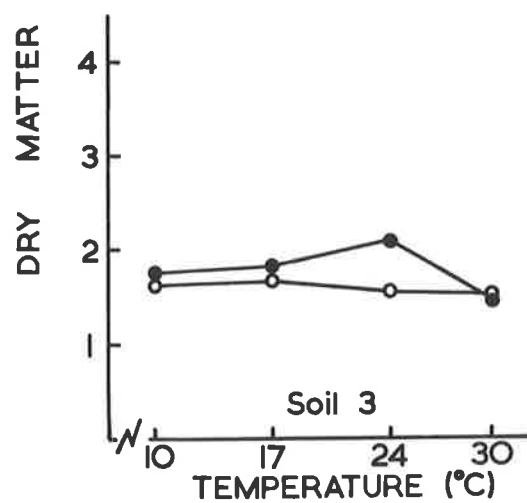
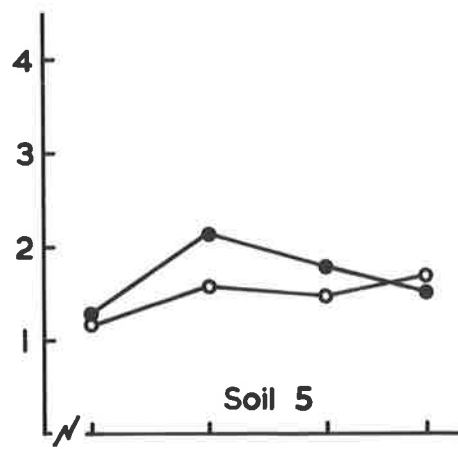
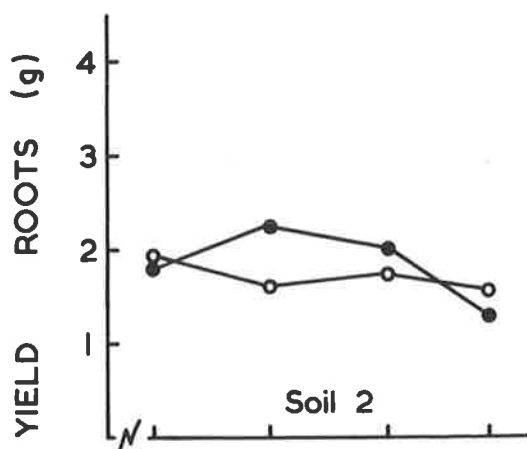
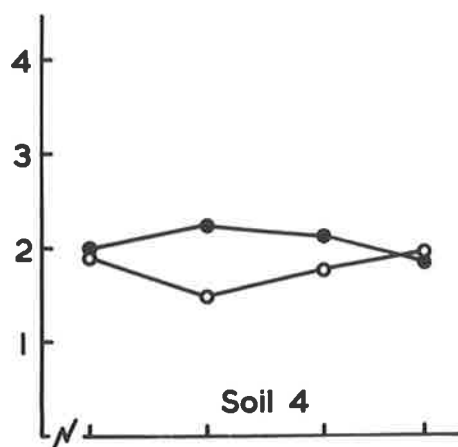
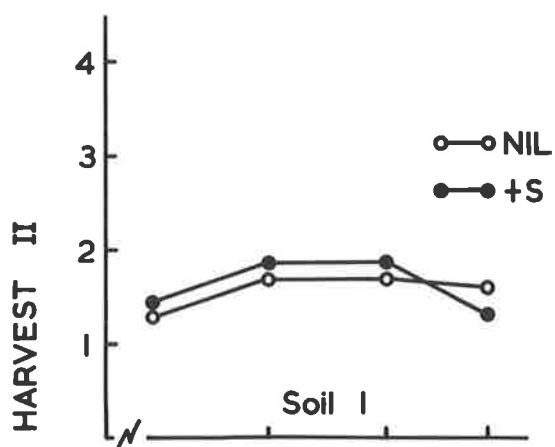


Figure 33

Pot experiment II - The influence of soil temperature, soil, and sulphate sulphur on the dry matter yields of subterranean clover roots at harvest II measured in g/pot.



small or non existent at 10°C and 30°C soil temperatures. However, at 17°C and 24°C variable responses to both soil and S treatments were obtained (Fig. 31). Highest yields at this stage (5 weeks from transplanting) occurred at 24°C ($P \leq 0.001$) but on most soils the best responses to added SO_4^{2-} -S were measured at 17°C. Yields from soils 2, 3 and 4 were better than those from soils 1, 5 and 6 but the significance of the differences varied with both S and soil temperature; hence the significant interactions between these factors (Table 19).

(iii) Dry matter yields of tops (harvest II) - At harvest II responses to added SO_4^{2-} -S were observed at soil temperatures of 10°C, 17°C and 24°C (Fig. 32). The effect of soil temperature on yield was greater in the +S treatment. Highest yields on the +S treatment occurred at soil temperatures of 17°C and 24°C ($P \leq 0.001$). The only significant effects of soil temperature in the nil S treatment were lower yields at 10°C and 30°C compared to 24°C ($P \leq 0.05$). Not all soils followed this general pattern and there was a significant ($P \leq 0.01$) interaction between soil and soil temperature in the nil S treatment.

Soils had a significant effect on plant yields from both S treatments ($P \leq 0.01$). As for harvest I, soils at an early stage of pasture development gave slightly better yields than soils that had been under continuous pasture for longer periods ($P \leq 0.05$ to $P \leq 0.01$).

(iv) Dry matter yields of roots - Root yields for all treatments are shown in figure 33. Soils, soil temperature and S all significantly ($P \leq 0.001$) affected root development but significant interactions between soils and soil temperatures ($P \leq 0.05$) and soils and S ($P \leq 0.001$) occurred. Separate analyses of variance for the two S treatments removed the interactions and revealed that soil was the only significant factor influencing root growth in the nil S treatment. Even so, examination of results showed no definite trend in response; soils 4 and 6 yielded better ($P \leq 0.5$) than soils 1, 3 and 5, while soil 2 gave an intermediate yield not significantly better than any other.

Where $\text{SO}_4^{=}\text{S}$ had been added, soil temperature effects were more pronounced ($P \leq 0.001$), 17 and 24°C being the best for root development. Again soils had a significant ($P \leq 0.01$) effect on root growth, but the trends were still not clear, soil 4 being the only one to yield significantly better than the others.

The roots were not sampled at harvest I, and differences in development at that stage may have disappeared by harvest II. The longer growth period, compared to tops, could have allowed slower growing roots at high and low temperatures to overtake those grown at 17°C and 24°C.

(c) Plant S Measurements

Measurement of % S content and plant uptake were made to enable preparation of S balance sheets and to relate yield responses to factors affecting S supply. The plant S contents indicated the severity of any S-deficiency or the presence of excesses of available S.

(i) Percentage S content of tops (harvest I) - There were highly significant ($P < 0.001$) responses in the ~~mean~~ S contents of tops at harvest I due to S and soil temperature treatments, but soils had a less significant effect. In the +S treatments the ~~mean~~ S content of tops (Table 21B) at a soil temperature of 10°C was higher ($P < 0.001$) than at any other temperature, while minimum S contents occurred at 17°C . On the nil S treatment minimum S contents occurred at soil temperatures of 17°C and 24°C . Increased S contents were measured at both 10°C and 30°C . Soil 3 of the nil S treatment yielded tops with a marginally higher S content than the other soils. The S contents of tops from the nil S treatments (Table 21A) ranged from a low of 0.08% indicative of severe deficiency to a high of 0.19% which should have been almost adequate for plant growth. The corresponding range of values from the +S treatments (Table 21B) was from 0.36% to 0.53% indicating ~~adequate~~ ^{adequate} S supply.

TABLE 21A

S Content of Tops - (Harvest I, Nil S Treatment)*

Soil Temp. (°C)	S content (%)				
	10	17	24	30	Mean
Soil No. 1	0.172	0.107	0.085	0.106	0.118
2	0.130	0.088	0.084	0.139	0.110
3	0.160	0.141	0.111	0.192	0.151
4	0.122	0.104	0.108	0.161	0.124
5	0.158	0.118	0.139	0.126	0.135
6	0.132	0.112	0.138	0.153	0.134
Mean	0.146	0.112	0.111	0.146	

L.S.D. soil temp. means - 5% = 0.014

1% = 0.018

0.1% = 0.024

L.S.D. soil means - 5% = 0.020

1% = 0.026

* All values in table are the means of four replicates.

S Content of Tops -(Harvest I. + S Treatment)*

Soil Temp. (°C)	S Content (%)				
	10	17	24	30	Mean
Soil No. 1	0.385	0.407	0.442	0.528	0.490
2	0.486	0.362	0.406	0.528	0.446
3	0.514	0.362	0.442	0.408	0.432
4	0.486	0.400	0.406	0.419	0.428
5	0.467	0.369	0.468	0.456	0.440
6	0.404	0.360	0.488	0.314	0.391
Mean	0.490	0.377	0.442	0.442	

L.S.D. soil temp. means - 5% = 0.042

1% = 0.056

0.1% = 0.073

L.S.D. soil means - 5% = 0.052

* All values in table are the means of four replicates.

(ii) Percentage S content of tops (harvest II) - At harvest II differences in the % S content of tops (Tables 22A and 22B) due to S, soils, and soil temperature treatments were more clearly defined than at harvest I. In the nil S treatment soils markedly influenced ($P \leq 0.001$) the S content of tops, whereas in the +S treatment soils had no significant effect, due no doubt, to the presence of large quantities of available S.

The S contents of tops from the nil S treatments were indicative of acute S deficiency at 10° , 17° and 24°C . At a soil temperature of 30°C there was a significant ($P \leq 0.001$) increase in the % S content of tops over that measured at 24°C . All tops from the +S treatments were apparently well supplied with S and most mean S contents exceeded 0.2%, the level normally indicative of adequate S supply (Bouma et al. 1969). Significantly higher values at soil temperatures of 24°C ($P \leq 0.05$) and 30°C ($P \leq 0.001$) showed that these increases in soil temperature resulted in "luxury" S uptake.

(iii) Percentage S content of roots - In most cases the % S contents of the roots (Tables 23A & B) were much higher than the corresponding values for the tops. In some samples small particles of organic matter were entwined in the roots and these may have caused contamination. Where top growth was limited by low or high soil temperatures (10°C and 30°C) significantly higher ($P \leq 0.001$) S contents

TABLE 22A

S Content of Tops - Harvest II, Nil S Treatment) *

Soil Temp. (°C)	S Content (%)				
	10	17	24	30	Mean
Soil No. 1	0.074	0.064	0.064	0.098	0.075
2	0.077	0.055	0.058	0.092	0.070
3	0.062	0.044	0.052	0.112	0.068
4	0.097	0.078	0.090	0.155	0.105
5	0.086	0.052	0.097	0.161	0.099
6	0.064	0.071	0.063	0.114	0.077
Mean	0.077	0.061	0.071	0.122	

L.S.D. soil temp. means - 5% = 0.014

1% = 0.019

0.1% = 0.025

L.S.D. soil means - 5% = 0.018

1% = 0.023

0.1% = 0.031

* All values in table are the means of four replicates.

TABLE 22B

S Content of Tops - (Harvest II₃ + S Treatment)*

Soil Temp. (°C)	S Content (%)				
	10	17	24	30	Mean
Soil No. 1	0.243	0.233	0.264	0.362	0.276
2	0.209	0.238	0.194	0.334	0.244
3	0.178	0.217	0.239	0.377	0.253
4	0.247	0.193	0.293	0.252	0.246
5	0.244	0.261	0.325	0.311	0.285
6	0.176	0.229	0.243	0.302	0.238
Mean	0.216	0.228	0.260	0.323	

L.S.D. soil temp. means - 5% = 0.029

1% = 0.039

0.1% = 0.050

* All values in table are the means of four replicates.

TABLE 23A

S Content of Roots - (Harvest II, Nil S Treatment)*

Soil Temp. (°C)	S Content (%)				
	10	17	24	30	Mean
Soil No. 1	0.111	0.086	0.079	0.124	0.100
2	0.074	0.084	0.071	0.113	0.085
3	0.105	0.102	0.092	0.180	0.120
4	0.104	0.123	0.129	0.158	0.128
5	0.141	0.107	0.144	0.176	0.142
6	0.139	0.111	0.115	0.217	0.146
Mean	0.112	0.102	0.105	0.161	

L.S.D. soil temp. means - 5% = 0.017

1% = 0.023

0.1% = 0.030

L.S.D. soil means - 5% = 0.021

1% = 0.028

0.1% = 0.037

* All values in table are the means of four replicates.

TABLE 23B

S Content of Roots - (Harvest II_r + S Treatment)*

Soil Temp (°C)	S Content (%)				
	10	17	24	30	Mean
Soil No. 1	1.011	0.515	0.254	0.551	0.583
2	0.635	0.444	0.421	0.499	0.500
3	0.705	0.660	0.596	0.802	0.691
4	1.170	0.797	0.497	0.818	0.820
5	0.780	0.434	0.567	0.691	0.618
6	0.690	0.474	0.455	0.595	0.554
Mean	0.832	0.554	0.465	0.659	

L.S.D. soil temp. means - 5% = 0.109

1% = 0.145

0.1% = 0.189

L.S.D. soil means - 5% = 0.134

1% = 0.178

0.1% = 0.231

* All values in table are the means of four replicates.

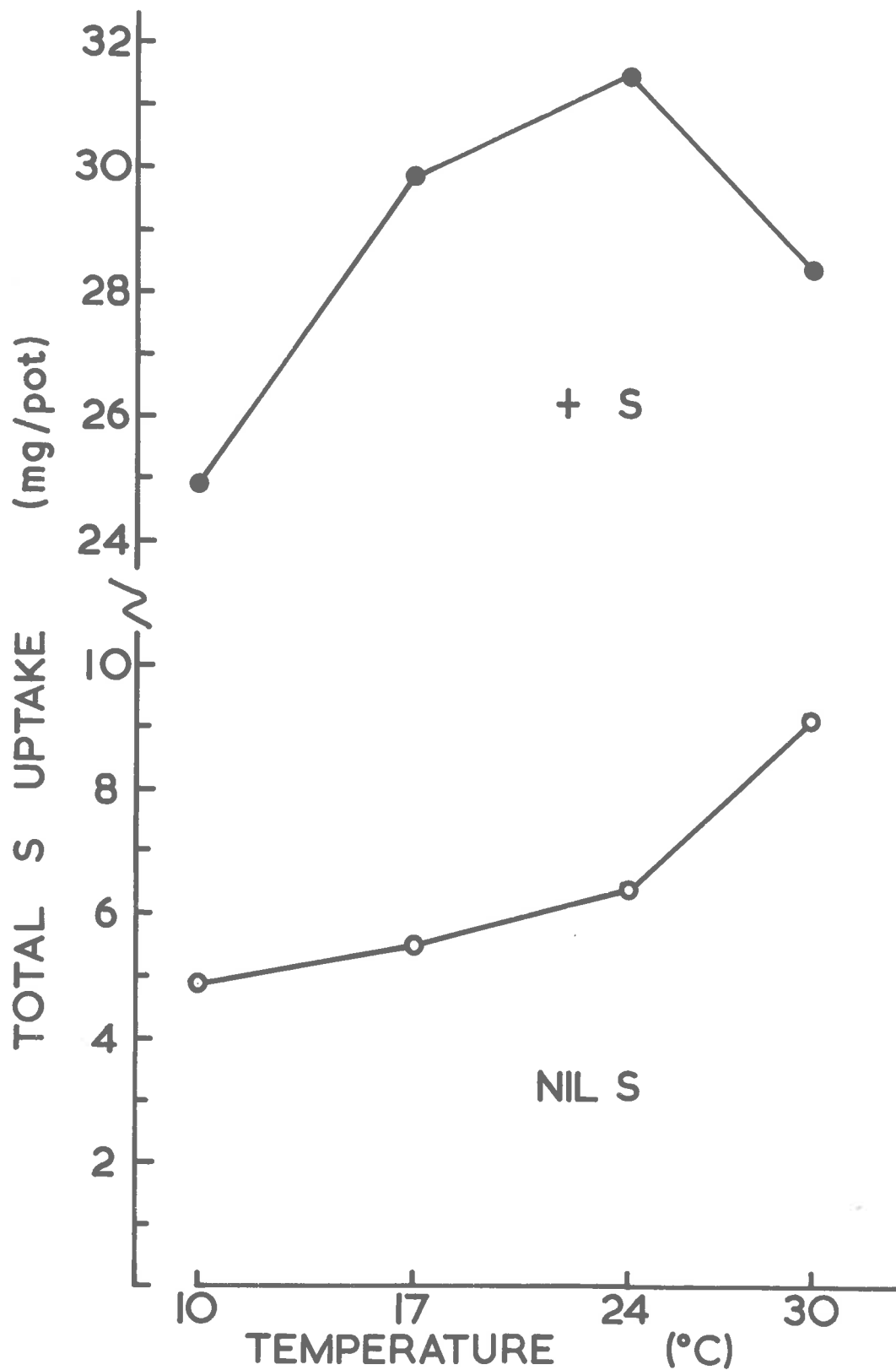
were recorded in roots. In the nil S treatments the differences due to soil temperature were not as marked except at 30°C, where a highly significant ($P \leq 0.001$) increase in the S content of the roots was observed.

(iv) Plant S uptake - S uptake values of tops tended to reflect total dry matter yield production, particularly from the +S treatment. At harvest I maximum yield and S uptake from both S treatments occurred at 24°C soil temperature but at harvest II the maximum yields on the +S treatments occurred at 17°C and 24°C, S uptake being slightly higher ($P \leq 0.05$) at 24°C. Results from the nil S treatment at harvest II deviated from this pattern, and the yields at 24°C were only slightly better than those at other soil temperatures. By harvest II $\text{SO}_4^{--}\text{-S}$ on the nil S treatments had become more limiting, and at this stage maximum S uptake occurred at 30°C soil temperature.

S uptake of roots differed markedly between the two S treatments. In the nil treatment where S was limiting, maximum S content in the roots occurred at 30°C, whereas in the +S treatment, maximum S content occurred at 10°C. In spite of 400% to 700% increase in root S uptake in the +S treatment, only small differences in total dry matter yield of roots resulted between the two S treatments.

Figure 34

The influence of soil temperature on plant S uptake
in the presence and absence of added $\text{SO}_4^{=}\text{-S}$.



Total S uptake values were obtained as follows:

$$TSU = SUT_1 + SUT_2 + SUR$$

where TSU = Total S uptake

SUT_1 = S uptake tops harvest I

SUT_2 = S uptake tops harvest II

SUR = S uptake roots

Patterns of mean total S uptake (Fig. 34) were quite different for each of the two S treatments. On the nil S treatment S uptake increased with soil temperature and the increases were larger at higher temperatures. Total S uptake values from soils of the +S treatment were much larger than those of the nil treatment, and to an exaggerated extent reflected growth responses at the various soil temperatures.

(d) Soil S and S Balance Measurements.

Total S recoveries and total $SO_4^{=}$ -S recoveries were computed and analysed. From these quantities it was possible to calculate net S balance or change in S content of the soil/plant systems, and net $SO_4^{=}$ -S balance. Complementary to the two latter quantities was a calculation of change or net balance of soil organic S. Table 24 summarizes the quantities presented in this section and their method of calculation.

TABLE 24

Formulae for Calculation of S Balances

$$\Sigma(\text{SO}_4^{\text{--}}\text{-S})_{\text{H}} = S_{\text{P}} + (\text{SO}_4^{\text{--}}\text{-S})_{\text{H}} \dots\dots\dots(1)$$

$$\Delta(\text{SO}_4^{\text{--}}\text{-S}) = \Sigma(\text{SO}_4^{\text{--}}\text{-S})_{\text{H}} - (\text{SO}_4^{\text{--}}\text{-S})_{\text{I}} \dots\dots\dots(2)$$

$$\Sigma S_{\text{H}} = S_{\text{P}} + S_{\text{S}} \dots\dots\dots(3)$$

$$\Delta S = S_{\text{H}} - S_{\text{I}} \dots\dots\dots(4)$$

$$\Delta S_{\text{O}} = [S_{\text{H}} - (\text{SO}_4^{\text{--}}\text{-S})_{\text{H}}] - [S_{\text{I}} - (\text{SO}_4^{\text{--}}\text{-S})_{\text{I}}] \dots\dots\dots(5)$$

Where:

$\Sigma(\text{SO}_4^{\text{--}}\text{-S})_{\text{H}}$ = Total recovery of $\text{SO}_4^{\text{--}}\text{-S}$ at harvest

S_{P} = Plant S uptake

$(\text{SO}_4^{\text{--}}\text{-S})_{\text{H}}$ = Soil $\text{SO}_4^{\text{--}}\text{-S}$ at final harvest.

$\Delta(\text{SO}_4^{\text{--}}\text{-S})$ = Change in $\text{SO}_4^{\text{--}}\text{-S}$ for soil/plant system.

$(\text{SO}_4^{\text{--}}\text{-S})_{\text{I}}$ = Initial soil $\text{SO}_4^{\text{--}}\text{-S}$.

ΣS_{H} = Total recovery of S at harvest.

S_{S} = Total soil S at harvest.

ΔS = Change in S for soil/plant system.

ΔS_{O} = Change in soil organic S.

(i) Net S balance - This quantity is dealt with first since most of the other items to be presented are components of it. Although only single determinations were made of each constituent quantity, an attempt was made to confirm the findings of pot experiment I regarding S losses from soil and soil/plant systems. Computations using equation (4)(Table 24) were made for each pot and mean values for all treatments are presented in Tables 25 A and B.

S ($P \leq 0.001$) and soil temperatures ($P \leq 0.01$) both affected S balance, but interpretation of the results was complicated by the significance of the interactions between soils and soil temperatures ($P \leq 0.01$) and soils and sulphur ($P \leq 0.01$).

Under most treatment combinations, net S losses occurred from the soil and soil/plant systems. A few net S gains were recorded in the nil S treatment, but these were small in size compared with the total S involved in the analyses. Since duplicate determinations were not carried out it is possible that these gains were due to analytical or sampling errors.

Where SO_4^{2-} -S had been added the mean S losses were much larger and in several treatments amounted to half of the applied S. In only one treatment to which S had been added was a net

TABLE 25A

Net S Balance (ΔS) -- (Nil S Treatment)*

Soil Temp. (°C)	mg per pot				
	10	17	24	30	Mean
Soil No. 1	-0.82	+3.84	-0.67	-3.06	- 0.18
2	-2.18	-1.29	-2.77	-4.64	- 2.72
3	-7.23	+3.49	-3.43	-0.48	- 1.91
4	-4.65	-1.29	-4.64	-9.50	- 7.52
5	-6.28	+3.00	+1.01	+0.63	- 0.41
6	-1.59	-1.26	-9.91	-2.08	- 3.71
Mean	-3.79	+1.08	-5.07	-3.19	

L.S.D. soil temp. means - 5% = 4.14

* All values in table are the means of four replicates.

Positive values indicate net gains and negative values
net losses.

TABLE 25B.

Net S Balance (ΔS) - (+S Treatment)*

Soil Temp (°C)	mg per pot				
	10	17	24	30	Mean
Soil No. 1	-12.89	-8.21	-14.04	-22.54	-14.42
2	-21.39	-9.40	-14.08	-21.85	-16.68
3	-23.72	-10.15	-10.41	- 8.56	-13.20
4	- 5.97	+ 0.52	-13.55	-14.54	- 8.39
5	-17.09	-13.22	- 8.53	- 8.74	-11.89
6	-17.53	-10.17	-23.60	-10.40	-15.43
Mean	-16.43	- 8.44	-14.04	-14.44	

L.S.D. soil temp. means - 5% = 5.22

* All values in table are the means of four replicates.

Positive values indicate net gains and negative values
net losses.

gain recorded, and this was very small. Smallest losses of S occurred at 17°C soil temperature.

(ii) Change in $\text{SO}_4^{=}\text{-S}$ - These changes were calculated using equation (2) (Table 24) and for this calculation plant S was regarded as recovered $\text{SO}_4^{=}\text{-S}$. Mean changes in $\text{SO}_4^{=}\text{-S}$ are shown in Table 26 A and B and it is clearly seen that net accumulation of $\text{SO}_4^{=}\text{-S}$ (indicated by +ve values) occurred in soils of all nil S treatments, whereas either immobilization or net loss of S occurred for all +S treatments.

Soils significantly affected accumulation of $\text{SO}_4^{=}\text{-S}$ in the nil S treatments, with most $\text{SO}_4^{=}\text{-S}$ being released from soils 4, 5 and 6. Soil temperature had a pronounced effect on accumulation of $\text{SO}_4^{=}\text{-S}$, which increased in both size and significance with temperature.

Net loss of $\text{SO}_4^{=}\text{-S}$ from the +S treatments was significantly influenced by soils ($P \leq 0.001$) and soil temperatures ($P \leq 0.001$). Lowest $\text{SO}_4^{=}\text{-S}$ losses occurred from soil 4 while the largest losses measured were from soil 6. Maximum mean $\text{SO}_4^{=}\text{-S}$ loss occurred at the lowest soil temperature (10°C). There were no significant interactions between treatments.

TABLE 26A

Changes in SO_4^{2-} -S (ΔSO_4^{2-} -S) - (Nil S Treatment) *

Soil Temp. (°C)	mg per pot				
	10	17	24	30	Mean
Soil No. 1	+2.96	+4.01	+3.73	+5.06	+3.94
2	+3.20	+3.38	+3.68	+6.03	+4.07
3	+2.40	+3.46	+3.47	+6.62	+3.99
4	+3.30	+3.43	+3.86	+8.53	+5.03
5	+3.17	+3.33	+6.08	+8.68	+5.32
6	+2.51	+3.66	+4.84	+7.52	+4.63
Mean	+2.92	+3.55	+4.44	+7.07	

L.S.D. soil temp. means - 5% = 0.64

1% = 0.84

0.1% = 1.10

L.S.D. soil means. 5% = 0.78

0.1% = 1.04

* All values in table are the means of four replicates.

Positive values indicate net gains and negative values
net losses.

TABLE 26B

Changes in SO_4^{--}S ($\Delta\text{SO}_4^{--}\text{S}$) - (+S Treatment)*

Soil Temp. (°C)	mg per pot				
	10	17	24	30	Mean
Soil No. 1	-13.42	-10.78	-10.44	-12.44	-11.77
2	-15.99	- 9.95	-10.74	-10.83	-11.88
3	-15.84	-10.85	- 5.71	- 8.46	-10.21
4	- 4.22	- 3.54	- 7.02	- 7.67	- 5.61
5	-15.69	-11.80	- 8.40	- 8.24	-11.03
6	-17.58	-12.47	-10.30	-12.83	-13.30
Mean	-13.79	- 9.90	- 8.77	-10.08	

L.S.D. soil temp. means - 5% = 2.46

1% = 3.27

0.1% = 4.24

L.S.D. soil means - 5% = 3.01

1% = 4.00

0.1% = 5.20

* All values in table are the means of four replicates.

Positive values indicate net gains and negative values net losses.

(iii) Total S recovery - This quantity was calculated using equation (3) of Table 24 and the values obtained reflect the total S status of the soil. As expected both soil and S treatment significantly ($P \leq 0.001$) influenced total S recovery. An interesting result was that there was a significant effect of soil temperature ($P \leq 0.05$) on total S recovered from the nil S treatment where best S recovery occurred at 17°C . This result was indicated previously by the net S balance values.

(iv) Total soil $\text{SO}_4^{=}\text{-S}$ recovery - Equation (1) (Table 24) was used to calculate this quantity which consists of two components, soil $\text{SO}_4^{=}\text{-S}$ and plant S uptake. This quantity was used to calculate net changes in soil/plant $\text{SO}_4^{=}\text{-S}$ and any variations in it were reflected in the results discussed previously.

(v) Changes in soil organic S - The above quantity was calculated using equation (5) (Table 24) and since four separate chemical determinations of soil S were involved in its calculation, the summation of errors, especially from total S analyses, led to variable results. Even so, highly significant differences ($P \leq 0.001$) due to S treatments were recorded. Added $\text{SO}_4^{=}\text{-S}$ reduced the size of changes in the organic S fraction compared with those measured in soils of the nil treatment. Many soils of the +S treatment, particularly those at a temperature of 17°C , showed slight increases

TABLE 27A

Change in organic sulphur (ASO) - Nil S Treatment*

Soil Temp. (°C)	mg per pot				
	10	17	24	30	Mean
Soil No. 1	-3.8	-0.2	-4.4	-8.1	-4.1
2	-5.4	-4.7	-6.4	-10.7	-6.8
3	-9.6	0	-6.9	- 7.1	-5.9
4	-8.0	-4.7	-19.5	-18.0	-12.6
5	-9.4	-0.3	- 5.1	- 8.0	- 5.7
6	-4.1	-4.9	-14.8	- 9.6	- 8.3
Mean	-6.7	-2.5	- 9.5	-10.3	

L.S.D. soil temp. means - 5% = 4.1

1% = 5.4

L.S.D. soil means - 5% = 5.0

* All values in table are the means of four replicates.

Positive values indicate net gains and negative values net losses.

TABLE 27B

Change in Organic Sulphur (ΔSO) - +S Treatment*

Soil Temp. (°C)	mg per pot				
	10	17	24	30	Mean
Soil No. 1	+ 0.5	+ 2.6	- 3.6	-10.1	-2.6
2	- 5.4	+ 0.6	- 3.4	-11.0	-4.8
3	- 7.9	+ 0.7	- 4.7	- 0.1	- 3.0
4	- 1.8	+ 4.0	- 6.5	- 6.9	- 2.8
5	- 1.4	- 1.4	- 0.1	- 0.5	- 0.9
6	0	+ 2.3	-13.3	+ 2.4	- 2.1
Mean	- 2.7	+ 1.5	- 5.3	- 4.4	

* All values in table are the means of four replicates.

Positive values indicate net gains and negative values net losses.

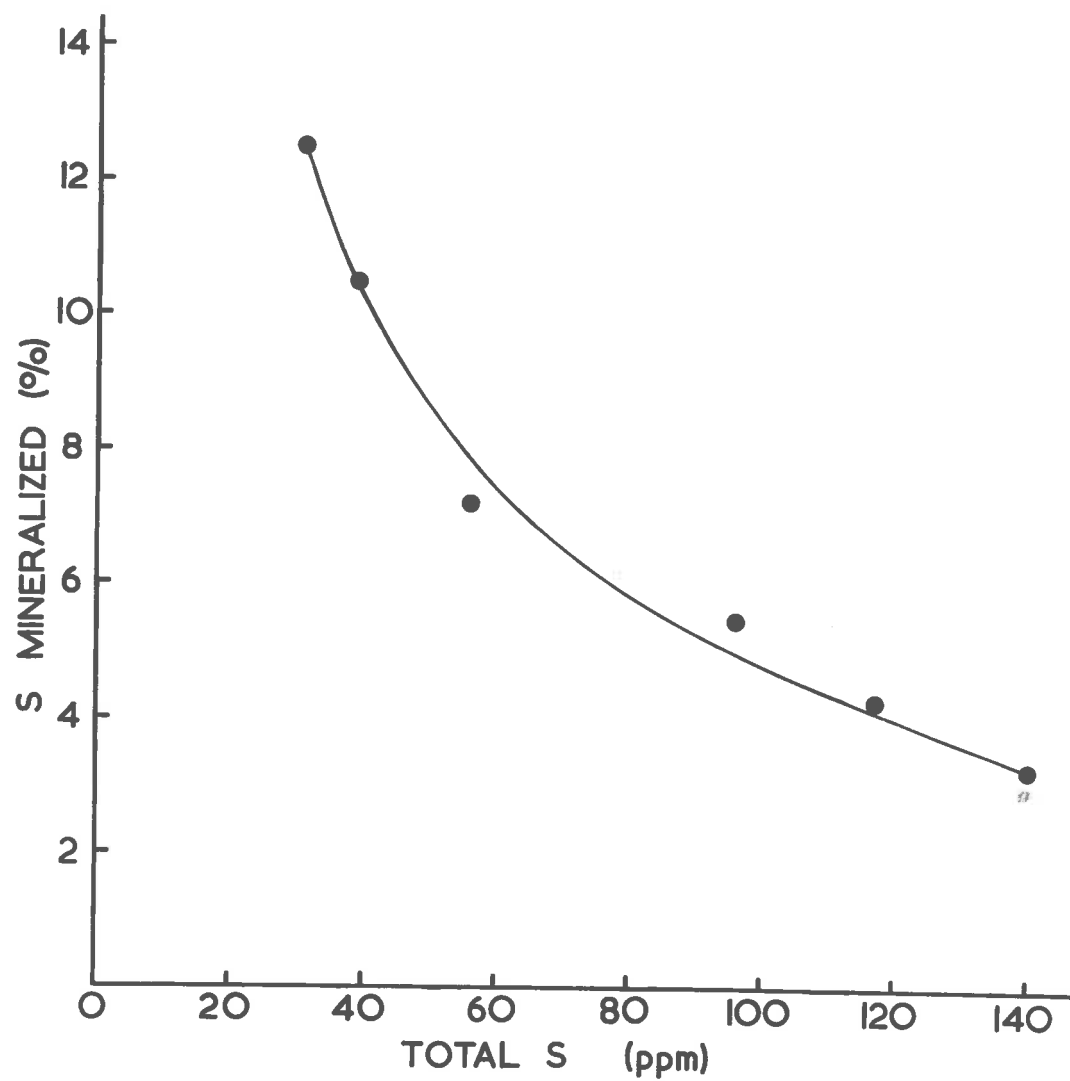
in organic S content, providing an indication that immobilization was occurring. By contrast, the relatively large reductions in the organic S contents of soils from the nil S treatment showed that S-mineralization was occurring, thereby supporting the total $\text{SO}_4^{=}\text{-S}$ recovery results. The lowering of organic S in the nil treatments was less marked ($P \leq 0.01$) at 17°C soil temperature. The biggest loss of soil organic S came from soil 4 ($P \leq 0.05$) but no other differences between soils were found. Mean changes in soil organic S are presented in Tables 27A and B.

(e) Pasture Development and S Mineralization

Table 17 shows that there was a rapid increase in soil organic matter following several years under pasture. Even so, soil 5, after 27 years of regular topdressing, contained less organic matter than soil 4 or 6. It was decided to compare effective S mineralization (i.e. $\text{SO}_4^{=}\text{-S}$ accumulation in plants and soil) with total S accumulation, using this as an index of organic matter accumulation. Only slight increases in effective mineralization occurred with increasing organic S content, and the S mineralized was expressed as a percentage of the total S present. Figure 35 shows percent S mineralized plotted against total S present in the soil and clearly demonstrates that as soil S accumulates on Laffer Sand a decreasing proportion of it is readily mineralizable.

Figure 35

Proportion of S mineralized from Laffer Sands of differing organic matter content (expressed as a percentage of the total S present), assuming no S lost by volatilization.



3. Discussion of Results

(a) Soil/plant S Balance

Evidence of S losses from the soil/plant system again appeared in this experiment. Although, for reasons previously noted, total S analyses were variable, statistically significant losses of S occurred from most treatments in the experiment. The main exception was the nil S treatment at 17°C soil temperature where a small mean net S gain (1.0 mg/pot) was recorded.

As in the previous pot experiment, additions of $\text{SO}_4^{=}\text{-S}$ led to increased loss of S, and for all +S treatments the mean net loss of S was 13 mg per pot, amounting to one third of that added. Soil temperatures other than 17°C had little or no effect on the size of S losses from either S treatment, but at 17°C there was a significant decrease ($P < 0.001$) in the loss.

It was suggested previously, (pot experiment I), that plants reduced S losses either by competing with micro-organisms for available S or through drying and improved aeration. Maximum plant yields occurred at soil temperatures of 17 and 24°C. Where $\text{SO}_4^{=}\text{-S}$ had been added the percentage S content of plants

was lowest at these temperatures, suggesting that competition for available S may have been a reason for increased recoveries of S at 17°C. However, to be consistent a similar effect should have been observed at 24°C. Soil micro-organisms responsible for losses of S may have optimum temperature ranges different from subterranean clover. At 24°C they could be more active than at 17°C, thereby becoming more competitive. At 30°C the total S uptake was still high in spite of declining plant yields and losses were similar to those noted at 24°C. Plant yields suggest that optimum soil temperature for subterranean clover growth lies between 17°C and 24°C, and an optimum range for soil micro-organisms responsible for S losses between 24°C and 30°C would explain the effects observed.

(b) Mineralization and Immobilization of S

In view of evidence of S losses, measurements of mineralization and immobilization were not possible. Net accumulation or loss of $\text{SO}_4^{=}\text{-S}$ were the quantities measured and as such are only indicators of mineralization or immobilization. The variability of total S determinations and their size relative to changes in $\text{SO}_4^{=}\text{-S}$ render them of little use for these measurements. The use of ^{35}S to enable the fate of applied S to be traced is described in the next section.

Addition of $\text{SO}_4^{=}\text{-S}$ caused net loss of S, while in all cases where no S was added, $\text{SO}_4^{=}\text{-S}$ accumulation was observed. This finding is consistent with the results of pot experiment I. A puzzling result from pot experiment I was the lack of response in $\text{SO}_4^{=}\text{-S}$ accumulation to soil temperature; over the wider range of this experiment $\text{SO}_4^{=}\text{-S}$ accumulation increased with increasing temperature. There was no significant difference between accumulation at soil temperatures of 10°C and 17°C , so it is not surprising that no response was detected in the previous pot experiment where the soil temperature ranged from 10°C to 20°C . Most $\text{SO}_4^{=}\text{-S}$ accumulated in the three soils that had been under pasture longest, but within this group there were only small differences between the soils in spite of the fact that soils 5 and 6 had been under pasture for 27 and 10 years respectively, while soil 4 had been under pasture for only 3 years. Those three soils contained the highest organic carbon contents and yet had the lowest C:N ratios. This suggests that the organic matter was closer to a stable equilibrium condition than in the other three soils.

$\text{SO}_4^{=}\text{-S}$ accumulation though significantly higher from these soils, than from soils 1,2&3, was only marginally so in terms of supplying plant S requirements, and it was shown (Fig. 35) that

there was a marked decline in the percentage of total S mineralized as organic S levels rose with lengthening top dressing history. A logical explanation of this is that the unstable proportion of recently incorporated organic matter decomposes leaving the resistant lignified and humified material behind. Over the years the material resistant to microbial attack accumulates, while annual increments of organic matter containing the easily decomposed fraction remain relatively constant. The build-up of a large reserve of resistant material slightly increases the amount of mineralizable S present, but at the rates of mineralization observed in the experiment, very large increases in total organic matter content would be needed to guarantee adequate supplies of soil $\text{SO}_4^{2-}\text{-S}$ by mineralization. Furthermore, it was noted that soil 5 with 27 years of pasture history had a lower organic matter content than either soil 4 or 6. This might be an isolated example, but from the present experiment, it appears that on these soils there is a factor limiting organic matter accumulation beyond that corresponding to a carbon content of about 1.5%, and that soil organic matter may decline after prolonged periods under pasture.

Inspection of Table 17 shows that the C:N ratios of all soils were wider than 14:1 in spite of the soils having been under clover pasture. Nitrogen losses by leaching may be one factor limiting

organic matter accumulation. Limited S supply in superphosphate coupled with high leaching losses may be another.

It is interesting to note that in this experiment the mean $\text{SO}_4^{=}$ -S accumulation from soil 6 (the soil from site of the field trial and similar to that used for all previous experiments) at 10° and 17°C soil temperatures over 74 days amounted to 2.9% of the total S originally present. The two soil temperatures chosen for this calculation covered most of the range measured in the top 5 cm of soil during the field experiment. The equivalent accumulation over 60 days is 1.8% of the total S present. This may be compared with values of 1.3% and 1.7% obtained from results of pot experiment I and the field experiment respectively. Removal of coarse organic matter containing some relatively fresh material during the preparation of soil for pot experiment I may be the reason for the lower value of $\text{SO}_4^{=}$ -S accumulation in that experiment. More of the coarse organic matter was incorporated into the soil used in the current experiment, and the initial total soil S content was higher than for pot experiment I.

(c) Plant Responses

Plant yield response and S uptake differed between harvests. In the nil S treatments compensatory effects appeared to operate

between harvests. Yield differences due to soil temperature at harvest II from the nil S treatments were small. Depletion of the limited quantities of available S at soil temperatures of 17° and 24°C by harvest I led to small yield differences at harvest III. Presumably, having established a root system, plants at lower soil temperatures were able to utilize the S conserved during the period of slow development, while plants at higher soil temperatures were dependent on supply by mineralization. On no soils examined in this experiment was S mineralization capable of supplying the S requirement of plants growing at their optimum soil temperatures. Percentage S contents in the plant tops of the nil S treatment, regardless of soil or soil temperature were indicative of S deficiency at both harvests.

D. Experiments Using ^{35}S Labelled Sulphate

1. Introduction

During the latter part of the project access to a liquid scintillation counter made possible the use of radioactive S. In view of the difficulties in measuring small changes in various S fractions relative to large backgrounds by chemical methods, the opportunity was taken to conduct two small experiments using ^{35}S labelled sulphate. One experiment was conducted within pot experiment II described in section C, and the other was a perfusion experiment. Both experiments were carried out to test the possibilities of using labelled S for further studies of this nature as time did not permit major experiments.

2. Soil/Plant S Balance Using $^{35}\text{SO}_4 = -\text{S}$

(a) Soil Preparation and Fertilizer Labelling

The experiment was carried out within pot experiment II. Soil 6 was chosen for the test since this came from the site of the field experiment and had been used in all previous experiments. The soil was prepared as were the other soils in pot experiment II, except that the fertilizer solution added to the +S treatment contained approximately $1\mu\text{c}$ per ml of ^{35}S in the form of sodium sulphate. This solution was prepared using $\text{Na}_2^{35}\text{SO}_4$ from carrier

free solution containing 10 mc/ml supplied by The Radiochemical Centre, Amersham, England. A dilute stock solution was prepared from the carrier free solution, and an appropriate aliquot was added to the fertilizer solution which was then made up to 1 l. In pot experiment II the 50 ml aliquot of fertilizer solution added to each 1 kg sample of soil contained 40 mg of S as Na_2SO_4 , in this case the aliquot contained 50 mc of ^{35}S as well.

(b) Treatments, Management, Harvesting and Chemical Methods

Soil temperature treatments of 10, 17, 24 and 30°C were replicated four times. Methods of management, harvesting, and chemical analyses for soils and plants were as described in pot experiment II.

(c) Measurement of Radioactivity

All activity measurements were carried out by liquid scintillation counting using a "Packard" Liquid scintillation counter fitted with automatic external standardization. The scintillation fluid used was prepared by dissolving 100 g of naphthalene, 7 g of PPO $\overset{0.5}{\text{g}}$, and $\overset{0.5}{\text{g}}$ of POPOP in 1 l of dioxan. The mixture was filtered and stored in an amber glass dispensing bottle. In all cases 0.1 ml aliquots of the samples to be counted were placed in 10 ml of scintillation fluid. All counts were made using the ^{14}C

channel and were terminated at a preset level of 1,000 ^{counts} or 10 _^ minutes, whichever came first.

Counts were carried out on total soil S extracts, soil $\text{SO}_4^{2-}\text{-S}$ extracts, and digests of plant tops from harvests I and II and plant roots. The activity of the fertilizer solution was determined at the same time as the activities of the soil and plant extracts. A quenching curve was constructed from counts obtained using increased volumes of blank extract added to scintillation bottles containing similar amounts of ^{35}S labelled sodium sulphate. A curve relating automatic external standardization (A.E.S.) to efficiency was obtained by using a set of prepared standards yielding 100,000 cpm on the ^{14}C channel at zero quenching. All experimental counts were corrected from this curve. Results obtained by radioisotope measurements were compared with those obtained by chemical methods.

(d) Results

At the time of counting the specific activity of the $\text{SO}_4^{2-}\text{-S}$ added to the soil was 33.5 cpm per μg of S. This was lower than planned because several previous attempts to obtain counts had failed, and the extracts had to be held for six months until a faulty scintillation counter was replaced.

Since the counts were carried out on samples of extracts prepared for chemical analysis, the problem of sampling errors and large dilution factors in the total S extracts was again apparent when attempting to measure ^{35}S extracted by this method. Very low count rates, in many cases near to background level, were obtained from total S extracts and the results from this section were of no use.

Results from plant material digests and soil $\text{SO}_4^{=}\text{-S}$ extracts were consistent between replicates, and in most cases followed the same trends as corresponding chemical analyses. Table 28 shows the mean values obtained for the S content of plant material by chemical and radiochemical methods of measurement respectively. The complete sets of paired data for tops from harvests I and II were tested for significant differences using the Wilcoxon signed-rank test for matched pairs (Siegel 1956). There was no significant difference between the S uptake of tops at Harvest I measured by chemical or radiochemical methods. By harvest II there was a highly significant ($P < 0.001$) difference between the results of the two methods, and it was apparent that the proportion of ^{35}S to total S that accumulated in tops between harvests I and II was lower than the proportion of ^{35}S in the $\text{SO}_4^{=}\text{-S}$ present initially in the soil. Figure 36 shows the mean total S uptake and the

Figure 36

Total S and ^{35}S contents in tops and roots of
subterranean clover grown on Laffer Sand
fertilized with $^{35}\text{SO}_4^-$ -S.

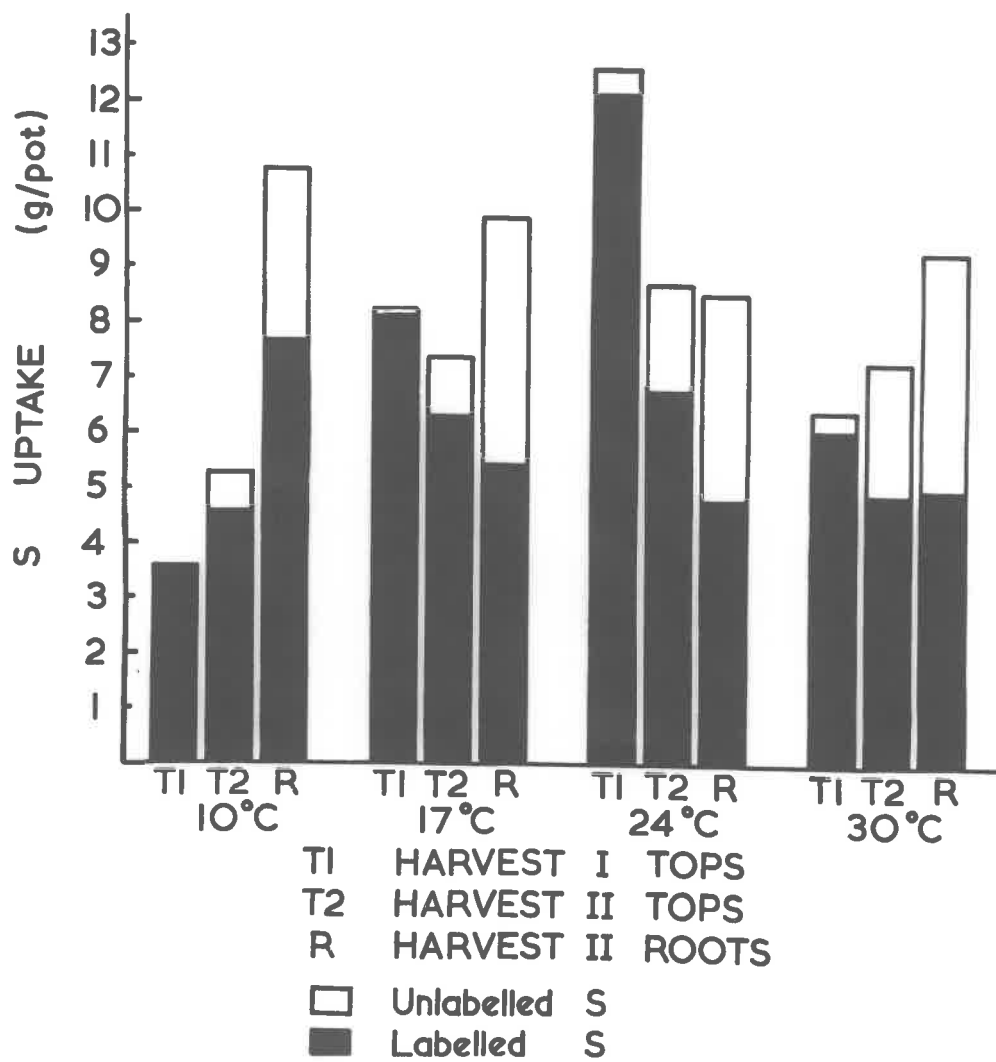


TABLE 28

Chemical and Radiochemical Measurements of S Uptake (mg/pot).

Soil Temp. (°C)	Tops HI		Tops HII		Roots	
	Chem.	³⁵ S	Chem.	³⁵ S	Chem.	³⁵ S
10°	3.61	3.95	5.31	4.59	10.78	7.67
17°	8.25	8.20	7.40	6.32	9.93	5.44
24°	12.64	12.17	8.72	6.77	8.54	4.81
30°	6.45	6.19	7.33	4.92	9.31	5.01
Means	7.74	7.63	7.19	5.65	9.64	5.73
	No sig. diff.		P<0.001		P<0.001	

TABLE 29

$\text{SO}_4^{=}\text{-S}$ Recovery by Chemical and Radiochemical Measurement (mg/pot)

Soil Temp. (°C)	Soil $\text{SO}_4^{=}\text{-S}$		Plant S Uptake		Total $\text{SO}_4^{=}\text{-S}$	
	Chem.	^{35}S	Chem.	^{35}S	Chem.	^{35}S
10°	5.2	11.4	19.7	16.2	24.9	27.6
17°	4.4	9.5	25.6	20.0	30.0	29.5
24°	2.3	4.4	29.9	23.8	32.2	28.2
30°	6.6	10.9	23.1	16.1	29.7	27.0
	P<0.01				No sig. diff.	

proportions of labelled and unlabelled S present in the plant tops and roots for each harvest at each soil temperature. Roots contained the highest proportion of unlabelled S (mean value of 40%) found in the plant material.

Soil $\text{SO}_4^{=}\text{-S}$ measurements (Table 29) provide an interesting contrast since all measurements by the radiochemical method exceeded those made chemically on the same extracts. When all fractions of each set of data (plant and soil) were summed (Table 29) to estimate total $\text{SO}_4^{=}\text{-S}$ recovery from the soil/plant system there was no significant difference (Wilcoxon test) between results obtained by the two methods. The "overestimate" of soil $\text{SO}_4^{=}\text{-S}$ obtained radiochemically balanced the corresponding "underestimate" of plant S uptake. In spite of internal discrepancies, both methods failed to recover a quarter of the $\text{SO}_4^{=}\text{-S}$ initially applied. The proportion of unlabelled S in the plant material provides a means of calculating the "effective" mineralization (i.e. that which supplied S for plant uptake) on this soil. Basing the calculation on mean results obtained at soil temperatures of 10° and 17°C , since these cover the range experienced in subterranean clover pastures on Laffer Sand during the growing period, it can be seen that 4.6 mg of unlabelled S/pot (equivalent to 4.6 ppm

from soil) were taken up during the experiment. This is 3.3% of the organic S initially present and it was released over 74 days.

(e) Discussion

Since the S contents of tops at harvest I by radiochemical measurements were similar to those measured chemically, the S taken up by plants was labelled with a level of activity similar to that in the applied $\text{SO}_4^{2-}\text{-S}$. It follows that the S uptake prior to harvest I came almost entirely from added $\text{SO}_4^{2-}\text{-S}$. A small quantity of unlabelled S was present in tops grown at soil temperatures of 17, 24 and 30°C indicating that a little mineralization had occurred at these temperatures.

At harvest II the proportion of unlabelled S present in the tops grown at all soil temperatures had increased. The proportion increased with increasing soil temperatures. This confirmed results previously obtained by chemical measurements on the nil S treatments (Pot Experiment II), that showed increased mineralization at higher soil temperatures. At soil temperatures of 17°C and 20°C uptake to the tops at harvest I was two and three times that at 10°C, but at harvest II, when the $\text{SO}_4^{2-}\text{-S}$ remaining in the soil of these treatments was lower, there was a decline in uptake, particularly of ^{35}S .

This may be regarded as a compensatory effect due to early depletion of S supply where high soil temperatures permitted faster initial growth rates. The large proportion of unlabelled S found in the roots at the end of the experiment may have been an artefact due to contamination. The roots were entwined around small pieces of organic matter, and contamination would have led to high values of unlabelled S. Error due to contamination does not change the conclusion from pot experiment II that losses of $\text{SO}_4^{=}\text{-S}$ occurred from the soil/plant system, since such an error overestimates the final S content of the plant.

Chemical methods measured less $\text{SO}_4^{=}\text{-S}$ in the soil than radiochemical methods. This is one of the more important findings of this experiment, for it shows that not all of the S extracted by CaCl_2 was present in reducible form (i.e. $\text{SO}_4^{=}\text{-S}$). A proportion of the $\text{SO}_4^{=}\text{-}^{35}\text{S}$ originally applied to the soil was apparently incorporated in non-reducible forms. Compounds containing S not detectable by reduction with hydriodic acid, including cystine, cysteine, methionine, vitamin B1, taurine, and glutathione (Freney 1958). Freney showed that many inorganic compounds containing S in partially reduced forms yielded low values of S when reduced with hydriodic acid. Adsorption of $\text{SO}_4^{=}\text{-S}$ to soil colloids did not prevent its measurement by the methylene blue method, so that

adsorption by soil organic matter would not account for the lowered recoveries of S in this experiment. The evidence suggests that a proportion of the $^{35}\text{SO}_4^-$ -S added to the soil in this experiment had become either fully or partially reduced and may have been incorporated in amino acids and microbial protein. Very little of the ^{35}S in microbial protein or partially reduced S compounds present in the CaCl_2 extracts would have been measured by the methylene blue method, but it would have been detected radiochemically. The S present in non-reducible forms at the time of extraction might represent only a small proportion of the amount converted since the processes involved are likely to be of microbial origin and to have been continuous in their operation.

The ultimate fate of reduced S is unknown. Reduction to H_2S or the production of volatile organic S compounds leading to losses of S from the soil/plant system or incorporation in soil organic matter are possibilities. One quarter of the SO_4^- -S originally present was not chemically or radiochemically detectable at the end of the experiment, showing that transformations to organically bound unavailable forms or to volatile forms had occurred. Total S balance measured chemically (pot experiment II) showed that a mean net loss of 9.5% of the total S originally present, occurred in this experiment.

The value of ^{35}S for further studies has been demonstrated in this experiment, and its use in completely enclosed soil/plant systems provided with means for trapping volatile products would enable direct verification of S losses. Using ^{35}S together with techniques such as chromatography and electrophoresis, identification of intermediate compounds involved in soil S transformations should be possible.

3. Perfusion Experiment

(a) Materials and Methods

A series of 11 perfusion units similar to those of Audus (1946), but with ground glass joints throughout, were filled with 75g (oven dry basis) of Laffer Sand and 100 ml of test solution. The compositions of the solutions used are shown in Table 30. Sulphate solutions provided a gravimetric concentration of 30 ppm of $\text{SO}_4^{2-}\text{-S}$ in the soils at any instant during perfusion. Solutions containing $\text{SO}_4^{2-}\text{-S}$ were labelled by the addition of a small quantity of $\text{Na}_2^{35}\text{SO}_4$ (to a final activity of approximately 0.4 $\mu\text{Ci/ml}$), obtained by diluting carrier free Na_2SO_4 solution from the Radiochemical Centre, Amersham, England. Sucrose was added to some solutions to test the influence of readily available energy on microbial transformations.

TABLE 30

Perfusion Experiment Treatments

Perfusion Unit No.	Solution
1, 6, 11	Distilled H_2O
2, 7	Na_2SO_4 (112 ppm S)
3, 8	Na_2SO_4 + 0.2% sucrose
4, 9	0.5% methionine
5, 10	0.5% methionine + sucrose

Radioactivity was measured by liquid scintillation counting using the method described previously. Since the counter broke down the experiment ended earlier than planned.

Three absorber towers (Stotzky 1965) containing 1N NaOH, 3% mercuric cyanide, and 4% mercuric chloride were connected in that order to the outlet of each perfusion unit. All inlet air was passed through NaOH absorbers to remove S compounds. The units were loaded and perfusion took place from 29th August 1967 until 2nd October 1967. During this period 9 samplings were taken from the perfusate of units containing $\text{Na}_2^{35}\text{SO}_4$, and the activity remaining in the solution was determined and compared with that from a reference sample of the original solution stored at -45°C .

On two occasions samples were taken from the absorber towers connected to perfusion units containing labelled sulphate. Before taking these samples the reservoirs were shaken to suspend any precipitate present. Activities of these samples were determined in the same way as activities of the perfusates, but breakdown of the counter prevented analysis of the final sampling.

S compounds likely to be precipitated by mercuric cyanide and mercuric chloride include sulphides that sublime on heating, thereby

causing volatile losses and preventing oxidative preparation for analysis. S-recovery tests were made on mercuric sulphide using the oxidation method of Steinbergs et al. (1962) followed by extraction and determination by the methylene blue method. Between 90 and 99% of the mercuric sulphide S was recovered suggesting that the method could be used to measure S compounds precipitated in the absorbers.

(b) Results

Only those results of immediate significance to the rest of the project are presented. Activity measurements showed that little change in the level of labelled S occurred in perfusion units to which $^{35}\text{SO}_4$ alone had been added, but where a metabolizable energy source (sucrose) was present there was a fall in the activity in the perfusate (Table 31).

TABLE 31
Recovery of ^{35}S in Perfusates

Time (days)	Mean ^{35}S Recovery (%)	
	Na_2SO_4	Na_2SO_4 + Sucrose
1	100.5	98.2
3	98.9	90.1
5	101.2	92.1
9	95.6	93.7
12	98.4	90.1
19	101.3	72.2
23	93.5	70.0
30	101.1	68.3
34	103.6	69.5

The falls in activities of perfusates containing sucrose were not accompanied by increased activities in the absorbers of these treatments except in unit 3. In this unit activity from 0.1 ml of mercuric cyanide absorber of 242 cpm was approximately six times that from a similar blank absorber. Unfortunately no further counts were obtained and conclusions drawn from this isolated example would be unreliable.

Wherever methionine was perfused, results similar to those of Frederick, Starkey and Segal (1957) were observed. Dense white precipitates appeared first in the mercuric cyanide and later in the mercuric chloride absorbers. The presence of sucrose hastened the process. Analyses of the precipitates indicated that between 56% and 75% of the S added in methionine was recovered in the absorbers after 34 days. Furthermore only traces of S were detectable in the NaOH absorbers indicating that most of the volatile S compounds produced were not absorbed in this solution.

(c) Discussion

Although few significant results were obtained from the experiment, it was clear that perfusion methods offer possibilities of examining S transformations in Laffer Sands. Disappearance of

labelled SO_4^{2-} -S from the perfusate was demonstrated by a decline in activity in the presence of sucrose. Volatilization of S from perfusates containing methionine was demonstrated by the formation of S containing mercurial compounds, when gases from the perfusion units were passed through mercurial absorbers. NaOH was shown to be an ineffective absorber of volatile S compounds that are precipitated by mercuric cyanide and mercuric chloride. The method of Steinbergs et al. (1962) offers possibilities for the recovery and determination of S compounds precipitated by mercury, but further tests need to be carried out using other materials besides mercuric sulphide. The use of labelled S compounds together with chromatography and electrophoresis may enable separation of intermediates from pathways involved in the production of volatile S compounds in soils.

IV General Discussion

Two types of S deficient soils were proposed by McLachlan and De Marco (1968). In the first type, S is lost from the soil/plant system, mainly by leaching (e.g. Powrie 1967, Barrow, 1966, Hingston 1959), in the second type, S is not lost, but accumulates in soil organic matter and is not readily available to plants (Donald and Williams 1954). Losses of S, applied as fine gypsum, from the topsoil in the field experiment suggest that Laffer Sand is in the first category. However, increased organic S contents in developed soils compared with undeveloped soils in pot experiment II showed that some applied S is incorporated in organic matter. Both processes limit S availability in Laffer Sand, thus placing these soils between the two extremes proposed by McLachlan and De Marco. The position of any S deficient soil in this classification depends mainly on soil texture and rainfall, although plant cover and grazing (May, Till and Downes 1968) also have some influence.

Leaching of SO_4^{2-} -S from these soils leads to low S uptake by plants and may limit organic matter accumulation. When phosphate dressings are lowered to post-development levels of 2 kg per ha applied bienially, S supply is further restricted. Limited input of S may be one reason why the accumulation of organic matter seen in the soils of pot experiment II reached an equilibrium in only four to seven years after development.

Balance studies (pot experiments I and II) showed that S may be converted to volatile forms and lost from the soil and soil/plant systems. Many workers have reported disappearance of $\text{SO}_4^{=}\text{-S}$ applied to soils (Bollen 1925, Freney and Spencer 1960, Williams, McKell and Reppert 1964), and the losses not accounted for by leaching or plant uptake have generally been attributed to immobilization. Without total S determinations, it is impossible to say whether or not immobilization occurred. Results from the present study suggest the loss of volatile forms of S, as well as immobilization contribute to the disappearance of $\text{SO}_4^{=}\text{-S}$ from soils.

Losses of volatile S, analogous to denitrification, have long been accepted, but it is generally supposed that they occur only under anaerobic conditions. Frederick, Starkey and Segal (1957) showed that losses from soils perfused with methionine involved reduced compounds such as dimethyl disulphide and methyl mercaptan. Conditions in the soils perfused did not necessarily favour reduction, for in a similar experiment cysteine was oxidized to sulphate. Assimilatory processes for the utilization of $\text{SO}_4^{=}\text{-S}$ by plants, animals and micro-organisms involve S reduction and incorporation in amino acids. Decomposition of amino acids may produce volatile end products including sulphides and mercaptans. The pathway postulated for the conversion of $\text{SO}_4^{=}\text{-S}$ into volatile products

(Section IIIB 2h) includes only the main intermediates, but should be possible in the presence of appropriate micro-organisms and energy sources.

Barrow (1960a, 1960c, 1961a) was aware of the possible losses of volatile S compounds from incubated soils. Using NaOH as an absorber (Barrow 1961a), he found that small quantities of H_2S were released from all soils examined in an incubation experiment. The perfusion experiment in this project showed that where losses of S from soils treated with methionine occurred, most of the S volatiles passed through NaOH absorbers and were precipitated in mercuric cyanide and mercuric chloride. H_2S is more likely to be produced by soil sulphur-reducing bacteria such as Desulfovibrio and Desulfotomaculum under anaerobic conditions, whereas organic disulphides and mercaptans may be released during organic matter breakdown under aerobic conditions. It is possible that many attempts to recover S lost by volatilization have been aimed at the wrong compounds.

As only half of the ^{35}S extracted from soil 6 by $CaCl_2$ at the end of pot experiment II was reducible, the applied SO_4^{2-} -S was probably being assimilated by micro-organisms and reduced to form

microbial protein. Since not all of the added $\text{SO}_4^{2-}\text{-S}$ was recovered from the soil/plant systems, the non-reducible ^{35}S may have been in intermediate compounds involved in immobilization or production of volatile S compounds. Early termination of the perfusion experiment prevented conclusions being reached about the fate of labelled $\text{SO}_4^{2-}\text{-S}$ added to soils, and further work using ^{35}S and biochemical techniques is suggested.

In view of evidence of volatile S losses, apparent mineralization or immobilization measurements really measure either positive or negative net S accumulation. Similar implications are involved with regard to N mineralization, since volatile losses of N occur following denitrification. Pot experiment II showed that increasing soil temperatures favoured mineralization of S. While there was little effect of temperature in the range from 10° to 20°C , there was a larger effect at 30°C . It appeared that two processes were involved. Firstly, at lower temperatures normally experienced in the field, responses were chiefly due to the influence on microbial activity. Secondly, at higher temperatures, breakdown of labile organic compounds may have occurred, providing more substrate for microbial activity or even direct release of $\text{SO}_4^{2-}\text{-S}$. Increased extraction of $\text{SO}_4^{2-}\text{-S}$ following heating has been noted by Williams

and Steinbergs (1959). The maximum soil temperature recorded during the field experiment was 25°C , and this occurred in late summer and was accompanied by a rise in CaCl_2 extractable $\text{SO}_4^{2-}\text{-S}$. The effect may have been due to drying (Freney 1958) rather than enhanced microbial activity, since the soils were dry during this period.

Plant responses to added S were lower at soil temperatures of 10°C and 30°C than at 15°C , 17°C and 24°C (pot experiments I and II), showing that factors other than S supply are likely to limit field responses during the winter growth period, when soil temperatures in the root zone are close to 10°C (Section IIIA 2(k)). Temperature may influence subterranean clover responses through effects on N fixation by Rhizobia and low metabolic rates within the plant. The results of the pot experiment suggest that while soil temperatures remain close to 10°C , mineralization of S can supply a greater proportion ^{of} plant S requirements than at higher temperatures. At soil temperatures from 15°C to 20°C plant growth rates were higher relative to mineralization than at 10°C .

Removal of soluble products by leaching increased the recovery of mineralized S (Section IIIB 4) and this cast doubt

on the validity of conventional incubation tests. Increased recoveries may have been due to removal of $\text{SO}_4^{2-}\text{-S}$ before volatilization occurred; this would have been consistent with findings in the balance studies. Removal of end products of reversible reaction, or toxins, could be equally valid reasons for enhanced S mineralization. The effects of leaching, and plants, have been shown to influence S transformations and if these factors, and others, are ignored, erroneous conclusions may be made. The results of incubation tests at 30°C cannot be realistically applied to soils under subterranean clover in the field. $\text{SO}_4^{2-}\text{-S}$ release at such high temperatures may be due to effects other than enhanced microbial activity. The main value of incubation tests has been to define the chemical and physical factors affecting S transformations. Extrapolation of results to field situations is not easy.

There is scope for the study of soil S transformations in dynamic systems. The use of ^{35}S and biochemical methods should prove rewarding as little work apart from that of Freney (1960) has been undertaken in this field.

Throughout this series of experiments, S mineralization has been unable to supply sufficient S to completely alleviate

plant S deficiencies. Low mineralization rates, and the apparently small amount of accumulated organic matter at equilibrium, coupled with leaching and other losses of $\text{SO}_4^{2-}\text{-S}$ keep the S status of Laffer Sands at a low level. Under current cultural and fertilizer practices there will be a continuing need for regular applications of S fertilizer. The material used should be sparingly soluble and become slowly available to ensure continued supply during periods of leaching. Elemental S and gypsum of appropriate particle sizes are the most promising materials presently available, but further work is needed to formulate practical recommendations.

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