GASEOUS LOSSES OF NITROGEN FROM SOILS BY DENITRIFICATION

A Thesis submitted
by
John Roland Burford, B.Ag.Sc., (Hons.), M.Ag.Sc.

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Department of Agricultural Biochemistry and Soil Science
Waite Agricultural Research Institute
University of Adelaide

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SUMMARY

The occurrence of denitrification products in the soil atmosphere of the Urrbrae fine sandy loam, a red-brown earth, has been investigated over the three year period 1966-68. Samples of the soil air were obtained from small, permanently installed reservoirs (30 ml capacity) and analyzed by gas chromatography for N_2, N_2O, CO_2, O_2 and A.

The detection of nitrous oxide in the soil atmosphere for periods of 2-6 months in each year demonstrated the normal occurrence of denitrification in this agricultural soil, and confirmed earlier predictions that aerobic denitrification can result in the gaseous loss of nitrogen from agricultural soils in the field: the mean oxygen concentrations in the large soil pores were always greater than 10% and usually greater than 15% in the B horizon and 18% in the A horizon. The mechanism responsible for the losses was that of biological dissimilation at anaerobic micro-sites within the generally well aerated soil.

A preliminary experiment indicated that the dissolution of evolved CO_2 in the soil water could create pressure gradients and the mass flow of soil air; it was shown that estimates of the losses as nitrogen gas could not be obtained from measurements of the nitrogen gas concentrations alone. The use of argon as a reference gas was investigated, but differences in the diffusion rates of N_2 and A prevented measurement of the evolution of N_2 gas from N_2/A ratios.

The nitrous oxide measurements indicated that there were two main sources of evolution in the soil profile: a zone in the A_1 horizon at about the 10 cm depth, and one in the B horizon at about the 60 cm depth. The occurrence of N_2O in the A_1 horizon was ephemeral and restricted to brief periods when the soil moisture content was high following rainfall. In contrast, N_2O concentrations were much less variable in the B horizon: in each season the gas was detected after the initial wetting of the subsoil in late autumn and levels increased to a peak in mid-winter then decreased in late winter and early spring.

Calculations based on transfer equations were unsatisfactory for accurately estimating losses, mainly due to uncertainties in the values for the air-filled porosity of the soils. However, such calculations indicated that losses were much greater from the A_1 than from the B horizon, despite the usually brief occurrence of N_2O in the A_1 horizon. The greatest losses from both horizons were in winter when soil temperatures were lowest (10 ± 5 C) and soil moisture contents highest.

Losses under a wheat crop were not markedly different on two areas with contrasting structure and organic matter status, but losses underneath a pasture were much smaller than under the wheat crop.

The application of nitrate fertilizer (100 lb/acre) increased N_2O evolution 3-10 fold in the A_1 horizon and up to 2.5 fold in the B horizon, but the estimated maximum diffusive losses were only 0.06-0.67 lb N_2O-N/acre/day from areas sown to wheat and fertilized with nitrogen.

STATEMENT

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 the author's knowledge and belief, the thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

September, 1969. J.R. Burford.

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CHAPTER I INTRODUCTION

I.1. PERSPECTIVES

The widespread importance of nitrogen in the agricultural utilization of soils is well known, and many aspects of the nitrogen cycle have been studied extensively. Increases in soil nitrogen levels due to biological fixation are well documented but rather less is known about the losses from soil systems. Nitrogen balances from lysimeter experiments have consistently shown deficits of 5-25% of added nitrogen fertilizer which could not be accounted for in analyses of the soils, leachates and plant products (Allison, 1955, 1966). Similar low recoveries have been obtained in experiments using N^{15} (Broadbent and Clark, 1965). It is generally agreed that these deficits represent losses of nitrogen by denitrification.

Denitrification can be defined as those reactions in which soluble inorganic nitrogen compounds are reduced to gases which, by escape through the soil pores to the atmosphere, result in a loss of nitrogen from the soil system. It is well established that such losses may occur by both biological and chemical pathways. While it has been proposed that denitrification should be used to describe only biological mechanisms (Soil Sci. Soc. Am., 1962), it is often not possible to assign an observed result to a particular mechanism. For this reason, it is more convenient to refer to all such gaseous losses as denitrification (after Broadbent and Clark, 1965) with the use of the qualifying adjectives chemical or biological (or the prefixes chemo- or bio-) where positive evidence indicates an operative mechanism.† This definition of denitrification does not embrace losses by ammonia volatilization.

The microbial utilization of nitrate, or reduced nitrogen compounds as terminal hydrogen acceptors during a deficit of oxygen, is usually termed '<u>nitrate respiration</u>' (Sato, 1956) or '<u>nitrate dissimilation</u>' (Fewson and Nicholas, 1961) in contrast to '<u>nitrate assimilation</u>', in which nitrate is utilized as a nutrient and the end products are nitrogeneous cell constituents. "(Biological) denitrification may be regarded as a special case of nitrate respiration, and is defined as the production of nitrogen gas or the oxides of nitrogen from nitrate, nitrite or any other suitable intermediate" (Fewson and Nicholas, 1961).

Chemo-denitrification cannot be well defined until the basic reactions are more clearly understood. Clark's (1962) definition "gaseous nitrogen losses associated with nitrite instability" represents the present state of knowledge. The term 'side tracking of nitrification' (Vine, 1962) has much merit, but only if nitrite produced by nitrate dissimilation does not participate in chemical reactions.

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†Even so, such descriptions can be somewhat arbitrary, since chemical losses are generally agreed to be dependent upon nitrite in solution (Broadbent and Clark, 1965), which is almost certainly of biological origin; and the nitrous oxide in solution has been suggested as emanating from a non-enzymic decomposition of hyponitrite (Nicholas, 1963), or imidonitric acid (Kluyver and Verhoeven, 1954).

Denitrification reactions are important agriculturally from two aspects. Firstly, a substrate for the denitrification reaction is usually that small fraction of the total nitrogen in the soil which is highly available for plant uptake. Additionally, the losses are not temporary such as those occurring in fixation or immobilization reactions, and in which the nutrient may be released at a later date, but are in the form of complete loss from the soil.

It is widely acknowledged that rapid denitrification will occur in waterlogged soils such as rice paddies (e.g. Patrick and Mahapatra, 1968). Previous laboratory results showing the importance of such factors as water content, pH, and available organic substrates and temperature will allow some predictions regarding the significance of such reactions in these soils.

The present need is for a full understanding of the losses in apparently well-aerated soils. The two established pathways of denitrification are firstly, that of denitrification at anaerobic micro-environments and secondly, the chemical losses from nitrite reactions - the 'side-tracking of nitrification' (Clark, 1962). The complete definition of such terms as anaerobic micro-environments, micro-sites, and micro-areas (Hauck, 1968), rests on further characterization of these highly reactive volumes of soils. However, these expressions are useful in indicating localized physical and chemical conditions in a soil which allow a particular transformation to proceed more rapidly than in the bulk soil. Anaerobic micro-sites are those favourable for bio-denitrification, and micro-sites may be used as a more general term, e.g. in discussing other micro-environments which may be highly favourable for chemo-denitrification such as those where high concentrations of nitrate or nitrite occur close to fertilizer granules (Hauck, 1968).

Broadbent and Clark (1965) suggest that losses at anaerobic micro-sites contribute more to total N losses from a soil than the denitrification occurring when the whole soil profile becomes temporarily anaerobic for a short period after exceptionally heavy rainfall. Furthermore, they suggest that the generally predicted order of losses of applied fertilizer nitrogen (10-30%) does not warrant the manufacture of specially formulated fertilizers which could minimize such losses, e.g. by the temporary inhibition of nitrification (Goring, 1962). It was stated that such products may be twice the cost of more commonly used fertilizers, and that it would be economically beneficial to allow for losses of 10-30% by compensatory larger fertilizer additions.

The estimated world usage of fertilizer N in 1968 was 20 m. tons, and it was estimated that this consumption rate would double within 10 years (Hauck, 1968). Using the 1968 values for consumption and the cheapest Australian source of nitrogen fertilizer (A$186 per ton urea N), such gaseous losses as 10-30% could represent a monetary loss of A$372-1,116 m. Accurate estimations of the magnitudes of losses are thus required from an economic viewpoint as well as for an understanding of the mechanisms and importance of losses.

Previous predictions of the principal factors responsible for losses have been largely based on the results of laboratory investigations. The present need is for measurement of losses under field conditions to assess the relative importance of these factors in different environments. A full knowledge of the mechanisms may enable losses to be minimized by changes in agronomic techniques rather than changes in fertilizer manufacture and use.

Prior research has accentuated the importance of losses of fertilizer nitrogen, and little is known of the losses in non-fertilized ecosystems. In Australia, relatively small amounts of nitrogenous fertilizer are applied to field crops. Land-use is largely based on the build-up of symbiotically fixed nitrogen under several years of legume-based pasture: the organic nitrogen is subsequently mineralized and utilized by one or more years of cultivation and cropping.

It is known that a large proportion of the beneficial effect of organic matter accretion under pastures is due to the increases in the amounts of partially-decomposed plant residues in the soil (Greenland and Ford, 1964). Such residues may comprise 20% of the total soil nitrogen, and supply 50% of the mineral nitrogen available to a crop (Ford and Greenland, 1968). The intense microbial activity on and around such pieces of organic material may well produce particularly favourable conditions for nitrogen losses by either bio-denitrification at anaerobic micro-sites, or 'side-tracking' of nitrification.

Recent Australian work has indicated that losses occur by bio-denitrification (Simpson and Freney, 1967) but not by chemo-denitrification (Simpson and Freney, 1967; Ross, Martin and Henzell, 1968). Further, from the work of Woldendorp (1963) with grass sods, it is possible that losses may even occur in pastures concurrent with N fixation.

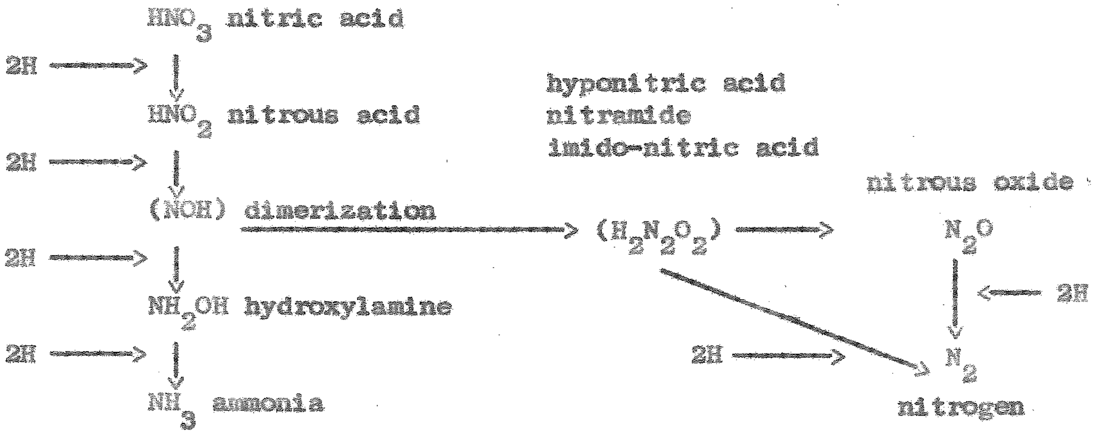
1.2. BIOLOGICAL DENITRIFICATION

1.2.1. Pathways, products and intermediates

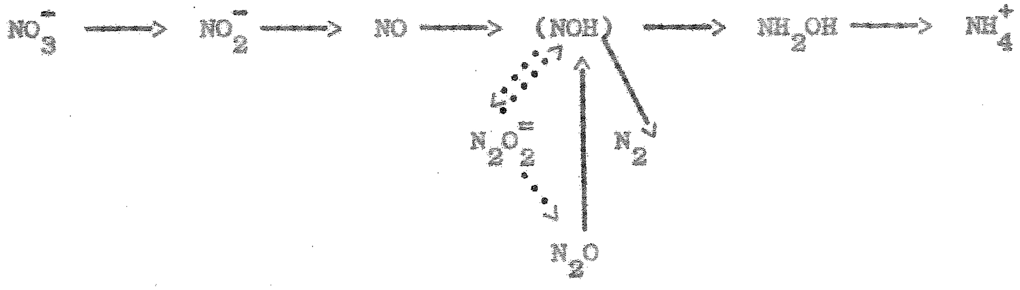
1.2.1.1. Biochemical and microbiological studies

Nitrate dissimilation can result in the reduction of nitrate through several successive oxidation-reduction states of N until either nitrogen gas, or the lowest oxidation-reduction state (ammonia) is produced.

The older schemes of dissimilatory nitrate reduction of Kluyver and Verhoeven (1954) and Delwiche (1956) have found reasonable acceptance in describing the reduction sequence:



More recent advances, resulting from an experimental emphasis on the enzymic processes involved, have indicated two changes in the above generalized scheme: the inclusion of NO as an intermediate and the non-enzymic formation of N_2O (Fewson and Nicholas, 1961):



<—— enzymic
<··· non-enzymic

There is good evidence for the existence of nitric oxide as an essential intermediate in the reduction pathway (Nicholas, 1963; Radcliffe and Nicholas, 1968), but little is known of the mechanisms of N_2O and N_2 formation from the postulated nitroxyl group. The known instability of imido-nitric acid (see Kluyver and Verhoeven, 1954) and hyponitrite do provide a logical basis for the non-enzymic liberation of N_2O, and an enzymic reaction for N_2O reversion into the pathway can explain the previously observed effect of inhibitors (Allen and van Niel, 1952; Sacks and Barker, 1952) in preventing N_2O dissimilation.

While Nicholas (1963) does not consider that N_2O is a usual intermediate, his side-reaction scheme is in agreement with previous biochemical and microbiological evidence indicating that N_2O is a common product of denitrification, that it may be dissimilated to N_2, and yet it is not an obligatory precursor (Allen and van Niel, 1952; Sacks and

Barker, 1952; Kluyver and Verhoeven, 1954; Delwiche, 1956; Sato, 1956; McNall and Atkinson, 1957). Further research on the reduction pathway from NO to N_2O and N_2 is obviously needed.

Denitrification represents the use of reduced N compounds as alternatives to oxygen as terminal hydrogen acceptors, but the level of oxygen at which denitrification occurrs has been the cause of much controversy. It has been clearly indicated (Skerman, Lack and Millis, 1951; Skerman and MacRae, 1957a, 1957b) that extremely low oxygen concentrations inhibit nitrate reduction; consequently it is conveniently assumed that an approximately zero concentration of oxygen is required to initiate microbial dissimilation of nitrate. In contrast, two reports indicate that the reduction of nitrite is not subject to such extreme inhibition by oxygen (Kefauver and Allison, 1967; Skerman, Carey and MacRae, 1958), although with the inherent experimental difficulties in such techniques, the overall effects of oxygen await further investigation.

Nevertheless, it can be concluded that dissimilation of nitrate substrates will require essentially a complete oxygen deficit, and that the denitrification products will be one or more of the three gases, NO, N_2O and N_2.

1.2.1.2. Soil incubations

Incubations of soils under laboratory conditions have clearly indicated that the usual denitrification sequence is $NO_3^- \longrightarrow NO_2^- \longrightarrow$

$N_2O \longrightarrow N_2$ (Wijler and Delwiche, 1954; Nommik, 1956; Cady and Bartholomew, 1960; Cooper and Smith, 1963; Laskowski and Moraghan, 1967). While Broadbent and Clark (1965) state that "most of the published evidence is in agreement with Nommik's (1956) conclusion that N_2O is an obligate precursor of N_2", in fact there seems to be little conclusive published evidence (including Nommik's original data) to confirm this. The normal occurrence of N_2O as an intermediate in microbiological and soil incubations indicates that it is a precursor, and the 'side-chain' mechanism (Fewson and Nicholas, 1961), if operative, would appear to be a somewhat tortuous pathway. Nevertheless, there is no satisfactory reason for rejecting the latter hypothesis. Further biochemical studies are necessary to resolve these differences.

Complete reduction to NH_4^+, the lowest oxidation state, does not seem to occur to any extent in soils (Broadbent and Stojanovic, 1952; Wijler and Delwiche, 1954; Nommik, 1956; Bremner and Shaw, 1958a), and so the common lowest nitrogen oxidation state produced has been gaseous nitrogen. Nitric oxide and NO_2 have not been reported in large amounts, and the small quantities detected (Wijler and Delwiche, 1954; Nommik, 1956; Cady and Bartholomew, 1960) have been generally attributed to the chemical self-decomposition of nitrite in acid soils ($2\ HNO_2 \longrightarrow NO + NO_2 + H_2O$). In partially aerated media, NO would be rapidly oxidized to NO_2 with subsequent dissolution in the soil moisture - $2\ NO + O_2 \longrightarrow 2\ NO_2$

$$2\ NO_2 + H_2O \longrightarrow HNO_2 + HNO_3$$

but in completely anaerobic systems the nett reaction would be

$$3\ HNO_2 \longrightarrow 2\ NO + HNO_3 + H_2O$$ (Reuss and Smith, 1965).

However, the presented evidence does not show conclusively the mechanism of NO production. The review by Nicholas (1963) indicated that nitrite reductases require a cytochrome system (i.e. an iron component) : Nommik (1956) obtained increased NO production from nitrite with the addition of reduced iron although with a lack of NO production from nitrate, the nitrite reactions were assumed to be non-enzymic. Cady and Bartholomew (1963) subsequently indicated other reactions and products were involved in NO metabolism than the chemical reaction usually given (e.g. Reuss and Smith, 1965). Evolution of NO in slightly alkaline soils has been observed (Wijler and Delwiche, 1954; Reuss and Smith, 1965) although this can be attributed to regions of lower pH close to clay surfaces (Bremner and Nelson, 1968).

Both Nommik (1956) and Wijler and Delwiche (1954) noticed that NO was dissimilated, but only slowly. This, together with the small amounts generally found, indicates that it is probably only of minor importance as a loss-product, and that N_2O and N_2 are the two-major gases concerned in losses of nitrogen from soils by biological denitrification.

The variable amounts of NH_4^+, NO, N_2O and N_2 produced during microbial dissimilation have often been discussed. While some effects are undoubtedly due to the physiological environment, there is little doubt that many can be attributed to genetic differences. Microbiological and biochemical studies have usually been accomplished with relatively pure strains of bacteria or isolated and purified enzymes, and it is probable that the reactions of these are more specific than those occurring within the wider spectrum of microflora occurring in soils. Verhoeven's (1952) results demonstrate this quite clearly; NH_4^+ was the predominant end product from some species (Bacillus licheniformis and isolates from ham), N_2O was the predominant end product from others (Bacillus nitroxus and isolates from garden soils), and with others NH_4^+ was never produced, and nitrogen was the main end product although variable quantities of N_2O were also produced (Pseudomonas spp. and Micrococcus spp.)

1.2.2. Occurrence in soils

1.2.2.1. Historical

The present concepts of biological denitrification occurring in agricultural soils have only been developed in recent years. Changes in general scientific opinion over the last century have been indicated by Verhoeven (1952), Bremner and Shaw (1958a), Delwiche (1956), Woldendorp (1963), Skyring and Callow (1962), Broadbent and Clark (1965) and Hauck (1968).

According to Warington (1897), the Rev. Angus Smith of Manchester in 1867 first demonstrated denitrification when nitrate added to sewerage was reduced to nitrogen gas. Investigations into the microbiology and chemistry of denitrification (Gayon and Dupetit, 1886) and into losses in soils (Wagner, 1895) initially caused much concern that losses could occur in well-aerated soils. This hypothesis was rapidly rejected by subsequent work (e.g. Vorhees, 1902; van Iterson, 1904), and it was generally considered for many years that denitrification was "of no economic significance in well-aerated, not too moist soils in the presence of moderate amounts of organic matter and nitrate" (Waksman, 1927). The generally high oxygen contents of the soil atmosphere (Russell and Appleyard, 1915; Russell, 1961) would have supported this conclusion.

Reports of 'aerobic denitrification' in bacterial cultures (Meiklejohn, 1940) and soils (Corbett and Wooldridge, 1940; Broadbent, 1951) revived interest in denitrification losses in agricultural soils. Additional stimulus was given by the establishment of nitrous oxide as an intermediate in the nitrogen cycle (Adel, 1946, 1951; Slobod and Krogh, 1950; Arnold, 1954; Goody and Walshaw, 1954) following reports of its presence in the atmosphere (Adel, 1939, 1941) and soil air (Kriegel, 1944).

After Broadbent's laboratory demonstration of denitrification in a well-aerated soil (Broadbent, 1951), Jansson and Clark (1952) postulated that the soil could consist of a micro-mosaic of anaerobic and aerobic spots and that, in such a system, denitrification and nitrification could occur in the one soil in the reduced and oxidized locations respectively. That denitrification required an almost zero oxygen level was shown by Skerman, Lack and Millis (1951) and Skerman and MacRae (1957a, 1957b) and the simultaneous occurrence of denitrification and nitrification was demonstrated by Loewenstein, Engelbert, Attoe and Allen (1957) and Greenland (1962).

Finally, the feasibility of such anaerobic micro-environments existing within a well-drained soil was established by theoretical and experimental considerations of oxygen diffusion rates in soils (Currie, 1961, 1962; Greenwood, 1963).

It is now accepted that denitrification can occur in a well-aerated soil. The previous bias in opinion resulted in defining the conditions necessary for optimum biological denitrification rates with respect to large volumes of mainly or totally anaerobic soils - important factors are water content, pH, temperature, availability and amounts of organic substrates, (Wijler and Delwiche, 1954; Nommik, 1956; Bremner and Shaw, 1958b; Cady and Bartholomew, 1960; McGarity, 1961).

However, little is known of the actual nature of the anaerobic micro-environments in soils, and of the relative importance of the above factors to such situations.

1.2.2.2. Anaerobic micro-sites

At present there is only one simple characteristic which can be used to define a micro-site suitable for bio-denitrification. This is the virtual absence of oxygen, which for a respiring population becomes oxygen demand > oxygen supply (Skerman and MacRae, 1957a).

Oxygen demand is determined by the respiration rate of the microbial population. Oxygen supply is determined by the diffusivity of oxygen in the soil - and in this aspect the much lower diffusion rate of oxygen in water than in air is of prime importance.

The expression "multitude of micro-environments" (Bacon, 1968) emphasizes the heterogeneity which can exist in a soil as a result of the large possible range of availabilities of organic materials of various sizes and chemical and positional availabilities, of pore sizes and shapes, and of microflora distributions. Greenwood (1968) has discussed Emerson's (1959) model of an aggregate in terms of temporal variability of respiration as influenced by various pretreatments; however, he has ignored the spatial variability in his predictions of oxygen distribution within aggregates (e.g. Greenwood, 1963).

An heterogenous array of micro-environments is in complete harmony with the earlier suggestions that continuous slow denitrification may occur by the formation of nitrate and nitrite in an aerobic spot of an aggregate, with subsequent diffusion into, and bio-denitrification in an anaerobic spot (Jansson and Clark, 1952; Broadbent and Clark, 1965). The factors considered important in creating anaerobic pockets were the restriction of O_2 diffusion by shielding due to inorganic particles, a long capillary water path, and the mass flow of CO_2 from the site of vigorous microbial activity. Greenwood (1968) has calculated that in a soil of 'normal' respiration rate, a 2 mm depth of water is sufficient to create such a micro-site. With localized greater microbial activity, such as around a piece of fresh plant material (Hauck, 1968) this distance would be greatly decreased.

The heterogeneity present in soils and soil aggregates must be the major reason for the failure of models to predict satisfactorily oxygen distributions in columns of natural soil aggregates (Greenwood and Goodman, 1967). The basic assumption for such models that respiration rates and microflora distributions within an aggregate are uniform (Currie, 1961) also cannot be tenable. Although uniformly packed columns of aggregates are extremely difficult to prepare (Greenwood and Goodman, 1967), such irregular packings must also occur in field situations.

Hauck (1968) has indicated the need for a better characterization of micro-sites : previous workers have indicated some of the difficulties, but it is apparent that this approach is essential to a better understanding of the nature of losses by bio-denitrification.

I.3. CHEMICAL DENITRIFICATION

I.3.1. Proposed mechanisms

It is generally agreed that losses by chemical mechanisms occur, and that these are associated with the nitrite ion. The transformation providing the source of nitrite is most likely to be nitrification, but the earlier suggestion of Jansson and Clark (1952) of chemical losses from nitrite formed during microbial dissimilation cannot be discounted.

The mechanisms proposed for the gaseous loss of nitrogen from nitrite in soils have been discussed in recent reviews (Broadbent and

Clark, 1965; Allison, 1966; Hauck, 1968). These comprise the reaction of nitrite with α-amino acids - the Van Slyke reaction, ammonium nitrite formation and decomposition, self-decomposition of nitrite in acid media, catalytic effect of transition elements such as Cu and Mn on chemical transformations of nitrite and nitrate, and nitrosation of phenolic groups of soil organic matter.

1.3.2. Occurrence in soils

As yet there is little well-established evidence to show the agricultural importance of these mechanisms in the soil. It has been argued that the first three reactions proceed effectively only at pH's much lower than those normally occurring in soils, and current opinion has largely rejected the importance of the Van Slyke reaction and ammonium nitrite decomposition (Allison, 1966; Hauck, 1968), despite the earlier evidence of Gerretsen and de Hoop (1957) and Allison (1963). Nitrous acid decomposition has not been considered to be of importance in soils at pH > 5, yet some results have indicated losses of NO at pH 7 (Wijler and Delwiche, 1954; Reuss and Smith, 1965; Bremner and Nelson, 1968), and Bremner and Nelson attributed this to a lowering of the pH at the clay surfaces (McLaren and Easterman, 1957; Harter and Ahlrichs, 1967).

There is little confirmation of the results of Wullstein and Gilmour (1964, 1966) indicating the catalytic effects of some transition elements on nitrite decomposition; although Nommik (1956) had previously reported an increased NO release in acid soils (by a chemical mechanism) due to the addition of ferrous iron.

The mechanism of nitrosation of phenolic groups (Bremner, 1957; Bremner and Fuhr, 1966; Bremner and Nelson, 1968; Stevenson and Swaby, 1964) of soil organic matter has only been recently proposed. Large losses of added nitrite (as N_2 and N_2O) occurred in acid to neutral agricultural soils (Bremner and Nelson, 1968), but the evolution of CH_3ONO in addition to N_2O, N_2 and CO_2 (Stevenson and Swaby, 1964) was obtained under extremely acid (pH 1.5) conditions.

The scheme proposed by Bremner and coworkers seems, for the first time, to provide a satisfactory chemical mechanism which can result in appreciable losses in normal, well-aerated soils. Their data is in agreement with previous reports indicating that losses were related to the degree of soil acidity (Clark and Beard, 1960; Clark, Beard and Smith, 1960, Reuss and Smith, 1965), soil organic matter contents (Clark and Beard, 1960; Reuss and Smith, 1965), and that losses occurred mainly as N_2, with only small amounts of N_2O (Smith and Clark, 1960; Tyler and Broadbent, 1960; Reuss and Smith, 1965; Greenland and Gasser, unpublished data). It has been shown that losses by this mechanism can result in large losses of nitrogen (30-40% of that applied) where nitrogen fertilizer is added to fertile moderately acid Iowa soils (Bremner, personal communication). However, further evaluation on a wider range of soils will be necessary to establish the full significance of this mechanism.

While the importance of losses as NO and CH_3ONO requires further investigation, it seems that losses as NO (or NO_2) will only be important in very acid soils, and that the major losses from chemical mechanisms in the majority of agricultural soils will occur as N_2 and N_2O (Broadbent and Clark, 1965; Allison, 1966).

I.4. DENITRIFICATION LOSSES IN SOIL-PLANT-SYSTEMS

1.4.1. Effect of plants

It has been proposed by Woldendorp (1963) that living plant roots significantly influence soil denitrification in two ways. Firstly, oxygen consumption by the plant root and the rhizosphere microflora results in lower oxygen concentrations in the rhizosphere than in the bulk soil. Secondly, the exudates from roots provide organic substrates which raise the biological demand for oxygen close to the plant root. A further suggestion was that the denitrifiers were stimulated by amino acids in the exudates. The combination of increased oxygen demand and lesser oxygen supply could provide increased possibilities for denitrification.

Woldendorp (1963) has indicated the relative importance of the respiration of the living roots and the microflora, and the effects of exudates from different species in stimulating denitrification losses. Living plant roots resulted in much greater losses than in grassland soils where the roots had been killed - losses of added nitrate were 17 and 9% respectively, and those of added ammonium 8 and 0%.

Much of Woldendorp's evidence was obtained by deficits in N^{15} balances. More positive evidence in the form of identification of gaseous products was desirable to provide firstly, information about the mechanisms involved, and secondly, as final conclusive proof that denitrification was involved. Stefanson and Greenland (1969) have recently provided such confirmation by the gas chromatographic measurement of nitrous oxide and nitrogen evolution in gas lysimeters (Ross, Martin and Henzell, 1964). A marked interaction with the soil moisture regime was observed - differences due to growing wheat plants occurred only in the moisture treatment approximating field capacity, and not at drier or wetter soil moisture contents.

There is now little doubt that the presence of plants in a soil can stimulate denitrification. However, while plant roots may provide a suitable anaerobic micro-environment for denitrification, rapid uptake of nitrate by the well-established plants could result in low or even negligible amounts of nitrate available for microbial dissimilation (Walker, Adams and Orchiston, 1956). Thus the effect of plants on denitrification could resolve into the result of competition between the plant and the dissimilatory microbial processes for nitrogen.

1.4.2. <u>Measurements under field and similar systems</u>

In 1954, Arnold reported the discovery of appreciable amounts of N_2O in the atmosphere of a soil. Despite criticisms which can be levelled at the techniques used, this was the first occasion on which the presence of denitrification had been satisfactorily demonstrated by the direct measurement of a gaseous product in a field situation.

Many losses have been reported from lysimeter and greenhouse experiments, based on the deficits in total nitrogen and N^{15} balances. Such evidence has shown that losses occur in well-drained agricultural soils : but the under-estimates of total nitrogen contents of soils have raised doubts on the accuracy of such methods (Martin and Skyring, 1962; Pack, 1965). Additionally, little information has been obtained regarding the mechanism of losses, and the applicability of such results to field conditions may be questioned.

Hauck (1968) has recently stressed that the measurement of losses under field conditions is of prime importance, and that such estimates should be made by direct measurement of all forms of nitrogen involved. The value of such measurements lies firstly in establishing the unquestioned occurrence of denitrification by the identification of a product, and secondly in the estimation of losses over short periods of time - with a greater opportunity of elucidating the operative processes.

The determination of denitrification products in the soil atmosphere has presented formidable difficulties (Cheng and Bremner, 1965). Nitrogen and nitrous oxide are the main products of both biological and chemical denitrification. In well-drained soils diffusion gradients will be established as soon as such products are evolved and there may be little opportunity for the accumulation of gases. Small concentrations of evolved gases must be measured, and in the case of nitrogen, against a large background of atmospheric nitrogen. Such determinations were extremely difficult with the older laborious methods of chemical analysis such as were used by Verhoeven (1952).

The techniques of mass spectrometry, infra-red spectroscopy and gas chromatography have provided accurate, sensitive, and reliable methods of gas analysis. Gas chromatography offers the most suitable method for the determination of nitrogen and nitrous oxide, as it has advantages of being rapid, sensitive, quantitative and comparatively simple (Cheng and Bremner, 1965). Furthermore, sample sizes as small as 1 ml allow the use of convenient small 'diffusion chambers' (Yamaguchi, Howard, Hughes and Flocker, 1962; Tackett, 1968), which overcome the sampling disadvantages of 'pumping' methods (Taylor and Abrahams, 1953) which were necessary in earlier work due to the large sample sizes required.

1.5. OBJECTIVES

The body of evidence suggesting the occurrence of denitrification in well-aerated agricultural soils, and the importance of nitrogen in Australian agriculture, has shown the need for measurements of denitrification products in field situations. The following objectives were chosen for this study:

(a) To determine whether denitrification losses occur in agricultural soils under field conditions, and whether these losses can be detected by measurement of the products under field conditions,

(b) and if losses do occur:

  (i) what are their magnitudes?
  (ii) under what field conditions, and by what mechanisms do they occur?

For such a system the most satisfactory initial approach appeared to be the sampling of the soil atmosphere, and subsequent analysis for the denitrification products, N_2 and N_2O, by the convenient technique of gas chromatography.

CHAPTER II. EXPERIMENT I (1966)

II.1. INTRODUCTION

The red-brown earths are a dominant soil group in the Australian landscape, and "are the mainstay of wheat growing in southern Australia..." (Stace et al., 1968). They are well drained, the B horizon being characterized by a prismatic to blocky structure, and with peds which separate easily and have well developed clay skins. As these soils have been well characterized by previous research, and are known to support an active population of denitrifying organisms (McGarity, 1961), they are a particularly suitable group on which to commence studies of denitrification in well-aerated soils.

II.2. EXPERIMENTAL

Small gas sampling devices, of the reservoir type (Tackett, 1968) were placed at 6 depths in the A, B, and C horizons of the soil profile of the Urrbrae fine sandy loam. The gas reservoirs (10 replicates at each depth) were installed immediately after the emergence of the wheat crop, which had been fertilized with nitrate of soda and superphosphate.

Measurements of soil gas composition were made during the growing season of the crop by removal of samples from the reservoirs, with subsequent analysis by gas chromatography.

II.3. MATERIALS AND METHODS

II.3.1. Experimental site

The site chosen was on an area of the Urrbrae fine sandy loam, a red-brown earth, which previously had been under pasture for several years. Some characteristics of the soil and climate are shown in Table 1; details of the experimental site and agronomic practices, and a full description of the soil are provided in Appendices I and II.

II.3.2. Gas reservoirs

II.3.2.1. Description

The gas reservoirs were manufactured in the Agronomy Workshop at the Waite Institute to the design of Dr. R.J. Millington. They consisted of a small tinned-iron can 4.1 cm diameter x 3.5 cm height, with a cone of stainless steel mesh for a base, and with a copper capillary tube (1 mm i.d.) leading from the top (Fig. 1a,b). The internal capacity of a reservoir was about 30 ml. The copper tube was sealed above the ground surface with a silicone rubber septum, and except while sampling, this sampling seal was protected from the weather with a small hood of black polythene tubing crimped at one end (Fig. 2).

II.3.2.2. Installation

The gas reservoirs were installed on the 14th July, one day after the commencement of seedling emergence.

TABLE I

Climate and soil characteristics, Experiment I

a. SOIL

Depth (cm)	Horizon	Texture	pH	% N
0-10	A	fine sandy loam	4.9	0.120
10-20	AB	clay loam	4.8	0.092
20-30	B_1	clay	5.2	0.090
30-45	B_2	clay	5.0	0.064
45-60	B_2	clay	5.5	0.065
60-75	B_2	clay	6.1	0.046
75-90	B_{ca}	clay	7.0	0.036

b. CLIMATE

Type	Mediterranean
Mean annual rainfall	24.16 in.
Growing season	
- duration	April-October
- mean rainfall	18.78 in.
Mean air temperatures (F)	
Winter (July)	51 (min.) - 57 (max.)
Summer (Jan.)	71 (min.) - 82 (max.)

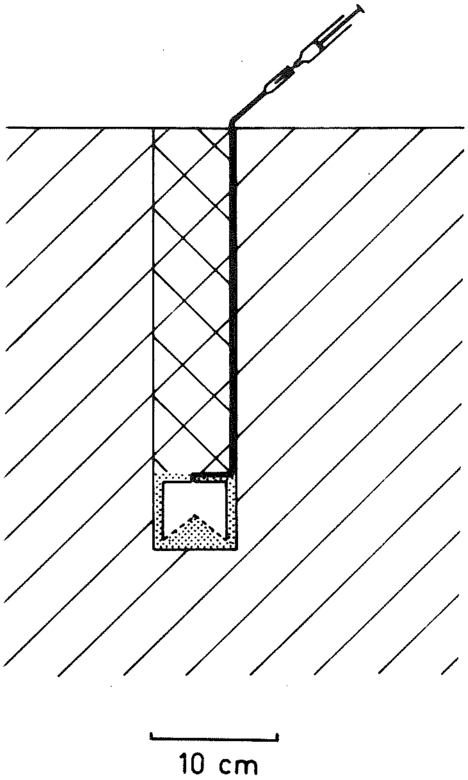
FIGURE 1. SOIL GAS RESERVOIR AND GAS SAMPLING

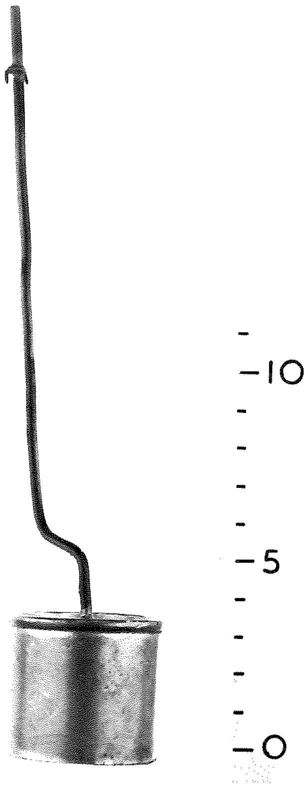
(a) Diagram of an installed reservoir.

(b) Reservoir (units of scale: cm).

(c) Sampling with 5 ml syringes (from Experiment II).

Note the rubber sheet used for transport of syringes.


10 cm


10
5
0


99

The reservoirs were placed on a thin (1 cm) bed of fine washed building sand at the bottom of a 6.1 cm diameter hole, made with a Stace-Palm modified Veihmeyer tube (Stace and Palm, 1962). Sand was used to pack the gap between the walls of the can and those of the hole. The soil was replaced with frequent tamping. Fig. 1a is a diagrammatic representation of these operations, and Fig. 2 is a photograph of one of the installed reservoirs.

II.3.2.3. Depth and location

Ten groups or replicates of reservoirs were placed over the experimental site. Each group consisted of 1 can at each of the 6 depths - 5, 10, 15 cm in the A horizon, 30 and 60 cm in the B horizon, and 90 cm in the C horizon. A different randomization was used for the position of the cans at the various depths within each group.

II.3.3. Gas sampling

"TUTA" brand disposable penicillin syringes of 2 ml and 5 ml capacity and with 25 gauge needles, were used for all gas samplings. The syringes and connections were flushed once by withdrawing a sample, of about the capacity of the syringe, from the reservoir. Then two samples, one each of 2.5 and 5.5 ml volume, were withdrawn. After removal from the septum, the syringe needle was thrust into a solid rubber sheet for transport to the laboratory. Sampling and transport techniques are demonstrated in Fig. 1c.

Figure 2. Soil gas reservoir at the 15 cm depth, partially excavated after the conclusion of Experiment I.

II.3.4. Gas analysis

A 'Shimadzu GC-1C' Gas Chromatograph, equipped with a thermal conductivity detector and employing helium as carrier gas, was used for all determinations. The columns were filled with Linde Molecular Sieve (M.S.) 5A, and operated at temperatures of 220 C and 60 C for separations of $(A+O_2+N_2) - N_2O - CO_2$ and $(A+O_2) - N_2$ respectively. At the lower temperature, N_2O and CO_2 were not eluted from the column. Further details of the gas chromatograph operating conditions are tabulated in Appendix VI.

The contents of the syringes, adjusted to 2 and 5 ml volume just prior to injection, were injected into the gas chromatograph using the normal injection port.

All analyses were computed from a basic calibration of peak height versus amount (μl) of gas present. From the 2 ml samples injected onto the columns at 60 C, the ratio of $N_2/(A+O_2)$ was calculated. The concentration of N_2O and CO_2 was calculated from the amounts of gas in the large syringe, assuming that exactly 5.00 ml of sample was injected. From this estimated concentration of $(N_2O + CO_2)$ the concentration of (argon + oxygen) and nitrogen were calculated by summation to 100%. The gas compositions were calculated only from the average peak heights for the 10 replicates at each depth, and compositions of samples from individual reservoirs were not determined. A correction for the loss of N_2O and CO_2 in the interval between sampling and injection (Appendix VII) was applied, but the time used was an approximate mean for all samples within a sampling.

II.3.5. Ancillary data

Soil samples were obtained, using the Stace-Palm modified Veihmeyer tube (5 cm i.d.) from the 0-30, 30-60 and 60-90 cm depths. Subsamples were oven dried at 105 C for gravimetric moisture contents, and at 45 C for mineral nitrogen determinations (Appendix III).

Additional estimates of soil moisture content were obtained by the neutron moisture meter method (Appendix IV) and volumetric values were converted to gravimetric basis, using approximate bulk density values based on a previous experiment conducted by Dr. R.J. Millington adjacent to the present site.

The records of the meteorological station at the Waite Institute have been used for all climatic data quoted in the text (see Appendix V).

II.4. RESULTS AND DISCUSSION

II.4.1. Composition of the soil atmosphere

II.4.1.1. Nitrous oxide

II.4.1.1.1. Occurrence

The total of 25 gas samplings were made over the period July 1966 to January, 1967. This period approximately coincided with that of crop growth, from emergence of the wheat crop in winter to maturity in early summer.

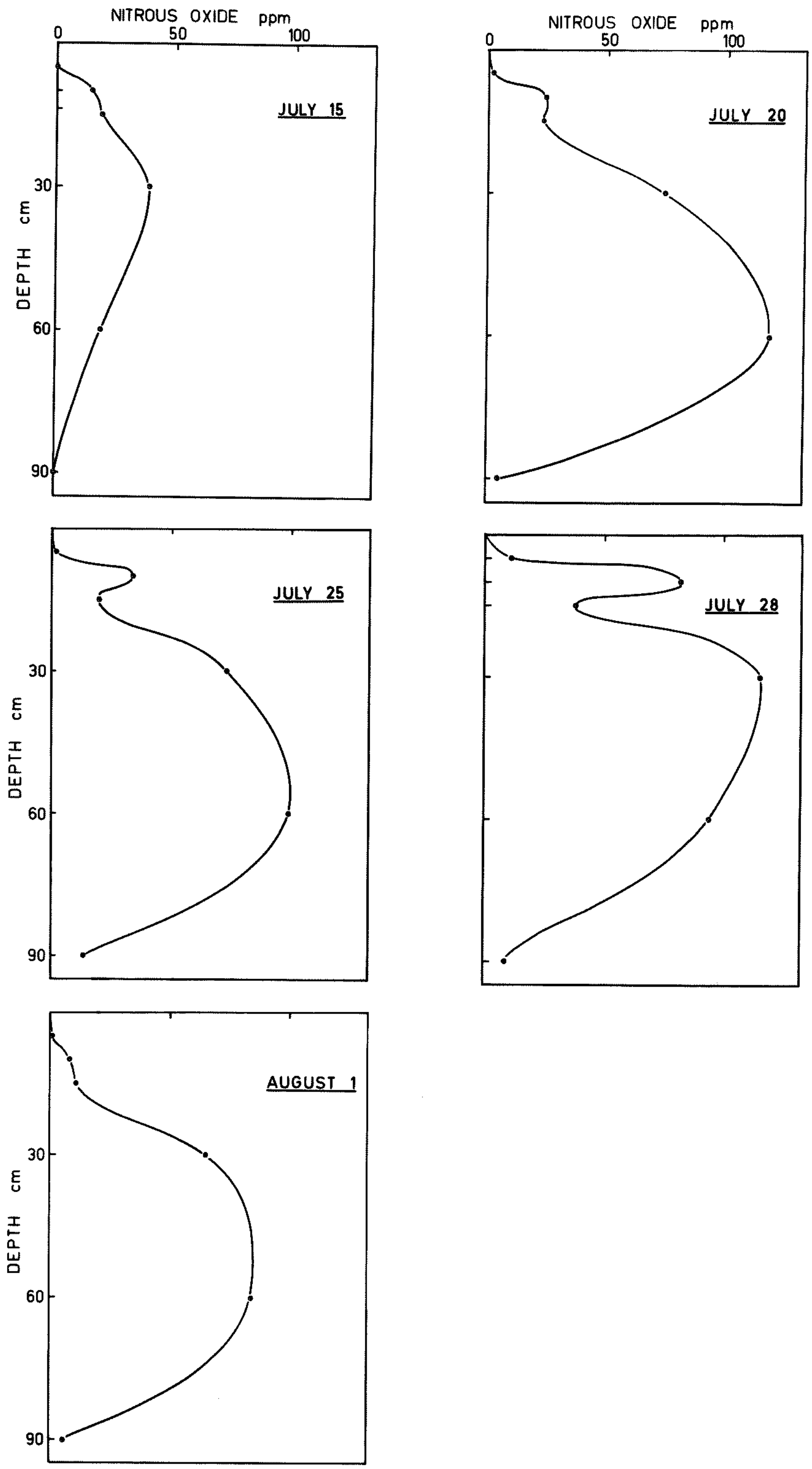
Nitrous oxide was detected in the soil profile on every sampling occasion, providing unequivocal evidence of the existence of denitrification in this soil. The very small amount of nitrous oxide, which could be detected by the gas chromatographic method (5 ppm in a 5 ml sample), allowed a great sensitivity for the detection of denitrification in the soil profile.

11.4.1.1.2. Distribution in the soil profile

Nitrous oxide was present at all except the shallowest depths at the first sampling (15th July), which was made within 24 hr of installation of the reservoirs. Wet field conditions prevailed both before this sampling, and for 14 days afterwards during which 3 additional samplings were made. The 5th sampling (1st August) was obtained after the soil had started to dry out following 2 days of fine weather.

The distribution patterns of nitrous oxide in the soil profile during this period are shown in Fig. 3. There were major accumulations of nitrous oxide at 2 depths in the soil profile; one at about 10 cm in the A horizon, and another between 30 and 60 cm in the B horizon. The concentration in the B horizon rose from about 20 ppm (v/v) for the first sampling to about 120 ppm on the second sampling, then steadily declined to 80 ppm at the fifth sampling. In contrast, the levels in the A horizon were more variable - increases occurred with the increasing soil moisture at sampling until the 28th July, then rapidly subsided, presumably due to drying of the soil between the

FIGURE 3. PROFILE DISTRIBUTIONS OF NITROUS OXIDE, 15th July-1st August, 1966.


NITROUS OXIDE ppm
0
50
100
DEPTH cm
30
60
90
JULY 15
NITROUS OXIDE ppm
0
50
100
JULY 20
DEPTH cm
30
60
90
JULY 25
JULY 28
DEPTH cm
30
60
90
AUGUST 1

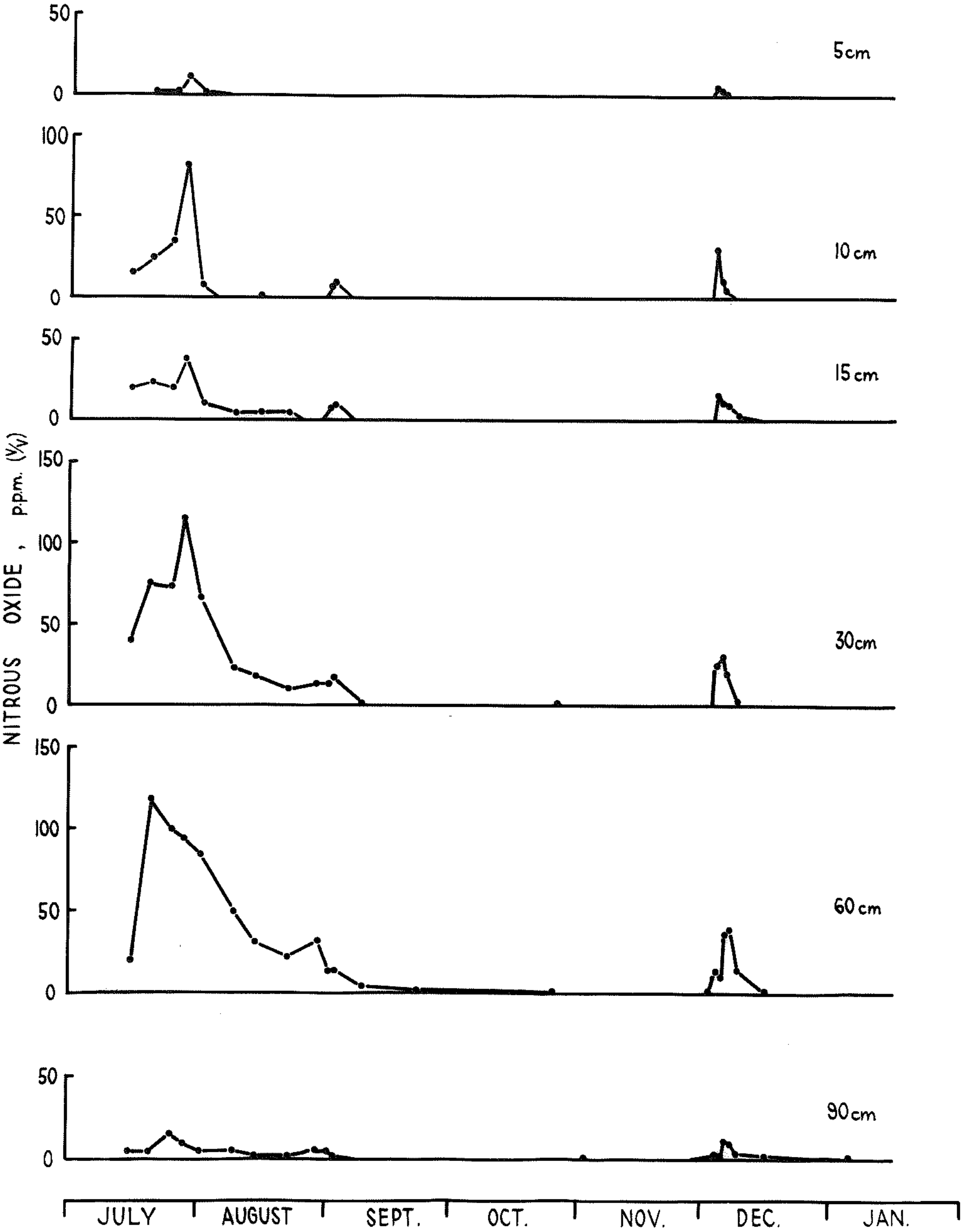
28th July and the 1st August.

The pattern of nitrous oxide concentrations in the profile for the whole year and the ancillary data of soil moisture content, soil nitrate levels, and rainfall, are shown in Fig. 4 and 5.

The contents of nitrous oxide in the atmosphere of the subsoil generally decreased over the rest of the year. The levels in the surface soil were much more variable, N_2O only being detected for brief periods of high moisture content after heavy falls of rain and the irrigation on August 31st to September 3rd. A fresh burst of denitrification occurred in December after a violent thunderstorm on 3rd December, which had been preceded by a week of rainy weather during which an additional 1 in. of rain fell. Once again, nitrous oxide was only present for a short period in the surface soil (3-4 days at the 10 cm depth), but persisted for about 30 days in the subsoil.

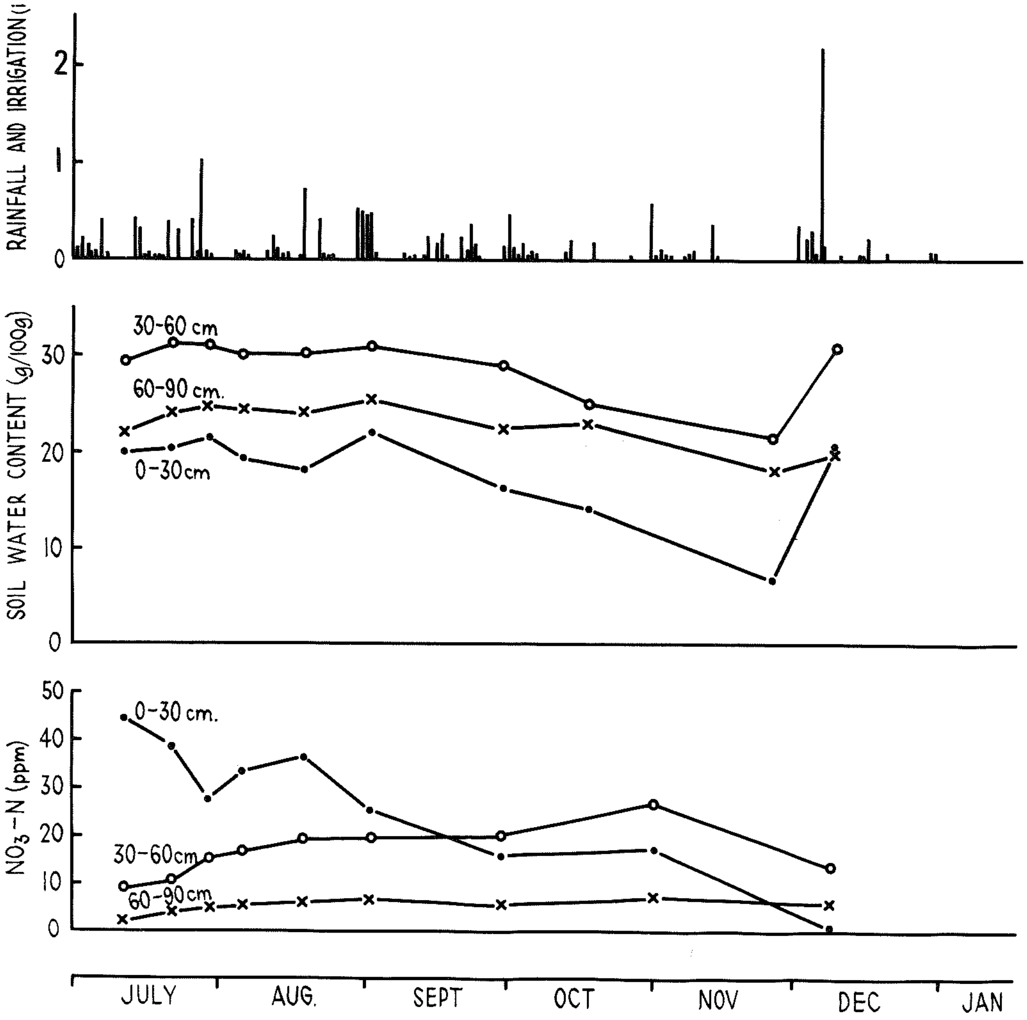
There was a pronounced tendency for nitrous oxide concentrations in the subsoil to exhibit maximum levels some days later than those in the surface. This is best shown during the flushes of denitrification occurring in the period July 15th-28th and during December. This lag in maximum N_2O levels in the subsoil may be attributed to the time taken for the soil water to move down the profile, and to organic materials dissolved in the water. The maximum water content at the 60 cm depth may not occur until several days after a heavy fall of rain (Kijne, pers. comm.).

FIGURE 4. TEMPORAL DISTRIBUTIONS OF NITROUS OXIDE IN THE SOIL ATMOSPHERE, 1966.



NITROUS OXIDE, p.p.m. (V/V)
50
0
5cm
100
50
0
10 cm
50
0
15 cm
150
100
50
0
30cm
150
100
50
0
60 cm
50
0
90cm
JULY
AUGUST
SEPT.
OCT.
NOV.
DEC.
JAN.

FIGURE 5. SOIL WATER AND NITRATE-N CONTENTS, AND DAILY PRECIPITATION, 1966.


RAINFALL AND IRRIGATION (in)
2
1
0
SOIL WATER CONTENT (g/100g)
30
20
10
0
30-60 cm
60-90 cm.
0-30cm
NO$_3$-N (ppm)
50
40
30
20
10
0
0-30 cm.
30-60cm
60-90cm
JULY
AUG.
SEPT
OCT
NOV
DEC
JAN

McGarity (1961) has shown that the low amounts of available organic substrates limit the rate of potential denitrification in samples from the Urrbrae B horizon, and that the addition of very small amounts of soluble carbohydrates produces a rapid increase in denitrification rates. Soluble organic compounds can be leached readily from plant materials and through a column of soil (Myers, 1966); and the very high, but transient, potential denitrifying activity in solodized solonetz subsoils may be due to the downward movement of small amounts of organic substrates (McGarity and Myers (1968). Additionally, the observed lag in maximum N_2O concentrations with depth in the profile may be partly due to the slower response of the subsoil to increased amounts of organic substrates and restricted oxygen supply. McGarity (1961) has shown that the length of the lag-phase is much longer and the eventual rate of denitrification is much slower for B horizon samples than for A horizon samples.

Over the period from mid-July to mid-August, about 9 ppm of the original 44 ppm of NO_3^--N in the surface 30 cm was leached into the 30-60 cm depth, causing a similar increase in soil nitrate content over the initial level of 9 ppm in this layer. There was thus only about a 20% decrease in the nitrate content of the surface 30 cm and a 100% increase in that of the 30-60 cm depth. Previous work has indicated that nitrate concentrations do not influence greatly the rate of denitrification (Wijler and Delwiche, 1954; Nommik, 1956; McGarity, 1961) although, of course, if the concentrations of nitrate negligible in the B horizon, the influx of nitrate by leaching would be important.

The ancillary data (Fig. 5) provide information on the decreasing amounts of nitrous oxide detected over the growing season. Soil moisture levels remained near field capacity during the winter (July-August) and, apart from the effect of the 2 in. irrigation in early September, decreased steadily during the rest of the growing season. Lesser amounts of rainfall were received in the spring, and the growth of the crop and increased evapotransporation associated with rising air and soil temperatures in spring resulted in a continual decrease in the soil water contents. It required a very large amount of rain (3 in.) in December to rewet the soil profile and allow denitrification to occur.

II.4.1.1.3. Estimated magnitude of losses

In a preliminary report of this experiment (Burford and Millington, 1968), an estimate was made of the losses as nitrous oxide by equating the change in mass of gas in the soil with the flux from the soil and the integrated source strength:

$$\Delta M_{t_{15}-t_{28}} = -J_{t_{15}-t_{28}} + S_{t_{15}-t_{28}}$$

where ΔM was the estimated change in total mass of gas in an acre-90 cm,
J was the estimated flux of gas to the atmosphere,
and S, the integrated source strength, was calculated from ΔM and J.

This estimate was made for the period July 15th to August 1st, when it appeared that the greatest losses were occurring, and the subscripts refer to this time interval. It was assumed that the flux to depths below 90 cm was negligible, and D, the gas diffusivity was constant with depth and time. D was calculated from:

$$D/D_o = (\varepsilon_A/\varepsilon_T)^2 \cdot \varepsilon_A^{4/3}$$

where D_o is the diffusivity in air, ε_T is the total porosity of the soil and ε_A is the air filled porosity of the soil (Millington, 1959b; Shearer, Millington and Quirk, 1966). Values of ε_A and ε_T were not measured, but from previous data values for soil at field capacity would be within the range of $\varepsilon_A = 0.05 - 0.10$ ml/ml, and $\varepsilon_T = 0.40$ ml/ml (Millington, unpublished data).

The estimates obtained for these two limits of ε_A indicated a total loss of 0.14 - 1.2 lb of nitrogen per acre as nitrous oxide. These reported values were erroneous due to use of an incorrect value for D_o : the correct estimates should have been 0.03 - 0.17 lb N/acre. However, this does not affect the main point of the calculations which was to assess the probable magnitude of the losses, and to show that these were small when compared with the 100 lb per acre of nitrogen applied as sodium nitrate at seeding.

The observed changes in soil nitrate levels (Fig. 5) also indicated that only small losses occurred. A temporary decrease in NO_3^--N, presumably due to microbial assimilation rather than dissimilation, occurred during the wet period in late July. Mineralization of nitrogen restored levels at the 13th August to only 10 ppm nitrate-N less than the initial level on 15th July, and this deficit can be accounted for by an increase of 9 ppm in the 30-60 cm depth. The decrease and subsequent increase in the 0-30 cm depth is not statistically significant ($0.10 > P > 0.05$, Appendix IX).

The method of calculating gaseous losses can be severely criticised since there were obviously two major sources of N_2O evolution in the soil profile - one in the A horizon, and the second a diffuse band in the B horizon at about 30-60 cms. The latter could be regarded as being in a quasi - steady state over the period July 20th-August 1st, as levels only ranged from 65-115 ppm of N_2O at the 30 cm depth and 84-118 ppm at the 60 cm depth. If this was the only source, it would be possible to estimate a diffusive loss from the subsoil. However, the ephemeral nature of N_2O occurrence in the surface with concentrations ranging from 8-82 ppm for these four samplings indicates that there was a variable contribution from the A_p horizon, and nitrous oxide at one sampling (July 28th) would have diffused towards the B horizon.

It is difficult to account for the apparent zone of minimum concentration at the 15 cm depth, as accretion of N_2O from the two sources in the A and B horizons should have resulted in its disappearance. The most likely explanations is that the production in the A horizon was so variable that steady state conditions for the attainment of this pattern were not achieved, and that the gas samplings were not sufficiently frequent to detect this. Alternatively, there could have been a 'sink' for N_2O at about the 15 cm depth. The most likely mechanism to account for such N_2O utilization would be further dissimilation. Biological fixation of nitrous oxide is known to occur, but gaseous nitrogen is a strong competitive inhibitor (Burris, 1956).

Despite such difficulties, it is desirable that some estimate be made of the relative contribution to losses by the A and B horizons. From the shape of the curves in Fig. 3, it can be assumed that the levels in the B horizon were in a quasi - steady state, and that there was a decreasing concentration of nitrous oxide with decreasing distance from the surface as this gas diffused to the atmosphere. Additionally, it can be assumed that the ephemeral evolution in the surface 10 cm caused a distribution pattern which became superimposed on that due to the B horizon evolution, and thus for a short period of time there was a bi-modal distribution of N_2O in the soil profile (e.g. 28th July, 1968). In this manner, an approximate estimate of the relative losses (by diffusion only) at a particular instant in time can be made, assuming independence of each source:

Horizon	A_p	B
Assumed N_2O level (ppm)	83	115
Assumed depth of source (cm)	10	45
Calculated diffusive loss of N as N_2O (lb N/acre/day)	$(0.37-3.7)\times10^{-2}$	$(0.11-1.1)\times10^{-2}$

The calculated maximum rate of loss is three times as great in the A horizon as in the B horizon. However, these calculations are based on estimated values of ε_T = 0.40 ml/ml and ε_A = 0.05 - 0.10 ml/ml. Measurements made at this site in 1967 and during Experiment II (Appendices XI and XIV) indicated that such values are satisfactory for subsoil horizons below the 10-15 cm depths. But for the surface 10 cm depth of soil, ε_A and ε_T values were much greater than 0.10 and 0.40 ml/ml respectively.

At a similar time of the year and season, values for the apparent density (ρ_A) of the 0-10 cm soil depth of sown cropland, which had previously been under long term pasture and continuous cropping, were 1.28-1.45 g/ml respectively (Experiment II); and at field capacity the moisture contents of soils were 24% and 21% (g/100 g) respectively. These values for ρ_A and moisture content from old cropland and old pastureland provide satisfactory limits for the surface soil in this experiment, based on a comparison of soil nitrogen contents (Table 1 and Fig. 19), paddock histories (Appendix 1) and previous field measurements by Millington (1959a) for a soil under crop in a 3 year pasture - 1 year wheat rotation. Thus, values of ε_T and ε_A and the estimated value for losses from the A horizon can be calculated:

<u>Assumed values for</u>

Max. N_2O at 10 cm (ppm)	83
Apparent density (g/ml)	1.28 - 1.45
Moisture content (g/100 g)	24.0 - 21.0

Calculated values

ε_W	(ml/ml)	0.31 - 0.30
ε_T	(ml/ml)	0.53 - 0.45
ε_A	(ml/ml)	0.22 - 0.15
Diffusive loss as N_2O	(lb N/a/day)	0.30 - 0.11

Therefore, for similar nitrous oxide levels, calculated maximum diffusive losses will be much greater from the surface soil than those from the subsoil, as a result of steeper diffusion gradients and greater air-filled pore space available for diffusion (Table 2).

TABLE 2

Calculated maximum losses from A and B horizons (July, 1966)

Horizon	ε_A (ml/ml)	ε_T (ml/ml)	N_2O (ppm)	Depth (cm)	N_2O diffusive loss (lb N/acre/day)
A	0.22 - 0.15	0.53 - 0.46	83	10	0.11 - 0.30
B	0.10 - 0.05	0.40	115	45	0.001- 0.011

The much higher calculated rates of losses of nitrous oxide from the A horizon indicate that the total seasonal losses are much greater from this horizon than from the B horizon, despite the more transient occurrence of nitrous oxide in the A horizon. Uncertainties in the porosity terms alone have indicated the difficulties involved in making an accurate estimate of the actual losses but the calculations indicate their order of magnitude.

In examining the losses, major attention has been paid to the mid-winter period July 15th to August 1st as it was assumed that losses were greatest at this time. The shape of the distribution at the 2 foot depth in the temporal graphs (Fig. 4) indicates that a surge of nitrous oxide production occurred in late July and that the decline in concentration in August and September was probably due to the slow diffusion of nitrous oxide from the subsoil, rather than to a slowly declining evolution.

Previous laboratory studies have indicated that nitrogen and nitrous oxide are the major products of denitrification: it is appropriate to discuss losses as nitrogen gas. Such a discussion will be clearer if considered jointly with the levels of CO_2 and O_2 in the soil atmosphere.

II.4.1.2. Oxygen, carbon dioxide and nitrogen

Profile distributions of (O_2 + A), CO_2 and N_2 for the first 5 samplings are shown in Fig. 6 and temporal distributions are shown in Fig. 7 and 8.

II.4.1.2.1. Oxygen

The oxygen levels throughout the year (Fig. 6 and 7) show trends that might be expected. Greatest departures from atmospheric levels occurred in the B horizon, where there was both a restricted diffusion due to a longer diffusion path to the atmosphere and a lower diffusivity due to the small air-filled pore space. Deficits

FIGURE 6. PROFILE DISTRIBUTIONS OF N_2O, CO_2, N_2 AND $(A+O_2)$ 15th July-1st August, 1966.

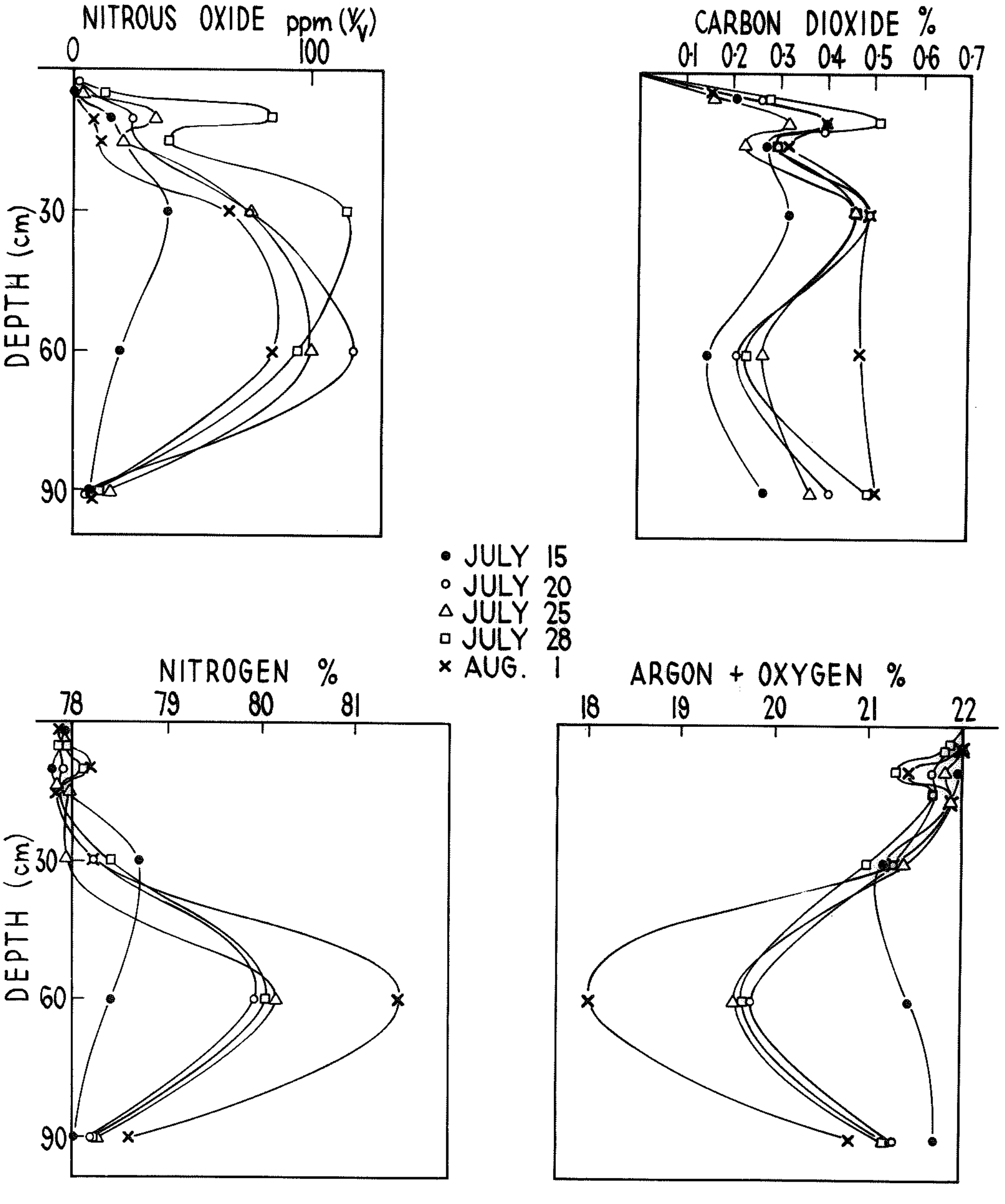
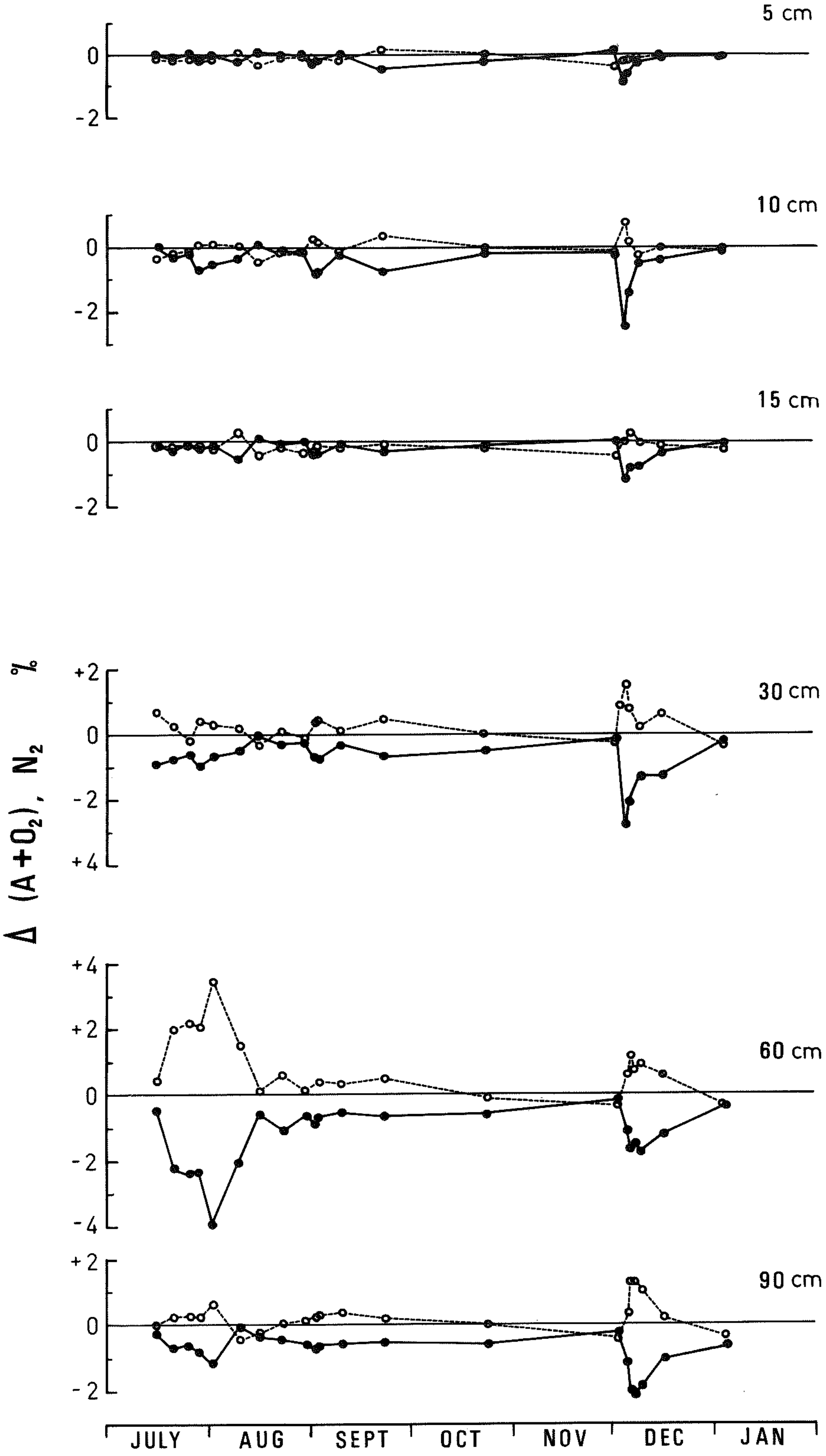

NITROUS OXIDE ppm (V/V)
0
100
CARBON DIOXIDE %
0·1
0·2
0·3
0·4
0·5
0·6
0·7
DEPTH (cm)
30
60
90
● JULY 15
○ JULY 20
△ JULY 25
□ JULY 28
× AUG. 1
NITROGEN %
78
79
80
81
ARGON + OXYGEN %
18
19
20
21
22
DEPTH (cm)
30
60
90

FIGURE 7. TEMPORAL DISTRIBUTIONS OF N_2 (O---O) and $A+O_2$ (●——●) IN THE SOIL ATMOSPHERE, 1966. VALUES PLOTTED ARE THE DEVIATIONS FROM ATMOSPHERIC CONCENTRATIONS.


5 cm
0
-2
10 cm
0
-2
15 cm
0
-2
Δ (A+O$_2$), N$_2$ %
30 cm
+2
0
-2
+4
60 cm
+4
+2
0
-2
-4
90 cm
+2
0
-2
JULY
AUG
SEPT
OCT
NOV
DEC
JAN

were greatest when the soil moisture contents were highest. The shape of the distribution patterns are indicative of soils with more restricted diffusion in subsoils than in the surface soils (Currie, 1962).

Oxygen concentrations in the A horizon were not, in general, greater than 1% below atmospheric levels in the winter period. The greatest oxygen deficit in the A horizon occurred following the heavy rain in December. This would seem to be the result of the stimulation of the microbial population by the high soil temperatures with consequent high demands for oxygen.

Oxygen levels at no stage were less than 4% below atmospheric levels (Figs. 6 and 7). The concentrations of nitrous oxide in the soil air of up to 115 ppm showed that small gaseous losses of nitrogen as nitrous oxide were occurring. Thus all denitrification must have occurred at very small anaerobic pockets within a generally well-aerated soil mass.

The close association between nitrous oxide levels and soil water content is indicative of bio-denitrification rather than chemo-denitrification. Small increases in soil water content when this is close to field capacity would have a large depressing effect on oxygen diffusion rates: the nitrous oxide levels indicate that, in some fine pores, the oxygen tension was essentially zero.

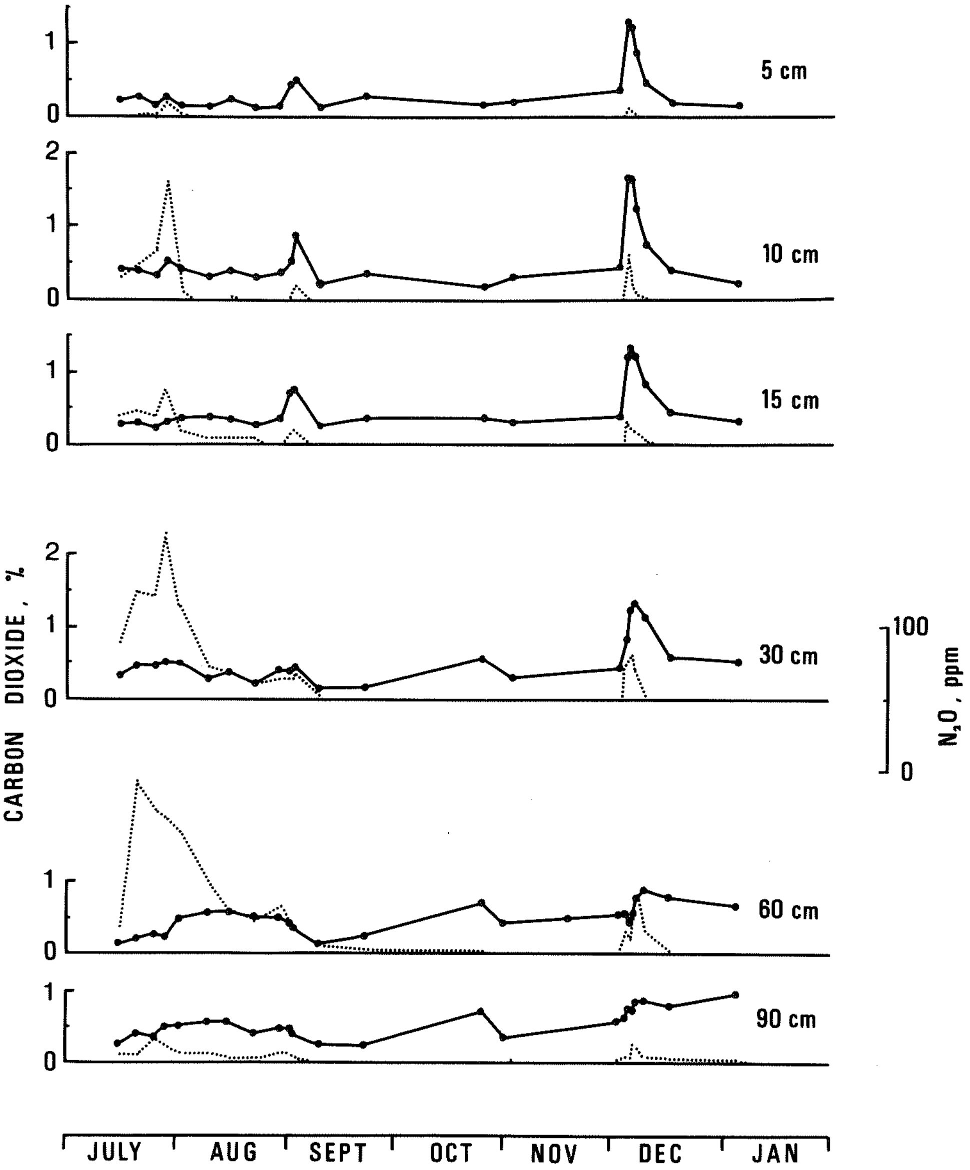
While the concept of 'aerobic denitrification' is well established, there is very little evidence available to indicate the correct methods of sampling the soil atmosphere in denitrification studies. It is possible that the detection of nitrous oxide under conditions of apparently small oxygen deficits may have been due to a fault in experimental technique: the reservoir may not contain an atmospheric composition representative of that in the soil. The column of disturbed soil above the reservoir is a possible source of error. Air channels may have been created during the repacking of the soil, allowing a more rapid diffusion between the reservoir and the above ground atmosphere. Alternatively, restricted diffusion due to excessive compaction during the repacking may have created anaerobic micro-sites in the column. There is thus a need for an examination of the soil gas sampling techniques.

II.4.1.2.2. Carbon dioxide

Carbon dioxide levels (Fig. 6 and 8) did not exhibit such variable distributions in the profile or during the year, as were noticed with nitrous oxide or oxygen. Levels in the A horizon were reasonably constant during the winter period but higher levels, associated with periods of high soil moisture content, occurred towards the end of the season. In the B horizon also, this phenomenon of increased response to soil water content with time of season was noticed, and in addition there was a very diffuse peak in late July-early August.

This behaviour of CO_2 would appear to be associated with increase in soil temperature over the experimental period (Table 3),

FIGURE 8. TEMPORAL DISTRIBUTIONS OF CO_2 IN THE SOIL ATMOSPHERE, 1966. NITROUS OXIDE DISTRIBUTIONS ARE INDICATED BY THE DOTTED LINE (••••).



CARBON DIOXIDE, %
N$_2$O, ppm
100
0
5 cm
10 cm
15 cm
30 cm
60 cm
90 cm
0
1
2
JULY
AUG
SEPT
OCT
NOV
DEC
JAN

and the large solubility of CO_2 in water. The relative amounts of CO_2 in the air and water phase contained in 1 cc of soil are shown in Table 4.

TABLE 4

Relative amounts of CO_2 contained in the air and water phase of 1 ml soil at designated temperatures and air-filled porosities (ε_T = 0.40 ml/ml)† All volumes of gases given as µl at S.T.P.

Porosity (ml/ml)		Temperature					
		10 C		20 C		30 C	
ε_W	ε_A	Soil water	Soil air	Soil water	Soil air	Soil water	Soil air
0.35	0.05	2.09	0.24	1.54	0.23	1.17	0.22
0.30	0.10	1.79	0.48	1.32	0.47	1.00	0.45
0.25	0.15	1.49	0.72	1.10	0.70	0.83	0.67

†Using values for CO_2 content of soil air of 0.5%, and the absorption coefficient of CO_2 for distilled water (Partington, 1951).

The ratio of CO_2 contained in the soil water to that in the soil air (ε_A = 0.05 - 0.10) varied from between 8.7:1 to 3.7:1 in winter (temperature = 10 C) to 6.7:1 to 2.8:1 for summer (temperature = 20 C). The large absorption of CO_2 in the soil water provides a considerable buffering capacity for the soil atmospheric CO_2 levels, and is no doubt a reason for the small effect of increased soil moisture content on CO_2 levels in the winter months.

After heavy rainfall CO_2 diffusion would be restricted; but only small increases in concentration in the soil air would result

TABLE 3

†Mean monthly soil temperatures, 1966 (°F)

Depth	Month						
	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
(Air)	54.8	50.2	52.1	55.4	59.4	67.1	66.7
2.5 cm	55.9	52.7	56.9	61.1	68.5	80.5	79.6
15 cm	54.1	51.5	53.4	57.4	63.4	72.8	73.1
30 cm	52.2	49.7	50.7	54.7	60.1	68.7	69.8
60 cm	52.4	50.1	49.9	52.8	56.8	63.4	65.4

†Source: mercury-in-steel recording thermometer, W.A.R.I. Meteorological Station.

from a large increase in a total unit volume of soil due to the buffering capacity of the soil water for CO_2. Additionally, the influx of water of lower CO_2 content and lower temperature would increase the absorptive capacity of the soil for CO_2. Carbon dioxide production would be sensibly constant with these varying water contents: the maximum oxygen deficit of 4% indicates that only a very small proportion of the soil volume was anaerobic, and Greenwood (1963, 1968) has indicated that respiration only decreases when almost complete anaerobicity occurs.

However, large increases in N_2O concentration can occur at this stage of incipient anaerobicity. The Bunsen's absorption coefficient of N_2O in water is similar to that for CO_2 (1.305 and 1.713 respectively at S.T.P. - Partington, 1951, p. 57, 574) and the solubility in soil water must have some damping effect on levels in the gaseous phase. This may be a reason for the 'tail' of nitrous oxide at subsoil depths in the spring months of September and October, and so the dissolved N_2O should be taken into account in transport equations for calculating losses of nitrogen from the soil. But dissolved N_2O may not be estimated readily from the measured gaseous concentrations and absorption coefficients. Verhoeven (1952) has shown that N_2O can be extracted from culture solutions in amounts greater than those predicted from solubility tables.

The high solubility of CO_2 in the soil water compared with that of oxygen, nitrogen and argon can explain an outstanding apparent discrepancy in the data. In the winter period (July/August) the oxygen deficits of 2-4% in the B horizon were much greater than the CO_2 concentrations of 0.3-0.5%. This would indicate a respiratory quotient of 0.08-0.25, which is much less than the values generally obtained for aerobic soils of close to unity, and values greater than unity for partially aerobic soils. Russell (1961) has previously attributed this effect to the greater solubility in water of CO_2 than that of O_2. There are numerous examples in the literature showing this effect. Calculation from the data (Table 3) clearly indicate that the deviations of O_2 and CO_2 contents, from those of a soil whose atmosphere is of the same composition as normal air, are of a similar magnitude when the comparison is made on the basis of a unit volume of soil, rather than a unit volume of soil air.

II.4.1.2.3. Nitrogen

Nitrogen concentrations of the soil air were up to 4% greater than that of the atmosphere (Fig. 7). As the standard error of the individual means was about 0.5%, such levels were indicative of an evolution of gaseous nitrogen in the soil profile. The time of appearance of the maximum concentration of nitrogen in the B horizon at the 60 cm depth in winter (1st August) was after the peak occurrence of nitrous oxide (28th July): this was in agreement with the usually expected sequential transformation of denitrification: $NO_3^- \longrightarrow N_2O \longrightarrow N_2$.

The previous calculations have demonstrated that a much greater volume of oxygen is being used by the soil than is apparently being

TABLE 5

Estimated amounts of soil gases in the soil air and soil water of 1 ml soil at a temperature of 10 C,[†] calculated for measured concentrations of the soil atmosphere (A) and soil air of standard atmosphere composition (B), assuming ε_T = 0.40 ml/ml and ε_A = 0.05-0.10 ml/ml.

	Gas			
	A + O_2	N_2	CO_2	N_2
ε_A ml/ml	0.05-0.10	0.05-0.10	0.05-0.10	0.05-0.10
FOR SOIL AIR OF COMPOSITION (%) (as at 60 cm, 1.8.66)	18.05	81.50	0.47	0.0084
‡ Absorption coefficients	0.038	0.0196	1.194	0.920
µl gas in 1 ml soil				
(a) water	2.40- 2.05	5.60- 4.79	1.96-1.68	0.027 -0.023
(b) air	9.03-18.05	40.75-81.50	0.24-0.47	0.004 -0.008
A. TOTAL WATER + AIR, µl	11.43-20.10	46.35-86.29	2.20-2.15	0.031 -0.031
FOR AIR OF STANDARD ATMOSPHERE COMPOSITION (%)	21.9	78.03	0.03	0.5 x 10^{-4}
Absorption coefficients				
µl gas in 1 ml soil				
(a) water	2.91- 2.49	5.35- 4.59	0.13-0.11	0.00019-0.00015
(b) air	10.95-21.90	39.02-78.03	0.01-0.03	0.00003-0.00005
B. TOTAL WATER + AIR, µl	13.86-24.39	44.37-82.62	0.14-0.14	0.00022-0.00020
A-B calculated difference	-(2.43- 4.29)	+(1.98- 3.67)	+(2.06-2.01)	+(0.031- 0.031)

[†] Actual mean temperature for August 1st, 1966, at 60 cm depth at Waite Agricultural Research Institute Meteorological Station was 9.4 C.

[‡] Partington, 1951. The absorption coefficient for O_2 has been used for (A + O_2), since the absorption coefficients for A and O_2 are very similar in magnitude, and the mixture would be dominantly O_2.

released as CO_2, the 'lost' CO_2 being dissolved in the soil water. This effect is most pronounced in the B horizon, and such a differential would create a volume decrease and a subsequent pressure drop, and so gradients favouring mass flow of soil air from the A and C horizons to the B horizon.

Thus, the distribution patterns of the components of the soil atmosphere are not the result of only transient-state diffusion. Obviously mass flow of gases occurred and this favoured oxygen and nitrogen transport to the B horizon and hindered the outward diffusion of N_2O, N_2, O_2 and CO_2. The observed increased N_2 levels may be attributable solely to mass flow.

Further evidence of such an effect is provided by the similar trends of increases in N_2 over atmospheric levels and the deficits of oxygen from atmospheric levels. However, it is more correct to compare the nitrogen increases with the differences between the CO_2 levels and the deficits of oxygen from atmospheric levels.

Such deviations in nitrogen content from expected atmospheric levels have been reported many times previously and usually without comment. It is therefore very surprising to find statements that, in general, the concentration of $(N_2 + A)$ in a soil atmosphere is more or less constant, or that of $(CO_2 + O_2)$ is almost constant at 21% of total gas pressure (van Bavel, 1965; McLaren and Skujins, 1968).

The difficulties caused by the mass flow of atmospheric gases in the soil profile indicate the need for allowing for this phenomenon in transfer equations. Additionally a method is needed for discriminating between the nitrogen gas evolved by denitrification, and that occurring naturally in the soil atmosphere, whether the latter is the normal 'background' 78% or the additional increment due to mass flow.

Two methods seem satisfactory: the use of N^{15} labelled fertilizers, or the comparison of N_2 levels with an inert component of the soil atmosphere, e.g. argon, which is present in the atmosphere in moderate amounts (0.93%).

The conduct of an N^{15} balance would allow accurate estimation of deficits, and so total losses of nitrogen from the soil. However, this would not allow accurate measurements of small losses over short periods of time. The cost of fertilizer N^{15} and the large experimental areas required would preclude measurement of evolved labelled N_2 in reservoirs such as those used in this experiment. The measurement of the N_2/A ratio of the soil atmosphere should be a satisfactory alternative, as it is known that the gas chromatographic separation of oxygen and argon is possible (Lard and Horn, 1960).

II.5. CONCLUSIONS

The detection of nitrous oxide in the atmosphere of the Urrbrae fine sandy loam has provided good evidence to support previous hypothesis that nitrogen can be lost, by denitrification, from an apparently well-aerated soil. High nitrous oxide concentrations occurred in the A horizon only at times of high soil moisture contents, but detectable concentrations were present in the B horizon for a total period of 3 months. The losses as N_2O were small, although accurate estimates of losses were not possible due to uncertainties in the values for air-filled and total porosity of the soil.

Further studies are needed to investigate the effects of a range of soil environments on denitrification losses from field soils. However, this initial experiment has also indicated the need for more vigorous testing of the method of sampling the soil atmosphere, and a method for detecting the nitrogen gas evolved by denitrification.

CHAPTER III. INVESTIGATIONS OF METHODS FOR DETERMINATION OF THE COMPOSITION OF THE SOIL ATMOSPHERE

III.1. INTRODUCTION

In the initial experiment, the entire experimental site had received the same cultural treatment. In further studies, it was intended to examine the effects of several treatments on the composition of the soil atmosphere. A simple and convenient gas handling system was an important prerequisite for the increased number of sampling points.

The reservoirs were very satisfactory for rapid sampling, but further work was required to test that the samples obtained were representative of the soil atmosphere. Analyses of the soil gas samples had been achieved in Experiment I within an average total time of analysis of about 5 minutes per reservoir: it was considered that this total time of analysis (including that of N_2/A measurement) was the maximum allowable where large numbers of samples were to be analysed.

III.2. GAS ANALYSIS

III.2.1. Introduction

A possible scheme of gas analysis was to use the existing gas chromatographic method for $(A + O_2) - N_2$ and $N_2O - CO_2$ analyses and to measure the N_2/A ratio by a separate measurement of A and O_2, using a third sample and a gas chromatographic column packed with molecular sieves and operated at -78 C (Lard and Horn, 1960). However, the most efficient scheme appeared to be one which would allow single-sample analysis, such as a multiple-column system of 3 columns in series, with a detection element after each (Jeffery and Kipping, 1964); and to use columns packed with molecular sieves at elevated, ambient, and subambient temperatures.

III.2.2. Experimental

Various combinations of column length, temperature, flow rate and column length were investigated for series arrangements of 3 columns packed with various sizes and types of molecular sieves.

III.2.3. Materials and methods

III.2.3.1. Column packings

Linde 4A, 5A and 13X Molecular Sieves were ground and sieved into 16-32, 32-60, 60-100 and <270 mesh sizes. After drying in air, initially at 105 C (16 hr) and subsequently at 350 C (16 hr), columns were packed with the coarse fractions. The fine material from some of these had been removed by washing with water (Fàrre-Rius and Guiochon, 1964) and removing excess water by an overnight drying at 70 C.

The finest fraction (<270 mesh) was rolled with non acid-washed 60-80 mesh Chromosorb (Bombaugh, 1963), and the excess fine material removed by gentle dry sieving with a 100 mesh sieve.

The packed columns were activated initially by heating in the carrier gas steam (dry helium) overnight at 350 C, but subsequently a period of about 40 hours was often necessary for complete activation.

III.2.3.2. Gas chromatograph modifications

The 'GC-IC Shimadzu' gas chromatograph, a dual column instrument, was used. The operating conditions are described in Appendix VI.

The inlet to the reference column was blocked. Other plumbing changes were made such that after the effluent from column 1 (inside the chromatograph oven) had passed through the sensing element of the detector, it was directed through the third column and/or the second column before passing through the old reference element of the detector. There were only two sensing elements in the detector, and a 6-way stopcock was used to change the inflow of gas to the detector 'reference' element from column 2 or column 3.

The elution patterns from each column were manipulated such that when a component was eluting from one column, there was not any eluting from the other column connected to the detector: this effluent acted as a reference. Such manipulations were achieved by varying the column lengths, packings, temperature and flow rate.

Column temperatures were controlled by the chromatograph oven at elevated temperatures (HT-column 1), a waterbath at just above ambient temperatures (MT-column 2) and a freezing mixture of solid-liquid methanol (LT-column 3).

III.2.4. Results and discussion

III.2.4.1. Chromatographic separation

Some resolution of (A + O_2 - N_2O - CO_2), (A + O_2) - N_2, and A - O_2, could be obtained with most of the prepared molecular sieve packings, but the optimum performance for each separation was obtained with MS 5A (<270 mesh/Chromosorb) (HT column, MS 5A (32-60 mesh) (MT column) and MS 13X (32-60 mesh, washed) (LT column) respectively.

The chromatogram of a soil air sample is shown in Fig. 9 and the gas chromatograph flow diagram in Fig. 10.

The compound peak of (A + O_2 + N_2) is eluted from the HT column and is resolved into (A + O_2) and N_2 by the MT column before N_2O and CO_2 elute from the HT column. Immediately after the nitrogen peak maximum has been observed, the 6-way column switching cock is operated so that effluent from the LT column flows through the detector. Argon and oxygen elute from this column after 3.8 and 4.8 minutes, and the next sample is injected 5 minutes after the previous one. The polarity of the detector is reversed for the effluent from the MT and LT columns.

This arrangement seemed quite satisfactory for use on a routine basis, and initial analyses indicated that the coefficient of variations were satisfactory (Table 6). These were determined

FIGURE 9. GAS CHROMATOGRAM OF A 5 ml SOIL AIR SAMPLE, 8th August, 1967. THE GAS CHROMATOGRAPH OPERATING CONDITIONS ARE DESCRIBED IN APPENDIX VI.

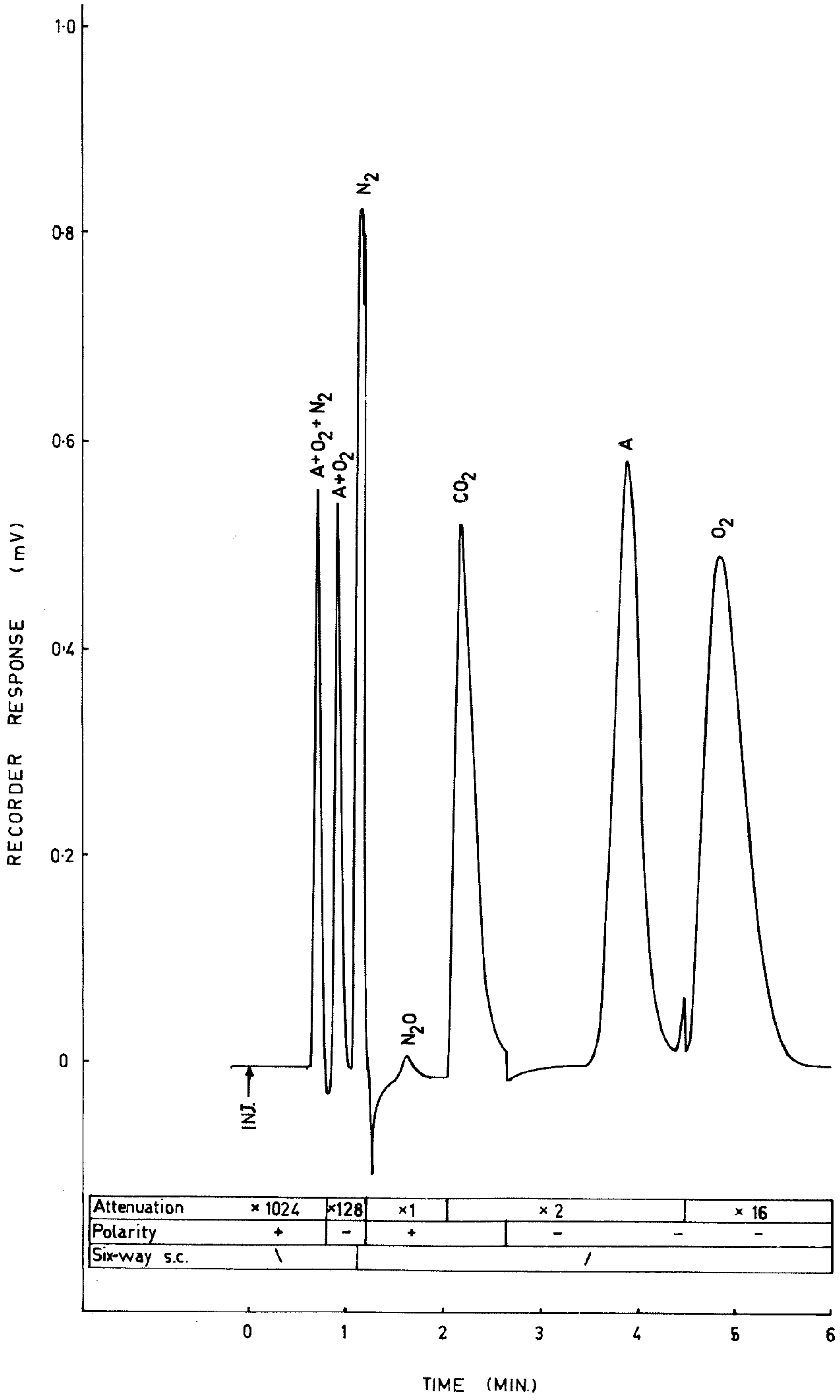
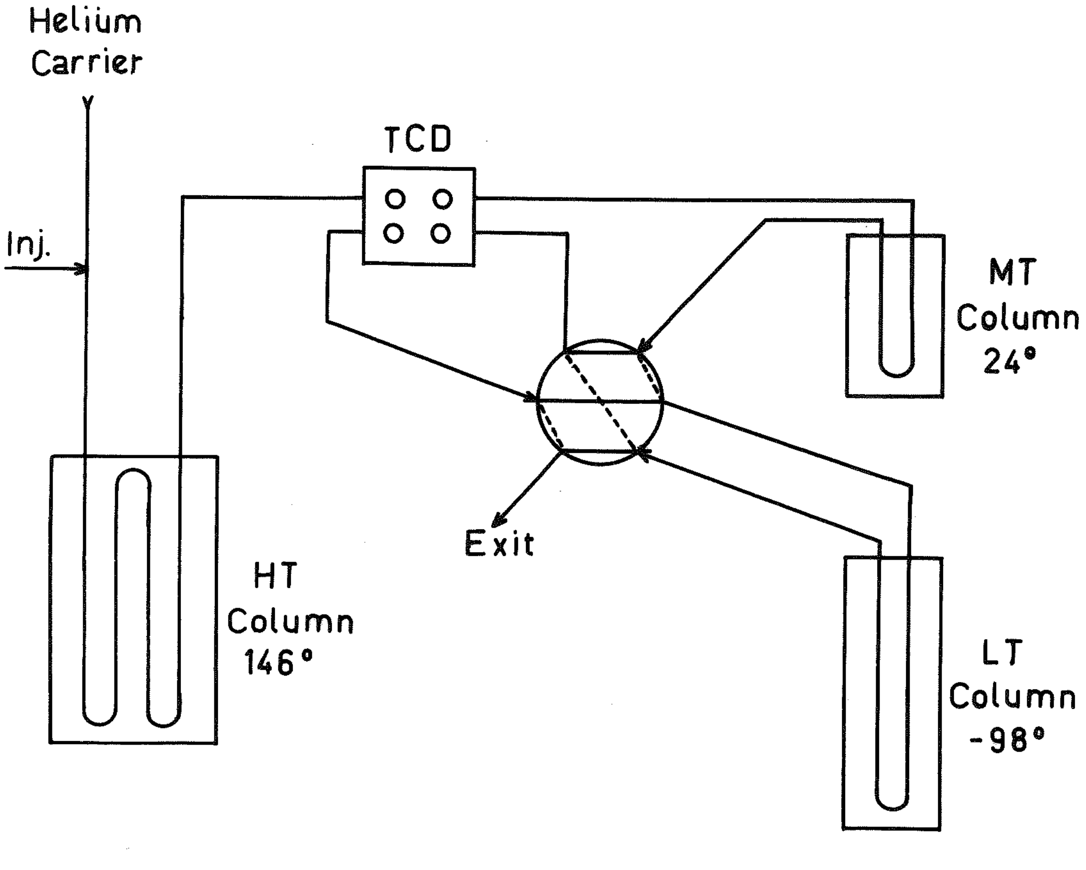

RECORDER RESPONSE (mV)
1·0
0·8
0·6
0·4
0·2
0
N2
A+O2+N2
A+O2
N2O
CO2
A
O2
INJ.
Attenuation
× 1024
×128
×1
× 2
× 16
Polarity
+
−
+
−
−
−
Six-way s.c.
\
/
0
1
2
3
4
5
6
TIME (MIN.)

FIGURE 10. FLOW DIAGRAM SHOWING THE SERIES ARRANGEMENT OF COLUMNS IN THE MODIFIED GAS CHROMATOGRAPH. THE DASHED LINE INDICATES THE FLOW PATH AFTER THE 6-way COLUMN SWITCHING COCK HAS BEEN TURNED.


Helium
Carrier
Inj.
TCD
MT
Column
24°
Exit
HT
Column
146°
LT
Column
-98°

on a peak area basis (weighing), and for this the 6-way column switching cock was not operated until all nitrogen had eluted from the MT column.

TABLE 6

Measured composition of air, and coefficients of variation†

	A	O_2	N_2	N_2/A
Composition, %	0.957	21.00	78.06	81.6
Coefficient of variation, %	1.7	1.7	0.49	2.4

† 7 determinations, peak area basis.

No column packing is yet available which allows the measurement, from a single column, of the five gases required for this study. It is well known that molecular sieves are most satisfactory for analyses of permanent gases, particularly of A, O_2 and N_2 (Jeffery and Kipping, 1964): in addition it has been found that the very finely ground material is quite suitable for N_2O and CO_2 separations. Preliminary investigations indicated that DMSO (Adlard and Hill, 1960; Trowell, 1965), porous-polymer beads (Hollis, 1966; Wilhite and Hollis, 1968) silica gel, activated charcoal alumina were not satisfactory, even for N_2O and CO_2.

III.2.4.2. Sources of error

Peak area measurements were not a convenient basis of measurement for large numbers of samples. However, several sources of error were important when analyses were made on the basis of peak height measurements.

During analyses of large numbers of samples, slight deterioration of the HT and MT columns occurred, presumably due to the adsorption of N_2O, CO_2 and water vapour. All columns were completely reactivated by heating overnight in the carrier gas stream at 350 C: but increases in the N_2 peak height of up to 5% for the same amount of gas injected occurred during a gas sampling.

Nitrogen accumulated on the LT column and produced a decrease in resolution, and an increase in peak height of oxygen and argon of about 1% per injection of 5 ml air. The column was reactivated by warming the column in a beaker of methanol at room temperature for 1 minute. The changes in the two peak heights were very similar: the error in the ratio of O_2:A, determined from volumes of gas calculated from mean calibration curves, was less than 1% for 6 consecutive samples.

These deteriorations in column performance were minimized by analysing samples in groups of 8 or 12 soil atmosphere samples, plus two air samples which were injected before and after the soil atmosphere samples. Nitrogen was removed from the LT column when one-half of the samples had been injected, and again after the 10th or 14th injection.

Soil gas compositions were calculated by firstly determining the nitrogen calibration curve, for each group of samples, from the two air samples; and by using the A and O_2 peaks to provide a ratio of O_2:A, the volumes of A and O_2 could be determined from the total volume of A + O_2, obtained from the (A + O_2) peak height calibration.

The percentage compositions of all samples were calculated by summation. Fuller details of the procedure are provided in Appendices VII and VIII.

Additional problems arose from the use of syringes in soil gas sampling: these are not normally recommended for use where accurate results are required (Jeffery and Kipping, 1964). Failure of the seal between the plunger and walls of the syringe often occurred as the syringe needle was inserted into the carrier gas stream which was at a pressure of 1.7-3.0 Kg/cm^2. Such failures were almost entirely eliminated by fitting a by-pass injector (Jeffery and Kipping, 1964) in which the carrier gas pressure could be reduced to atmospheric level by briefly opening through a one-way valve.

Nitrous oxide and carbon dioxide are lost from the air space of the syringes during the interval between sampling and injection. This was mainly due to the adsorption of N_2O and CO_2 by the walls (polystyrene) of the syringes, and the proportion lost could be estimated from the recorded interval of time (Appendices VII and VIII).

A computer program was developed to facilitate calculations of the soil gas composition by this method (Appendix VIII).

III.3. CONFIRMATION OF THE GAS CHROMATOGRAPHIC IDENTIFICATION OF NITROUS OXIDE

III.3.1. Introduction

The positive identification of peaks attributed to N_2, O_2 and A is not a serious problem since these gases are present in the atmosphere in large amounts, and other components which are likely to have similar retention times are few. The most likely is methane, which has been observed in small amounts in the atmosphere of flooded Urrbrae loam samples (Stefanson, pers. comm.), and which elutes after nitrogen from the MT column and so is not detected.

However, N_2O and CO_2 can occur in very small amounts and contributions from other components might be serious. There are two possible gases which would cause interferences: NO and CO. Carbon monoxide would elute with CO_2. Nitric oxide, like CO_2, is reactive with molecular sieves (Jeffery and Kipping, 1964) and would only give a small contribution to the CO_2 peak. In larger amounts, it should be immediately evident since its unusual peak shape (Jeffery and Kipping, 1964) prevents confusion with N_2O, which has a symmetrical peak.

The retention time of N_2O was reproducible (1.45 minutes) and was used as an identification check for small peaks in soil atmosphere samples. However, verification was required that the peak observed at this retention time, for soil air samples, was nitrous oxide.

III.3.2. Experimental

A sample of soil air was obtained from a gas reservoir at the 60 cm depth on 8th August, 1967, by connecting an evacuated flask to a sampling point. The flask was of 1160 ml total capacity, and contained P_2O_5 as a desiccant. The resultant sample containing 60 ppm N_2O was connected to the evacuated gas cell (volume 1 litre, path length 1 metre) of a Perkin-Elmer 237 infrared spectrograph, and the spectrum examined over the range of 2.5-16μ.

III.3.3. Results and discussion

The small characteristic doublet-absorption band at 4.5μ (Cheng and Bremner, 1965) was observed (Fig. 11). This was found to occur at the same wavelength, and to be of similar shape to those of a standard nitrous oxide sample, and of samples of gas in which N_2O had been identified by gas chromatography from a gas lysimetry experiment (Stefanson and Greenland, 1969). For the latter samples, the levels of nitrous oxide were higher than that present in the sample from the soil atmosphere, and the other major band at 7.8μ and the subsidiary bands at 2.9, 3.9, and 8.5 microns were also observed. These spectra are shown in Fig. 12.

While the amounts of N_2O present were too small to allow a quantitative corroboration of the nitrous oxide levels, the infrared identification has positively confirmed the presence of N_2O in the soil atmosphere.

FIGURE 11. INFRARED SPECTRUM OF A SAMPLE OF THE SOIL ATMOSPHERE. SOURCE OF SAMPLE: 60 cm DEPTH, CC ROTATION, 8th August, 1967.

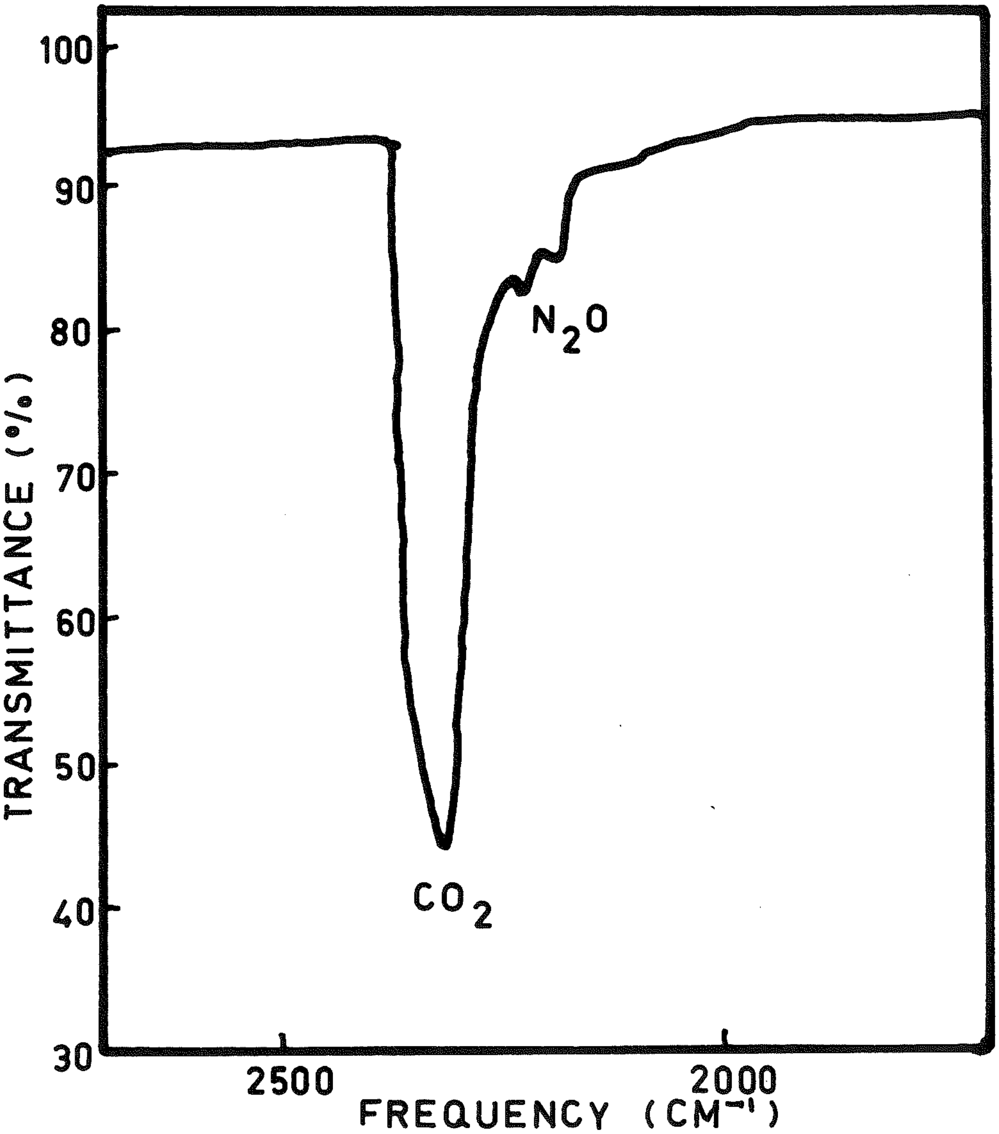
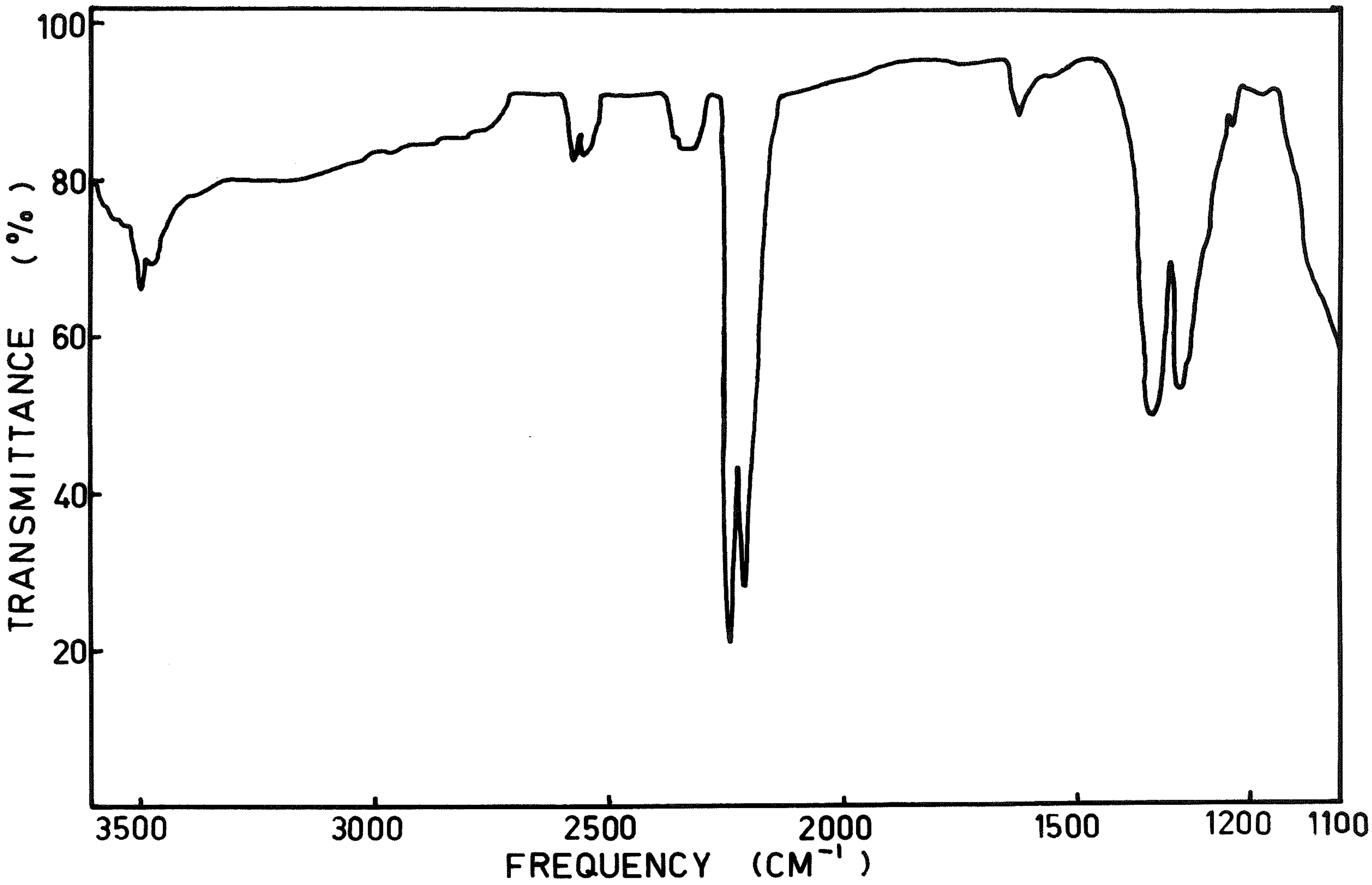

100
90
80
70
60
50
40
30
TRANSMITTANCE (%)
N$_2$O
CO$_2$
2500
2000
FREQUENCY (CM^{-1})

FIGURE 12. INFRARED SPECTRUM OF A SAMPLE OF THE ATMOSPHERE IN A GAS LYSIMETER. WATER VAPOUR AND CARBON DIOXIDE REMOVED FROM THE SAMPLE WITH P_2O_5 AND 'CARBASORB'. (REPRODUCED BY PERMISSION OF Dr. R.C. STEFANSON).



TRANSMITTANCE (%)
100
80
60
40
20
3500
3000
2500
2000
1500
1200
1100
FREQUENCY (CM^{-1})

III.4. GAS SAMPLING

III.4.1. Introduction

The gas reservoirs initially installed were similar to those proposed by other workers for measurements of the composition of the soil atmosphere (Yamaguchi, Howard, Hughes, and Flocker, 1962; Tackett, 1968). The main advances in recent years have been the reduction in size of such reservoirs associated with the development of gas chromatography and a greatly increased sensitivity of the methods for the measurement of gases. Accurate analyses can be made on samples as small as 1 ml (Yamaguchi et al., 1962; Tackett, 1968), whereas with other methods for gas analysis, samples of 30 ml (Russell and Appleyard, 1915) to 1 litre (Arnold, 1954) were used.

The reservoir must be of larger volume than the sample required as there is little doubt that "pumping" methods may lead to erroneous results (Taylor and Abrahams, 1953) and that some form of "diffusion equilibrium" method is necessary. Such a reservoir must be small enough not to exert a considerable buffering effect and introduce a time lag in reaction to changes in the soil atmosphere. This is a function of the volume and shape of the chamber in respect to the area available for exchange with the soil. In brief, the reservoir should have dimensions which minimize such buffering effects (low volume:contact area with soil), and be large enough to form a small reservoir which reduces pumping effects at sampling. In practice, of course, an ideal situation may not be completely realised, but it should be possible to reach a satisfactory compromise. The effectiveness of such reservoirs can be tested by observing the soil gas composition of samples obtained by successive extractions.

It was possible that samples obtained in Experiment I were not representative of the soil atmosphere, due to the disturbed column of soil above the reservoir. It is difficult to test this suggestion rigorously, as most sampling methods involve some soil disturbance but a suitable method appeared to be the use of alternative sampling devices.

Little mention has been made of the diurnal fluctuations in soil gas composition, due to the diurnal changes in soil temperature: this was examined to establish whether it was necessary to standardize sampling times.

III.4.2. Experimental

An examination was made of the variation in composition of samples of the soil atmosphere due to successive sampling from a reservoir, type of sampling device, and diurnal changes in temperature.

III.4.3. Materials and methods

III.4.3.1. Sampling

III.4.3.1.1. Successive sampling

Successive 5 ml aliquots were withdrawn from a gas reservoir, and some of the samples were retained for analysis. Measurements were made during both Experiment I (1966) and Experiment II (1968).

III.4.3.1.2. Sampling devices

Three types of sampling devices were investigated - the now standard reservoirs used in Experiment I, probes (Tackett, 1968) and a horizontal reservoir, a modification of that used by Myers (1966). These are shown in Fig. 13.

The horizontal reservoirs were installed by digging a small trench, making a horizontal core hole of diameter the same as the body of the actual reservoir (2.2 cm) and driving the reservoir in so that the expanded section (3.2 cm o.d.) formed a seal with the soil. The open face was lined with a plastic sheet (0.004 in. thickness) and the soil from the trench replaced. These reservoirs were installed concurrently with the replacement of the reservoirs in the A_1 horizon of the experimental area in 1968, and were placed at a depth of 10 cm on the +N plots of the CC area.

Measurements were made during June and early July, 1968. There were 8 replicates of each sampling device.

III.4.3.1.3. Diurnal fluctuations

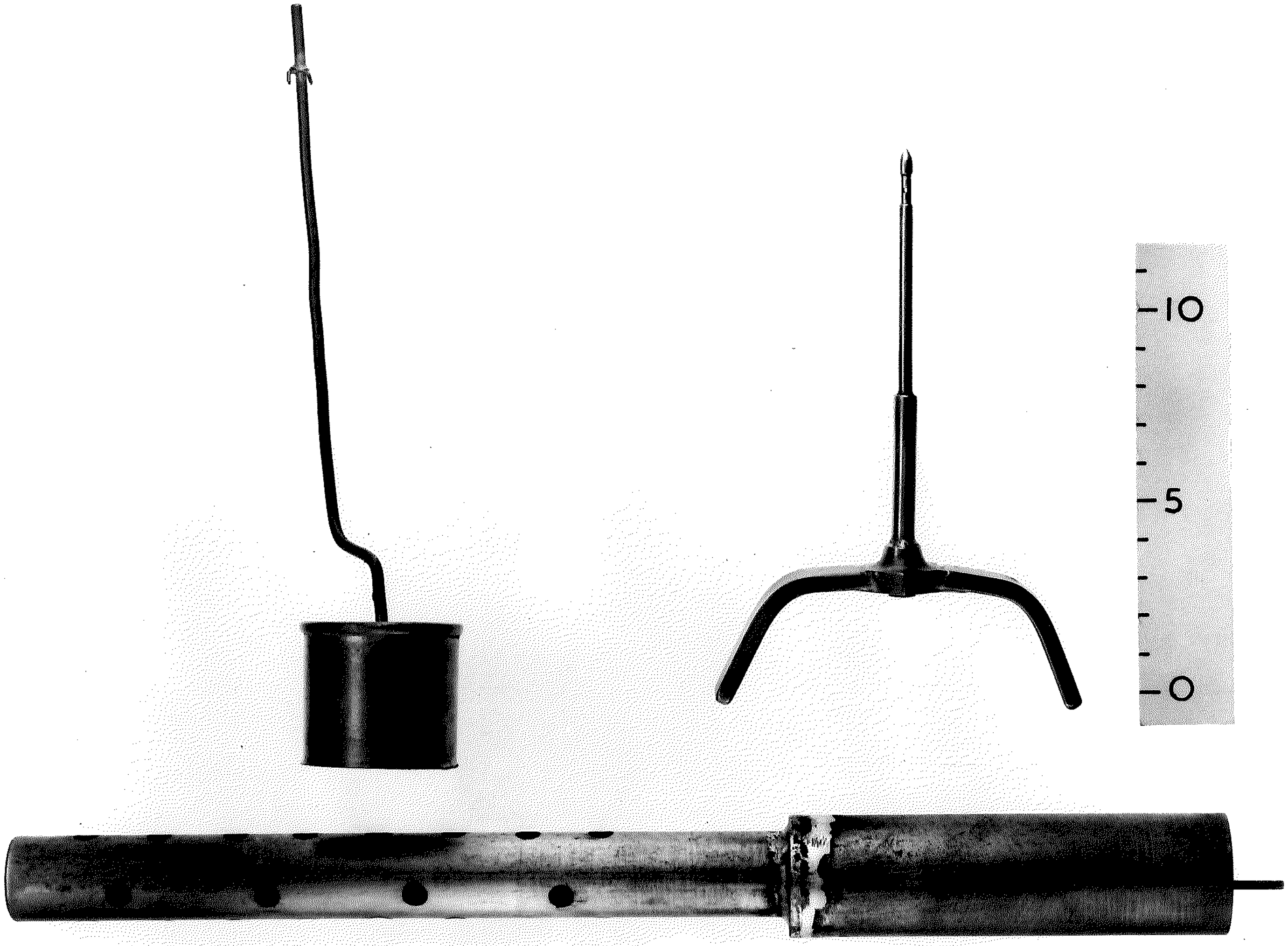
During Experiment I (1966) samples were withdrawn from the normal gas reservoirs at only 2 occasions, during a day when there was a large diurnal fluctuation in soil temperatures. Samples were taken at 10.00 a.m. and 2.45 p.m. on the 6th December, 1966, and were withdrawn from 5 reservoirs at each of the 5, 10, and 15 cm depths. Temperatures at the 2.5 cm and 15 cm depths in bare soil at the Waite Institute Meteorological Station were 20 C (both depths) at 10.00 a.m.

FIGURE 13. DEVICES FOR SAMPLING THE SOIL ATMOSPHERE (UNITS OF SCALE: cm).

(a) Standard reservoir

(b) Probe

(c) Horizontal reservoirs.


10
5
0

and 38C (2.5 cm) and 26 C (15 cm) at 2.00 p.m.

A second set of readings were taken in mid July, 1968 (Experiment II) on one reservoir at each of the 10, 30 and 60 cm depths on plot 38. Readings were obtained over a 30 hr period.

III.4.3.2. Gas analysis

All samples of the soil atmosphere were analysed by gas chromatography, using either the initial method (1966) or the improved method (Section III.2.)

III.4.4. Results and discussion

III.4.4.1. Successive sampling

The initial results obtained in 1966 (Appendix XIII) indicated that the sampling reservoirs did provide a reasonably representative sample of the soil atmosphere. However, the results were rather variable as no correction was applied for the losses of N_2O and CO_2 in the sampling syringes.

Subsequent results obtained in 1968 (Table 7) verified that the previous result was substantially true. Concentrations of CO_2 remained reasonably constant with the volume extracted, and N_2O levels were also sensibly constant until more than 1.5 times the reservoir volume had been extracted. Thus both nitrous oxide and CO_2 levels in the reservoir were representative of the atmosphere in the close vicinity of the reservoir - the decline observed in a 60 ml sample (N_2O) must be attributed to a "pumping" effect. The soil was drying

TABLE 7

Successive sampling from a soil gas reservoir at the 10 cm depth, 25th June, 1968: N_2O and CO_2 concentrations and deviations of N_2, O_2, and A concentrations from atmospheric levels†‡

Total volume removed (ml)	Sample for analysis from (ml)	Deviations from atmospheric concentrations			Levels	
		N_2 (%)	O_2 (%)	A (%)	N_2O (ppm)	CO_2 (%)
10	5-10	+0.2	-0.8	-0.004	53	0.66
20	15-20	+0.2	-0.9	-0.008	51	0.66
30	25-30	+0.6	-0.9	-0.030	50	0.61
45	40-45	+0.2	-0.9	-0.014	48	0.68
65	60-65	+0.2	-0.8	-0.022	30	0.63

† Internal volume of reservoir = 30 ml;

‡ N_2O and CO_2 values corrected for syringe losses.

at this stage, N_2O levels were declining rapidly and CO_2 values less rapidly (Section IV.3., Fig. 24 and 27) and it might be expected that the soil closer to the surface would contain much lesser amounts of N_2O but not necessarily a greatly decreased amount of CO_2. As the observed N_2O levels did not show signs of diminishing before, or as soon as, a volume equal to the capacity of the reservoir had been withdrawn, it can be assumed that the reservoirs do not exert a great buffering effect.

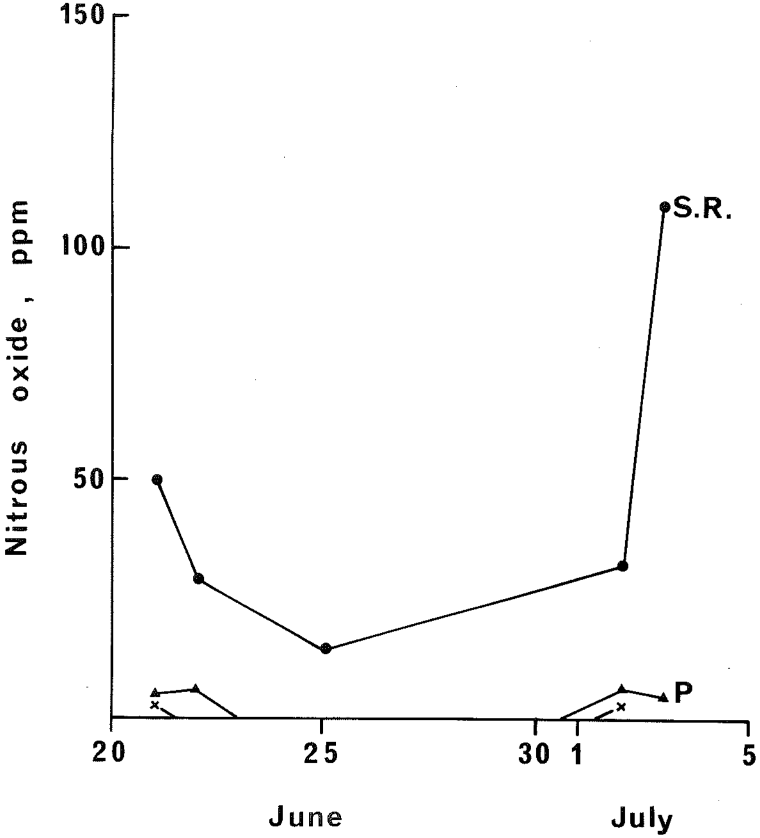
III.4.4.2. Sampling devices

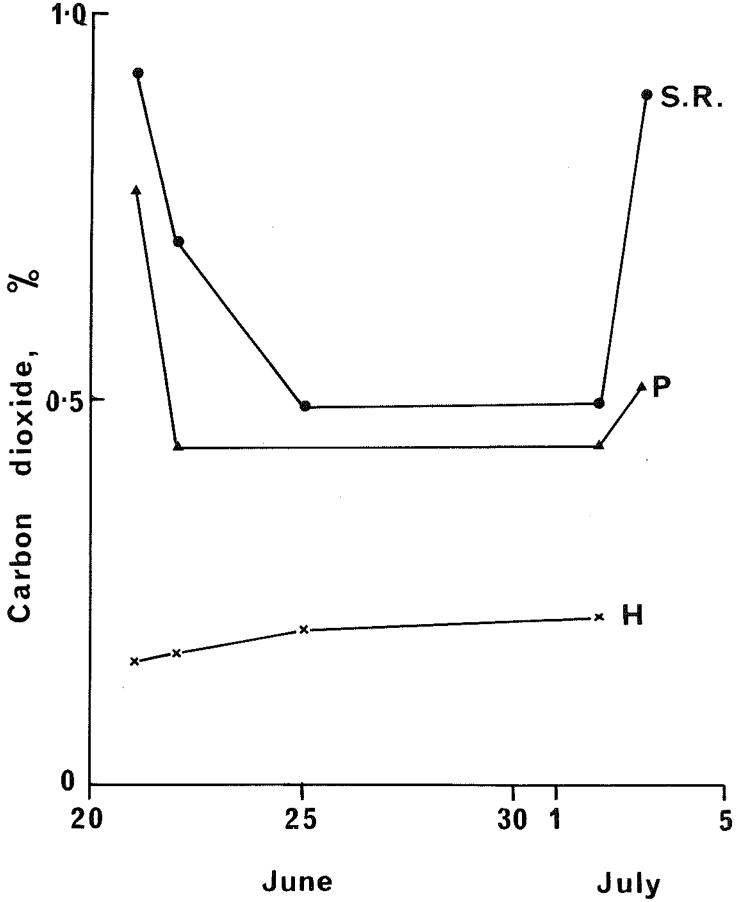
The mean values for the N_2O and CO_2 composition of the samples obtained by these three methods are shown in Fig. 14.

The low values for the horizontal reservoirs, and lack of change in levels, may be attributed to the poor sealing in the soil and the presence of large channels which allow the much greater diffusion rate of CO_2 to the atmosphere. Such channels may not become flooded even during rainfall.

The slightly lower CO_2 levels for the probe with respect to the reservoirs could in part be attributed to an imperfect seal in the soil and a tendency for mass flow of air between the probe and the soil. However, the much lower N_2O levels were a cause for concern. The above results were obtained by inserting the probe, removing a 1 ml aliquot which was discarded, then withdrawing 3 ml sample for analysis after an interval of 10 minutes.

FIGURE 14. NITROUS OXIDE AND CARBON DIOXIDE CONCENTRATIONS IN SOIL ATMOSPHERE SAMPLES FROM STANDARD RESERVOIRS (SR), PROBES (P) AND HORIZONTAL RESERVOIRS (H).


150
100
50
Nitrous oxide, ppm
S.R.
P
20
25
30
1
5
June
July


1·0
0·5
0
Carbon dioxide, %
S.R.
P
H
20
25
30
1
5
June
July

Tackett's (1968) equation predicts that the time taken for essentially complete equilibrium to be reached is approximately 2 minutes for a reservoir of height 3.5 cm. The corresponding value for the probes is 16 minutes. However, Tackett's equation does not allow for the soil gas diffusivity or for the shape of the sampling instrument (Taylor and Abrahams, 1953); the effect of the latter would be to greatly increase the differences in equilibrium times.

Subsequently samples were obtained by inserting the probe, removing an aliquot to flush the probe and syringe, withdrawing 1 ml increments of sample into the syringe at 1-2 hr intervals until the syringe was withdrawn to the 3 ml mark (Table 8). As the sampling time was protracted, measurements were made during normal gas samplings and only 5 comparisons were obtained. However, the results indicate a great improvement over the more rapid sampling, and that there is reasonable agreement between the probe and conventional gas reservoirs measurements.

It was expected that the sampling error would have been much greater for the probes than for the reservoirs, due to the much smaller area of contact with the soil; but this effect could only be shown for the nitrous oxide levels.

III.4.4.3. Diurnal fluctuation

The results from the 1966 sampling are shown in Table 9. After allowance for the loss of N_2O and CO_2 in the syringes between sampling and injection, the difference in CO_2 levels due to time of

TABLE 8

Comparison of reservoir and probe samplers: mean concentrations of N_2O and CO_2, and mean deviations from atmospheric levels of N_2, O_2 and N_2/A†. Parentheses indicate $\log_e$ transforms.

Measurement	Sampler		L.S.D. (P = 0.05)
	Reservoir	Probe	
N_2 (%)	−0.42	0.04	1.27 (NS)
O_2 (%)	−2.18	−1.40	1.56 (NS)
N_2/A	−0.56	0.43	1.76 (NS)
CO_2 (%)	1.73	1.25	0.78 (NS)
‡ N_2O (ppm)	26	57	–
	(3.18)		(S.E. = 0.45)
		(3.14)	(S.E. = 1.60)

† Paired measurements on 5 plots.

‡ S.E. standard error ($s/\sqrt{n}$)

The variances of the transformed N_2O levels were significantly different ($P < 0.05$).

TABLE 9

Nitrous oxide and carbon dioxide contents of the soil atmosphere at 3 soil depths at 10.00 a.m. and 2.45 p.m., 6th December, 1966.

	Depth (cm)			Mean			
	5	10	15				
NITROUS OXIDE (ppm)							
Time 10.00 a.m.	1	3	5	3			
2.45 p.m.	1	5	5	4		†N.S.D.	
Mean	1	4	5				
						L.S.D.	
CARBON DIOXIDE (%)						0.05	0.01
Time 10.00 a.m.	0.80	1.15	1.15	1.03	DxT	0.39	0.51
2.45 p.m.	0.99	1.54	1.27	1.27	D	0.28	0.38
Mean	0.90	1.34	1.21		T	0.22	0.30

† No significant differences.

of day is significant. The N_2O levels were too close to the limit of detection to provide any significant information.

The results from 1968 indicate that the diurnal variation was small - even at the 10 cm depth where diurnal temperature fluctuations were largest (Fig. 15). The data show that soil conditions such as soil moisture content, have much greater effects than those temperature fluctuations.

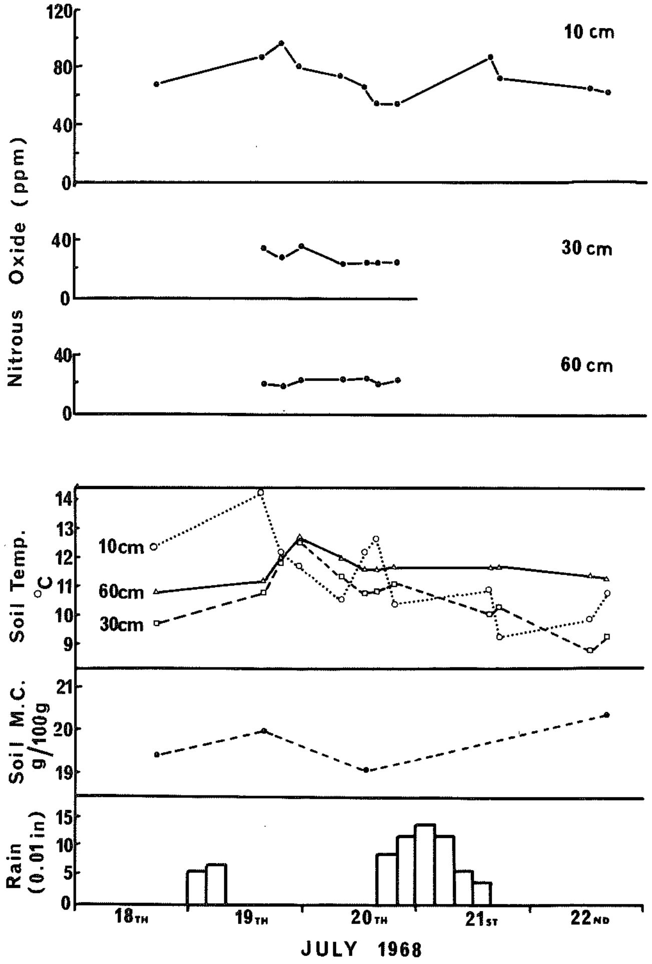
III.4.5. Conclusions

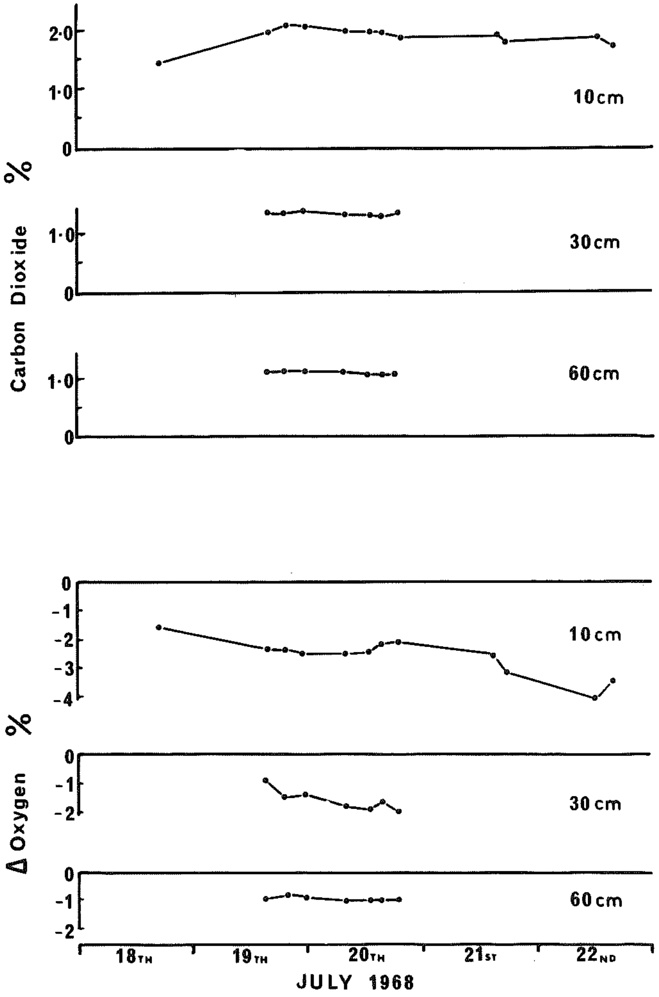
The results have indicated that the diurnal variation in soil gas composition is small, particularly during the winter months when the daily range in temperatures is not great. Prior to the establishment of this fact sampling times had been standardised between the hours of 10.00-12.00 a.m., except when prevented by unfavourable weather conditions.

These simple experiments have indicated that the 'standard' reservoirs used in the initial experiment provided a satisfactory estimate of the composition of the soil atmosphere for the purposes of this investigation.

They are convenient to install, and sampling can be achieved rapidly. Sampling error due to spatial heterogeneity of the soil is reduced: the area in contact with the soil atmosphere is much larger than that for the probes. In addition, in observations of the sequential changes in the soil gas composition, sampling errors are further minimized by the use of permanent sampling points.

FIGURE 15. DIURNAL FLUCTUATIONS IN N_2O, CO_2 and O_2 CONCENTRATIONS IN THE SOIL ATMOSPHERE, SOIL TEMPERATURES AND MOISTURE CONTENTS, AND RAINFALL OVER FOUR HOURLY PERIODS, JULY, 1968.


Nitrous Oxide (ppm)
120
80
40
0
10 cm
40
0
30 cm
40
0
60 cm
Soil Temp. °C
14
13
12
11
10
9
10cm
60cm
30cm
Soil M.C. g/100g
21
20
19
Rain (0.01 in)
15
10
5
0
18TH
19TH
20TH
21ST
22ND
JULY 1968


% Carbon Dioxide
2·0
1·0
0
10cm
1·0
0
30cm
1·0
0
60cm
Δ Oxygen %
0
-1
-2
-3
-4
10cm
0
-1
-2
30 cm
0
-1
-2
60 cm
18TH
19TH
20TH
21ST
22ND
JULY 1968

CHAPTER IV. NITROUS OXIDE CONCENTRATIONS AND NITROGEN:ARGON RATIOS IN THE SOIL ATMOSPHERE OF A RED BROWN EARTH

IV.1. INTRODUCTION

The initial experiment indicated that a study of the losses of nitrogen in gaseous forms over a range of soil environments was needed to establish relative values for the losses occurring in an agricultural soil, and to indicate the mechanisms responsible for these losses. The Urrbrae fine sandy loam is well known for its rapid decline in structural stability and organic matter contents when cultivated (Clarke and Marshall, 1947), and improvement in these properties by several years of pasture (Greenland, Lindstrom and Quirk, 1962): thus this soil provides a range of soil conditions suitable for the present study.

Previous work has shown that changes in organic matter contents of the Urrbrae series are accompanied by greater changes in the contents of partially decomposed plant residues (Greenland and Ford, 1968). These make an important contribution to the total rate of mineralization of the soil nitrogen: the rapid mineralization of such materials during the opening of an old pasture should provide conditions suitable for the 'side-tracking' of nitrification, or might provide centres of rapid respiration at which bio-denitrification could occur. Alternatively, the poor structure and thus severe consolidation of seed beds on old cultivated land (Millington, 1959a) may be more effective in creating anaerobic micro-environments, despite the lower organic matter levels in such soils.

Thus, in addition to including measurements of the N_2/A ratio in further experimentation, it was decided to compare the compositions of the soil atmospheres underneath wheat crops grown on old cropland and old pasture land, and from underneath wheat crop and pasture grown on old pastureland; and additionally to measure the effects of irrigation and applications of nitrogen fertilizer.

IV.2. PART I, 1967 SEASON

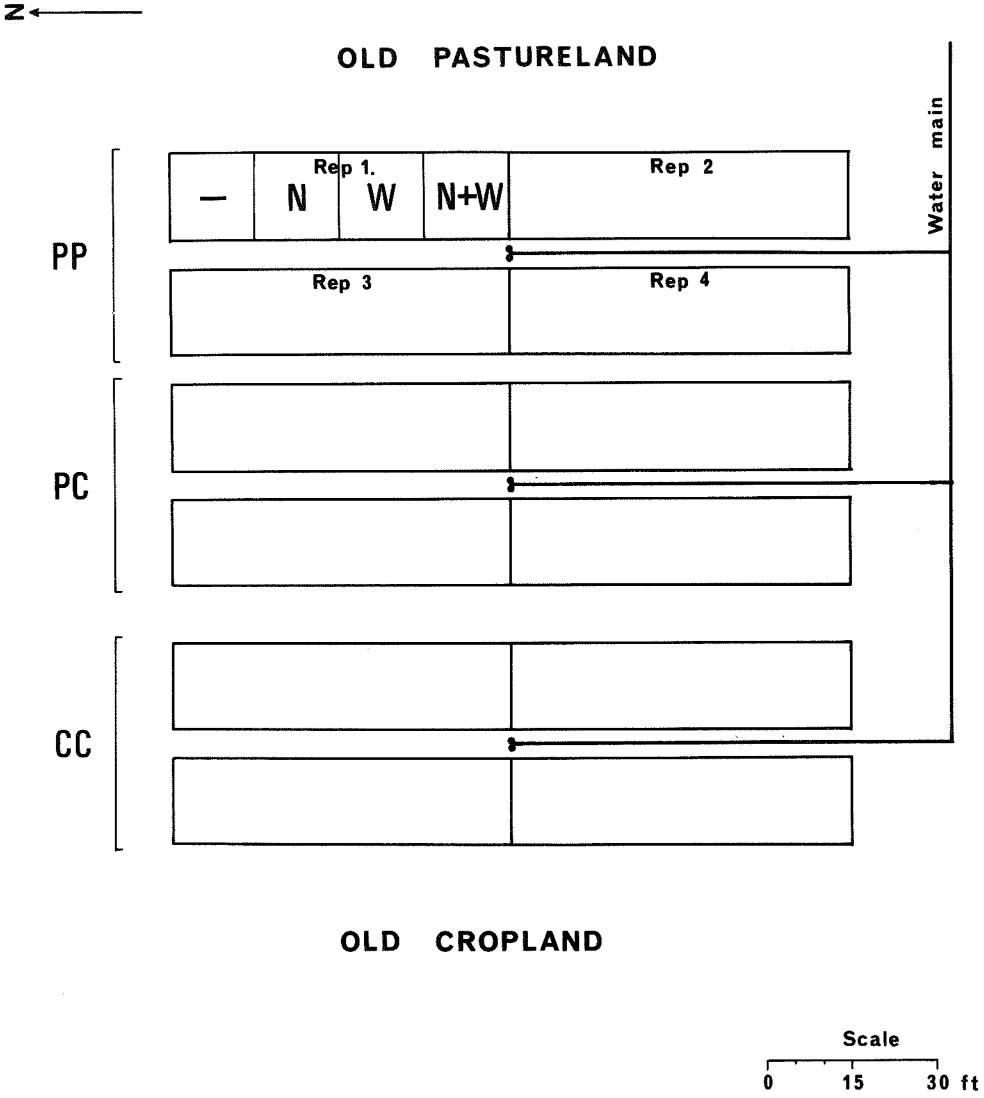
IV.2.1. Experimental

Gas reservoirs were established within the soil profile in three parallel, rectangular, adjacent areas each 1.1 square chains in area. The three areas were located on adjacent paddocks, which had long histories of predominantly pasture and crop management (Fig. 16). Two areas were sown to 'NAPO' wheat, a variety recommended by Dr. A.J. Rathjen for its response to environmental variables such as water and nitrogen. The third area, from the pasture paddock, was maintained as a permanent pasture. These three rotational treatments have been subsequently denoted:

PP - former pasture area, under pasture (annual)
PC - former pasture area, under wheat crop
CC - former cropped area, under wheat crop

Treatments of irrigation and fertilizer nitrogen were applied to each area in a 2x2 factorial with four replicates. Each plot so

FIGURE 16. EXPERIMENTAL LAYOUT: 1967/68.


N
OLD PASTURELAND
Water main
PP
Rep 1.
—
N
W
N+W
Rep 2
Rep 3
Rep 4
PC
CC
OLD CROPLAND
Scale
0
15
30 ft

formed contained one gas reservoir at each of the three depths: at 7.5-10 cm in the A_1 horizon, 30 cm in the A_2 horizon and 60 cm in the B horizon. This soil had a deeper A horizon than that in Experiment I, and these locations were chosen as representing the two depths of maximum N_2O concentration and the one of apparent minimum concentration previously observed.

The composition of the soil atmosphere was estimated by gas chromatography, and some ancillary measurements of soil moisture content, and nitrate levels were also obtained.

IV.2.2. Materials and methods

IV.2.2.1. Experimental site

The basic unit in the experiment was a 15 ft square plot, containing 3 gas reservoirs, one at each of the designated depths, and a neutron moisture meter (NMM) access tube. The NMM access tube and gas reservoirs were all situated on a circle of 4'6" radius from the centre of the plot.

Treatments were applied on a plot basis, and consisted of a 2x2 factorial of plus nitrogen fertilizer (+N) and irrigation (+W) treatments arranged in a randomized block design, with four replicates. Nitrogen was applied as nitrate of soda (100 lb NO_3-N/acre), and irrigation was achieved by installing a sprinkler in the centre of each (+W) plot.

Figures 16, 17 and 18 illustrate the field and plot layout.

FIGURE 17. PLOT LAYOUT, 1967/68 (THERMISTORS WERE INSTALLED IN 1968 ONLY).

KEY

○	soil gas reservoir
●	NMM access tube
•	thermistor
---	thermistor leads
■—	capillary tube from soil gas reservoir
*	plot centre; sprinkler on +W plots (1967 only).

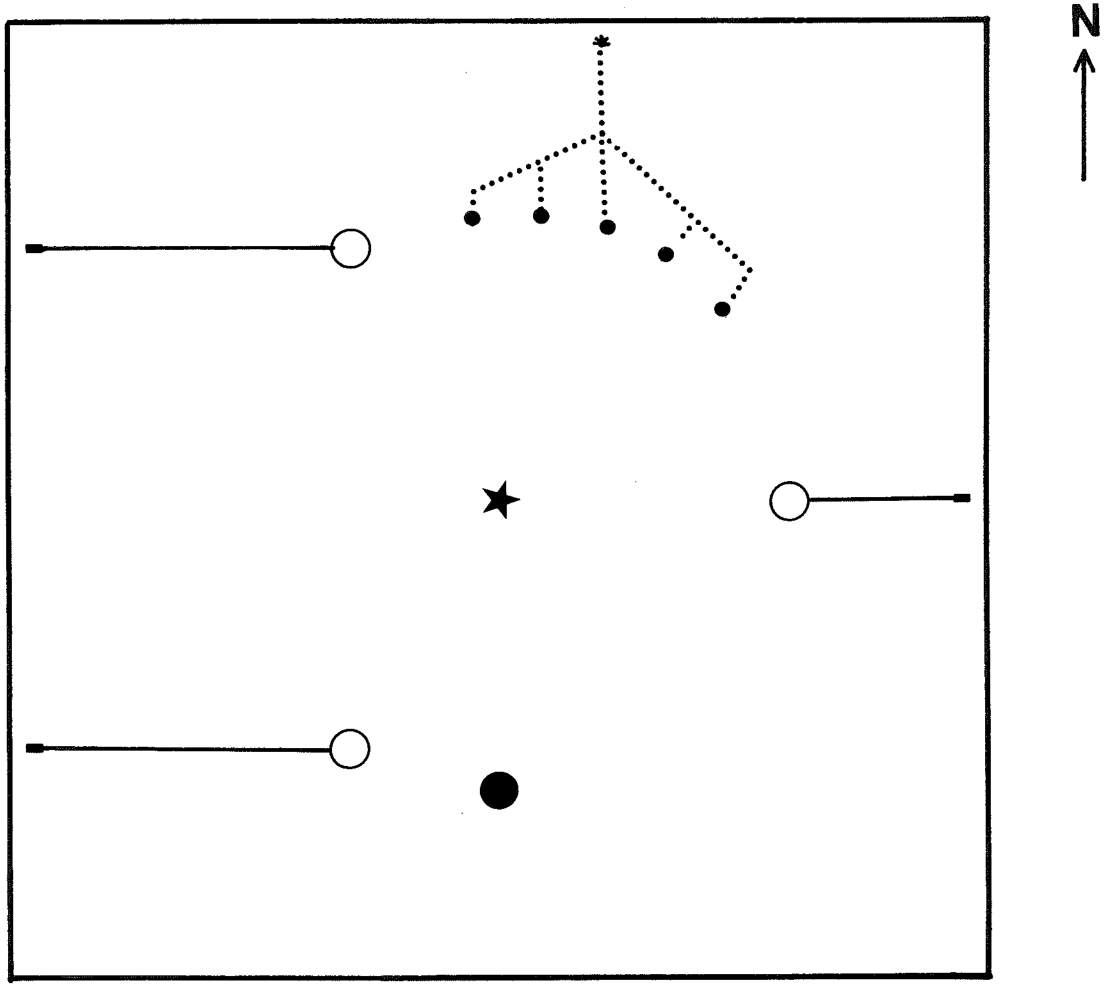

N

FIGURE 18. PHOTOGRAPHS OF THE 1967/68 EXPERIMENT, FACING:

(a) South towards the CC rotation

(b) South-east towards the CC rotation

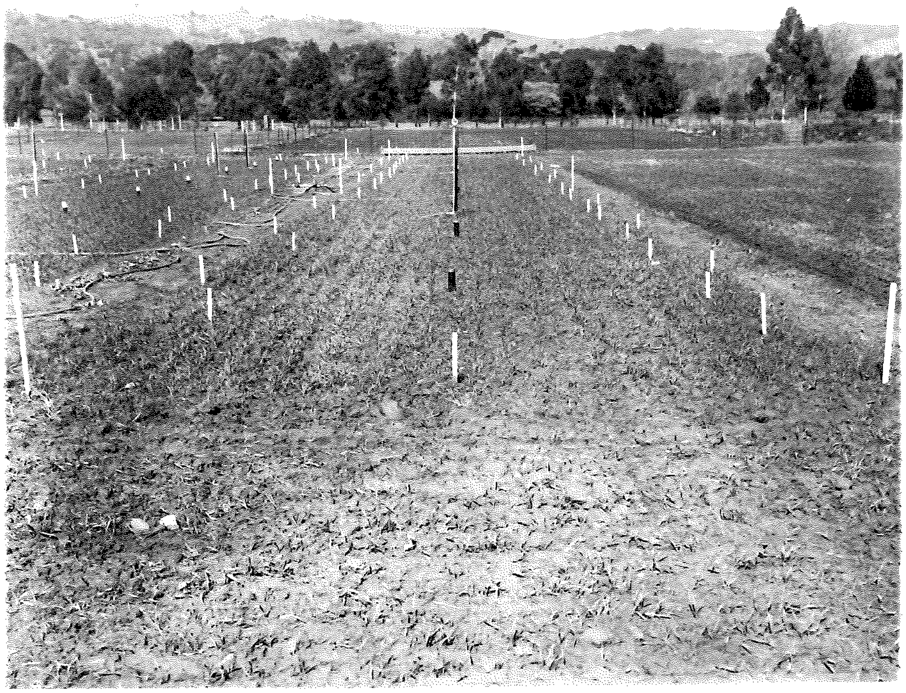
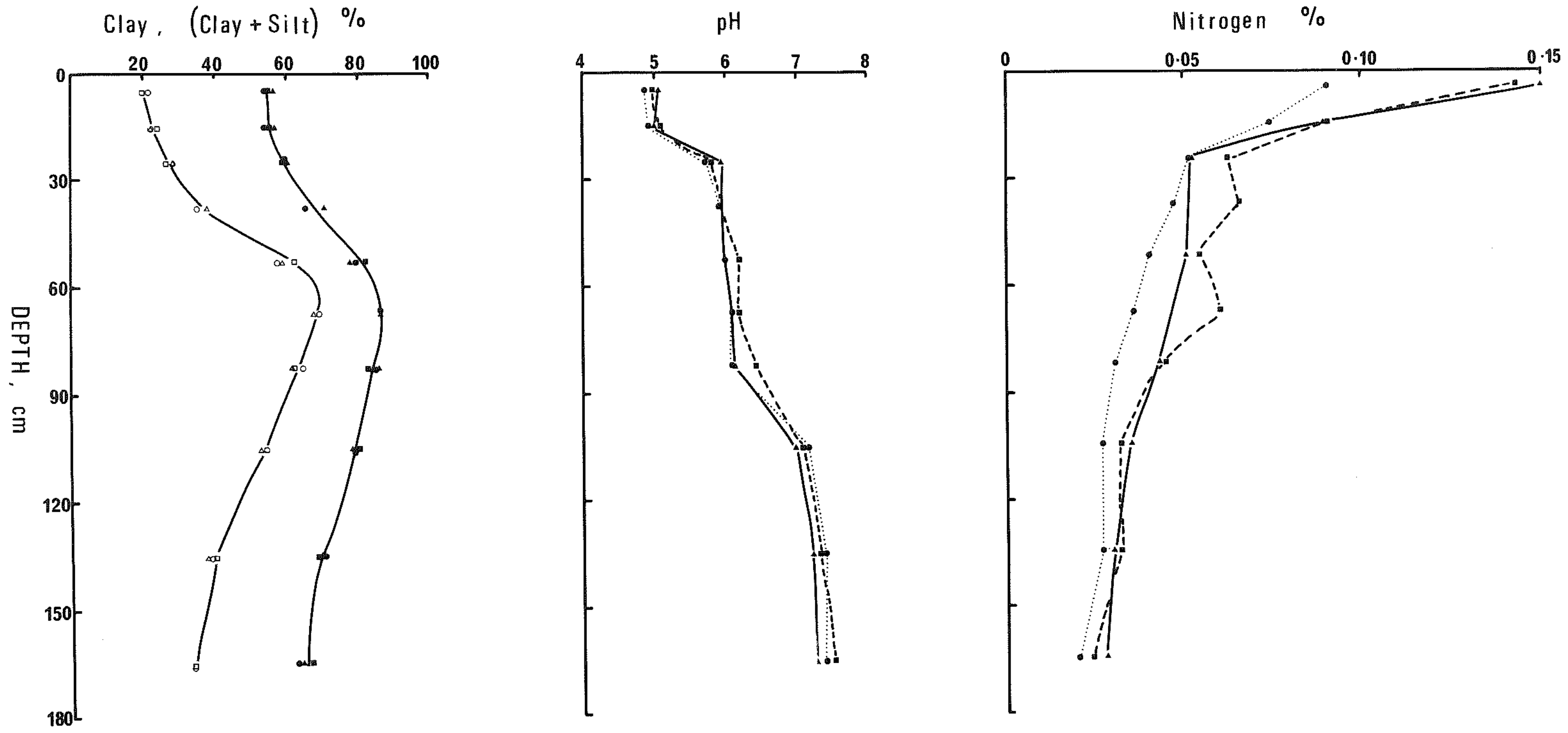


FIGURE 19. PROFILE DISTRIBUTIONS OF CLAY (<2μ e.s.d.) AND CLAY + SILT (<20μ e.s.d.), pH, AND TOTAL NITROGEN CONTENT OF THE URRBRAE FINE SANDY LOAM AT THE SITE OF EXPERIMENT II.

Legend

	PP	PC	CC
clay	△	□	○
clay + silt	▲	■	●
pH, N	▲	■	●


Clay , (Clay + Silt) %
20
40
60
80
100
pH
4
5
6
7
8
Nitrogen %
0
0·05
0·10
0·15
DEPTH , cm
0
30
60
90
120
150
180

Details of the location and past history of the site and agronomic practices are supplied in Appendix I. Some characteristics of the Urrbrae fine sandy loam at this site are shown in Fig. 19.

IV.2.2.2. Measurement of composition of the soil atmosphere

IV.2.2.2.1. Installation, location and depth of gas reservoirs

The gas reservoirs and installation techniques were similar to those used previously, except that much longer copper capillary tubing (200 cm total) was employed to allow sampling from pathways without trampling the plots.

The reservoirs were placed on a circle of 4'6" radius from the centre of each plot, and on the apices of an equilateral triangle with one axis running east-west (Fig. 17). The position of the cans within each plot was randomised separately for each block of each rotational treatment. The cans were located at the 30 cm (A_2 horizon) and 60 cm (B_2 horizon) depths for all treatments, and at 7.5 cm (PP) and 10 cm (PC and CC). Previous work has shown that a large consolidation of the seedbed occurs after sowing a crop (Millington, 1959a). As the reservoirs were installed immediately after seeding, allowance for this was made by a deeper placement on the cropped areas.

IV.2.2.2.2. Gas sampling and gas analysis

The sampling technique was similar to that used in Experiment I except that after flushing the capillary and the syringe, only one sample (of 5 ml capacity) was withdrawn from each reservoir. Immediately prior to injection excess sample was discarded and 5 ml injected into the gas chromatograph. Using the modified system of 3 columns in series (Section III.1; Appendix VI) the gas composition (A, O_2, N_2, N_2O and CO_2 concentrations and the N_2/A ratio) could be determined. Corrections were made for the time lapse between sampling and injection and the change in N_2 calibration curves during a sampling (Appendix VII and IX).

IV.2.2.3. Ancillary data

IV.2.2.3.1. Soil water content

Soil moisture contents of the 0-10 cm depth were obtained at each gas sampling (except for the first three). The use of a small core sampler (4 cm i.d.) allowed calculation of bulk or apparent density. Usually the cores from individual plots were bulked to provide a pooled value for each treatment.

For greater depths, measurements were made by the neutron moisture meter method (Appendix IV). The neutron moisture meter access tubes were installed on a north-south line running through the centre of each plot, and additionally were all situated on the south side of the plot at the same distance (4'6") as the gas reservoirs from the plot centre (Fig. 17). The tubes, of 2 in. PVC tubing with a collar of polyethylene tubing at the upper end to facilitate a good seal with the soil surface, were 90 cm deep on Reps 2, 3 and 4 and 180 cm deep on Rep 1.

IV.2.2.3.2. Soil samples

During the installation of NMM access tubes using the core sampling tube, soil samples were obtained over depth intervals of 0-10, 10-20, 20-30, 30-45, 45-60, 60-75, 75-90, 90-120, 120-150 and 150-180 cm intervals. The cores from each replicate were bulked, the total weighed, and one subsample taken for moisture content determination, and another (100 ± 1 g) immediately placed in normal Na_2SO_4 for soil mineral nitrogen determination (moist soil:solution ratio = 1:2.5). The soil from each treatment was then bulked to provide an average sampled profile for each of the rotations (PP, PC and CC). Some characteristics of the soil profile, based on analyses of these samples, are shown in Fig. 19.

IV.2.2.3.3. Soil and plant analyses

Soil inorganic nitrogen contents (NO_3^- - N and NH_4^+ - N) were determined by semi-micro distillation (Appendix III).

Total nitrogen contents on plant materials were determined on 100 mg subsamples by a modified-Dumas method, with a Coleman Nitrogen Analyser, Model 29A (Stewart, Porter and Beard, 1964).

Total nitrogen contents of soils were determined on 10 g subsamples by Kjeldahl digestion, using the salicylic acid modification described by Bremner (1965a).

Soil pH was measured in an equilibrium suspension of soil: M/100 $CaCl_2$ (1:2.5).

Bulk density was calculated from the field weight of soil from the core sampler, the moisture content of the soil (oven dried at 105 C) and the cross-sectional area of the Stace-Palm modified Veihmeyer tube. Allowance was made for quartz and slate stones, assuming a density of 2.65 g/ml.

All meteorological data was obtained from the Waite Institute meteorological station (see Appendix V).

IV.2.2.4. Agronomic treatments

The whole experimental area was irrigated, using a portable irrigation system, in June. The rainfall prior to this date had been insufficient to bring the soil to a moisture content suitable for the initial cultivation of the areas to be cropped.

Nitrate of soda (615 lb/acre = 100 lb N/acre) was handspread onto the +N plots immediately before seeding on the 6-7th July.

The irrigation treatment (+W) was achieved by placing a sprinkler, of the circular throw type, in the centre of each plot. Water from the Adelaide mains supply was used.

Further details of the agronomic practices are provided in Appendix I.

IV.2.3. Results and discussion

IV.2.3.1. Seasonal conditions

In 1967, the total rainfall for the year (12.82 in.) was the lowest since recordings were commenced at the Waite Institute in 1925, and was only a little over one half of the total average rainfall of 24.33 in. In only two months, February and July, did the monthly rainfall exceed the long term average (Appendix X). Even when due allowance is made for the 1.05 in. of irrigation applied in June to facilitate seedbed preparation, the total precipitation still remains the driest on record.

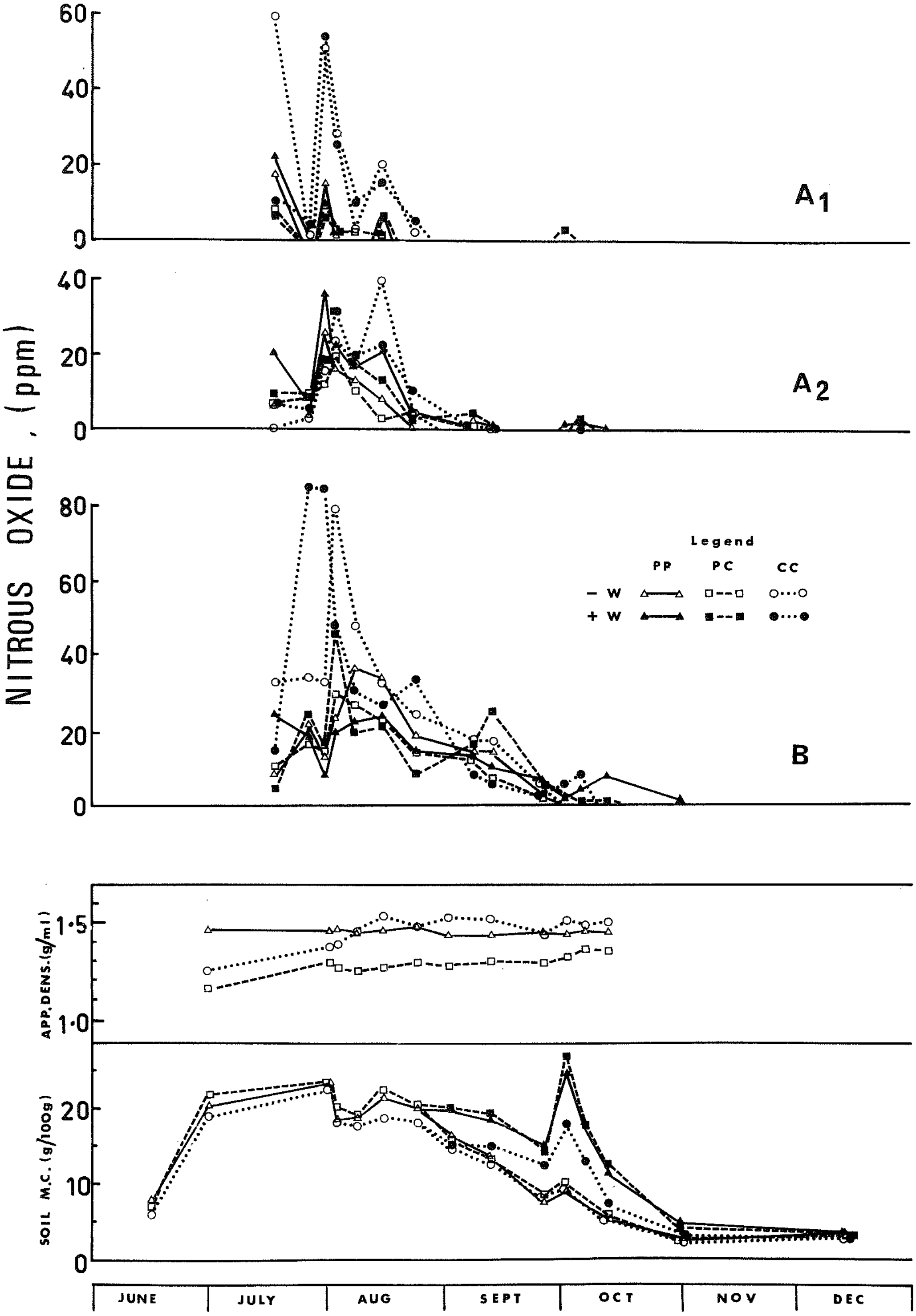
It would seem that such a year would provide a good occasion for establishing the causal relationship between soil moisture content and denitrification via the irrigation treatments. However, it was found that only slight winds strongly biased the spray from the sprinklers towards adjacent plots. While a large droplet size minimized wind-drift, the large rate of delivery (1 in./hr) caused excessive run-off on the CC rotation. It was not possible to reduce the rate of watering by using a finer spray. The late irrigation applied during warm weather could not adequately simulate rainfall due to a high concurrent evaporation rate.

IV.2.3.2. Composition of the soil atmosphere

IV.2.3.2.1. Nitrous oxide

The pattern of occurrence of nitrous oxide in the soil atmosphere was similar to that observed in the previous year (Experiment I), except that it did not persist as long and the concentrations were smaller (Fig. 20). This can be attributed to the generally dry soil conditions (see Appendix XIV for moisture contents).

FIGURE 20. TEMPORAL DISTRIBUTIONS OF NITROUS OXIDE IN THE SOIL ATMOSPHERE OF THE A_1, A_2 AND B HORIZONS; AND MOISTURE CONTENTS AND APPARENT DENSITIES OF THE 0-10 cm SOIL DEPTH, 1967.


NITROUS OXIDE , (ppm)
60
40
20
0
A1
40
20
0
A2
80
60
40
20
0
B
Legend
PP
PC
CC
− W
+ W
APP. DENS. (g/ml)
1·5
1·0
SOIL M.C. (g/100g)
20
10
0
JUNE
JULY
AUG
SEPT
OCT
NOV
DEC

The ephemeral production in the A_1 horizon was confined to the 3 periods following the 3 major falls of rain for the winter period. The changes in concentration with respect to time in the A_2 and B horizons were in general much less pronounced than in the Experiment I, and exhibited only a diffuse peak in the late July-August period. The sharp peaks at the 60 cm depth on the CC rotation were mainly due to very high N_2O concentrations (up to 500 ppm) in one hole at each of the -W and +W treatments. In contrast to the 1966 results, there was no evidence that levels at the 30 cm depth were considerably less than those at the 60 or 7.5-10 cm depth. However, there was an observed tendency for the lag in maximum levels with depth down the profile following the heaviest rainfall on the 29th July-1st August.

The frequency distribution of the N_2O concentrations was so skewed that analyses of variance were not possible. The variability in the data is shown by the large differences in the -W and +W treatments in July and August: irrigation was not commenced until early September. It was therefore necessary to use non-parametric statistics, and the data was examined using the Mann-Whitney U test (Siegel, 1956).

The only significant differences detected were higher concentrations of N_2O in the A_1 horizon of the CC area compared with those in the PP and PC areas: CC > PP, PC ($P < 0.01$). The effects of the heavy irrigation in early October on N_2O levels approached significance when the data for the PC and CC rotations at the 60 cm depth on the 6th October was pooled ($0.05 < P < 0.10$).

All values for the nitrogen treatments have been pooled. There were no observable differences, although the levels of nitrate in the subsoil at the 60 depth were less than 2 ppm NO_3-N at the initial soil sampling (Appendix XV).

The irrigation of the 29th September-1st October produced very little effect on N_2O levels, although the moisture content of the surface soil on the PP and PC plots rose temporarily to very high values. The relatively lower value for the moisture content of the CC plots reflects the poor physical condition of this soil and its ability to shed water. The soil on this area formed a strong surface crust (Fig. 21a, b) following the rain in mid July and subsequent drying. This subsequently restricted the entry of water, such that even after very light rain (0.01-0.02 in.) pools of water were evident on the surface (Fig. 21c, d). The poor structure also resulted in a considerable consolidation of the seedbed.

It is of interest to note that the irrigation applied to Experiment I in 1966 also produced only small effects on the N_2O levels. Both irrigations were conducted during spells of weather which was relatively free from wind, but warm: the appreciable evapotransporation during this period (potential tank evaporation approximately = 0.10 in./day) would have resulted in much more rapid drying of the soil than those after the winter winds (about 0.05 in./day) (Appendix X). Another factor may have been that the soil nitrate levels were depleted. This does not seem really likely, as the pasture was harvested on the 10th

FIGURE 21. PHOTOGRAPHS OF THE SOIL SURFACE ON THE CC AND PC ROTATIONS (NIL TREATMENT), 21st AUGUST (a,b) AND 5th SEPTEMBER (c,d), 1967.

(a) CC rotation, 21st August.

(b) PC rotation, 21st August.

(c) CC rotation, 5th September.
(Note the pools of water in the centre and centre foreground).

(d) PC rotation, 5th September.

(a)

(b)

(c)

(d)

October and the N uptake on the (+N) plots was only 19-31 lb/acre greater than that on the (-N) plots (Appendix XII). Finally, the irrigation water used was from the Adelaide mains water supply. The chlorination[†] of this probably had an important effect on the soil micro-organisms.

As the nitrous oxide levels were all low it would seem that the extremely dry season made effects due to the treatments difficult to detect.

IV.2.3.2.2. Nitrogen:argon ratios and nitrogen

Nitrogen concentrations in the soil atmosphere did not differ from atmospheric concentrations by more than 0.5, 1 and 2.5% in the A_1, A_2 and B horizons respectively, and there were no significant increases in the N_2/A ratio (Fig. 22). Coefficients of variation of 0.2-0.5% (N_2) and 1-2% (N_2/A) were frequently obtained in the A_2 horizon: these indicate that the methods of gas sampling and analysis are sufficiently accurate to allow measurement of moderate losses as nitrogen gas.

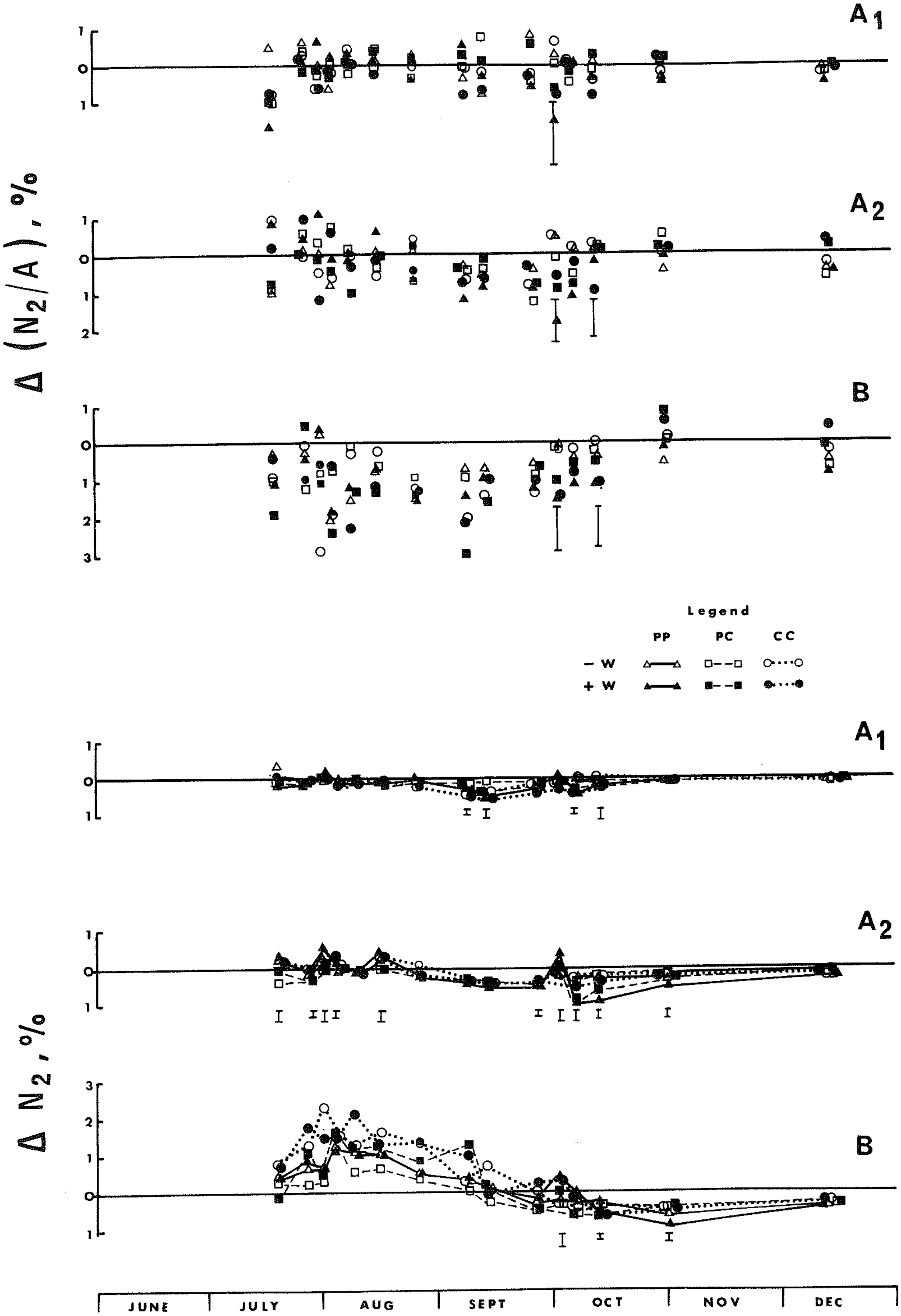
The largest increases in the N_2 concentrations occurred in the B horizon. These values were very variable, and it is suspected that this was the result of localized areas of high soil moisture content resulting from an uneven distribution of water during the general pre-

---

[†] Usual chlorine content at the Waite Institute is 0.1-0.5 ppm Cl (Engineering and Water Supply Department, Adelaide).

FIGURE 22. N_2/A RATIOS AND NITROGEN CONCENTRATIONS IN THE SOIL ATMOSPHERE, PLOTTED AS DEVIATIONS (Δ) FROM ATMOSPHERIC VALUES, 1967.

Least significant differences at $P = 0.05$ are indicated by I.


A1
A2
B
Δ (N2/A), %
Legend
PP
PC
CC
− W
+ W
A1
A2
B
Δ N2, %
1
0
1
2
3
JUNE
JULY
AUG
SEPT
OCT
NOV
DEC

seeding operation.

Although significant increases in the N_2/A ratio did not occur, significant decreases were observed on a number of occasions, principally after the irrigation in early October. This could indicate an utilization of gaseous nitrogen by fixation, but such effects were apparent at all three depths in the soil. It is more likely to have resulted from a greater diffusion coefficient for N_2 than for A: if mass flow of gases occurred then the greater rate of diffusion of nitrogen against the pressure gradient would have resulted in an enrichment of argon in the atmosphere close to the source of biological activity. The diffusivity of argon in air is not tabulated in International Critical Tables, but on the basis of †Graham's Law, the ratio of the diffusion coefficients would be 1.2:1 for N_2:A.

The departures of the nitrogen concentrations from atmospheric levels can be generally accounted for by the differences between the O_2 deficit and the CO_2 levels (Fig. 23). The oxygen deficit was usually greater than the CO_2 concentration in winter and vice-versa in spring. This agrees with the predicted behaviour from the solubility data (Chapter II), as nitrogen increases generally occurred in winter and

---

†Graham's Law states that the rates of diffusion of two gases is inversely proportional to the square roots of their densities (Partington, 1951).

the deficits in the spring: this trend was most pronounced in the B horizon.

IV.2.3.2.3. Oxygen and carbon dioxide

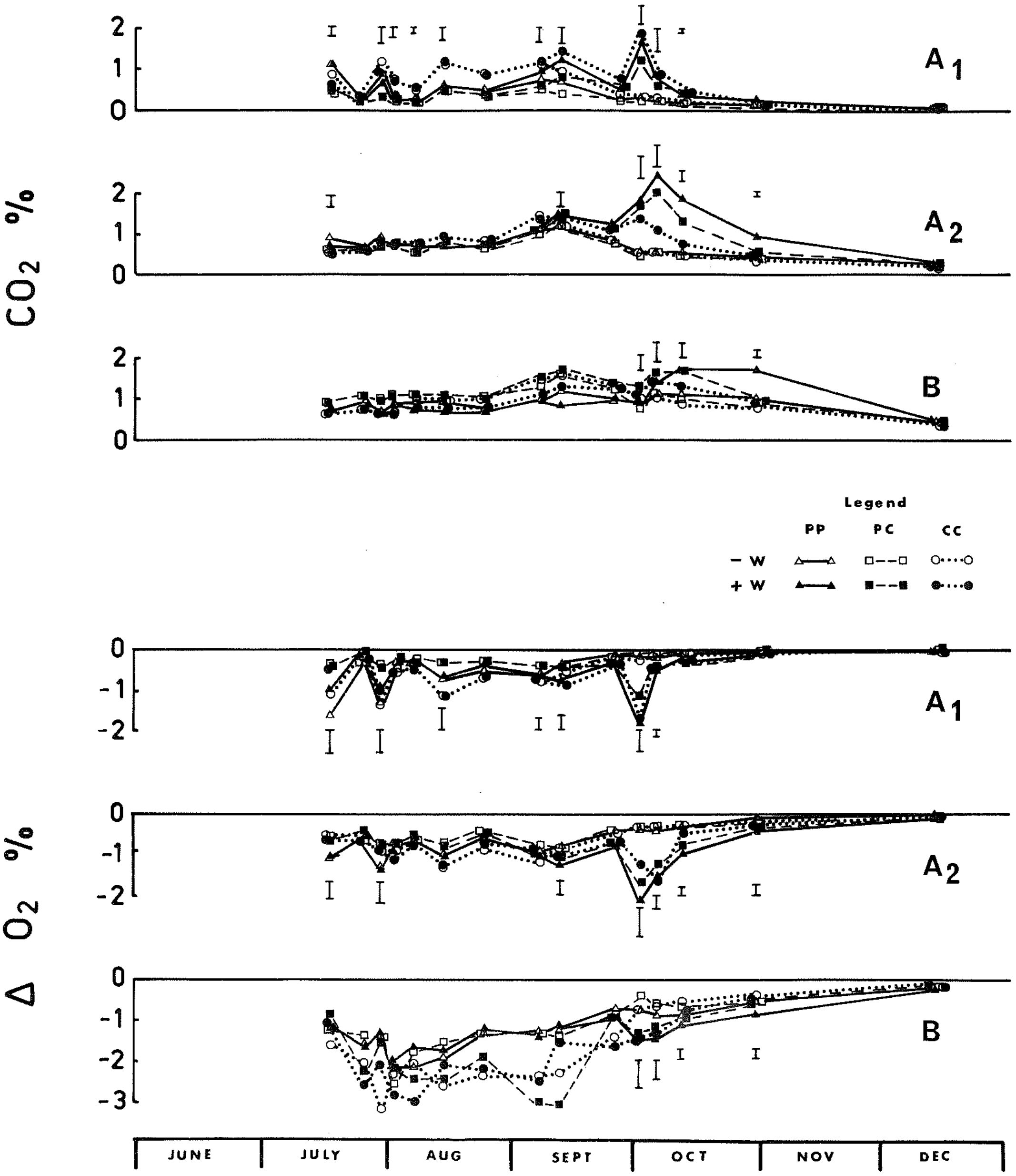
Oxygen and carbon dioxide concentrations (Fig. 23) under the non-irrigated plots exhibited similar trends to those previously observed: the levels of CO_2 in the A_2 and B horizons changed only slowly during the winter with a diffuse peak in late winter, whereas the oxygen concentrations were more variable with greater deficits occurring at higher soil moisture contents.

Greater responses to soil moisture contents occurred in the spring than in the winter. After the irrigation in early October there was an immediate decrease in oxygen concentrations throughout the profile: but the CO_2 levels in the A_2 and B horizons did not reach maximum concentrations until about 4 and about 20 days later respectively. This can be attributed to both the slower decrease in evolution due to drying with depth in the soil, as well as the slower expulsion of CO_2 from the soil water due to drying and increases in temperature.

The effect of nitrogen fertilizer on the composition of the soil air was very slight and results from all N treatments have been pooled. There was a tendency to lower CO_2 concentrations and O_2 deficits on the +N plots, presumably due to slightly lower soil moisture content and so higher diffusion coefficients.

FIGURE 23. CARBON DIOXIDE AND OXYGEN CONCENTRATIONS IN THE SOIL ATMOSPHERE, 1967: OXYGEN CONCENTRATIONS PLOTTED AS DEVIATIONS (Δ) FROM ATMOSPHERIC VALUES.

Least significant differences at P = 0.05 are indicated by I.


CO₂ %
Δ O₂ %
A 1
A 2
B
2
1
0
-1
-2
-3
Legend
PP
PC
CC
− W
+ W
JUNE
JULY
AUG
SEPT
OCT
NOV
DEC

The differences in the O_2 and CO_2 levels of the soil air under the different rotations can be mainly attributed to the restriction on diffusion in the soil. Greater departures from atmospheric levels in the surface soils occurred under the PP and CC rotations: these surface soils had higher apparent densities and so lesser air spaces available for diffusion than the PC soil. In the spring, the higher soil water contents would have had a two-fold effect in increasing the biological activity as well as reducing the volume of air spaces available for diffusion.

IV.2.3.3. Magnitudes of losses

Losses as nitrogen gas could not be demonstrated, but the concentrations of nitrous oxide in the soil were lower than in the initial experiment, indicating that losses in this form were again small. Due to the differences in the apparent density (Fig. 20) and porosity values for the surface soil of the three rotations, estimates of the rates of losses for the measured N_2O concentrations are necessary to provide a sensible companion between the rotational areas. The calculated rates of losses from the surface soil on the 31st July become:

	PP	PC	CC
N_2O concentration (ppm)	15	8	52
Diffusive loss, lb N_2O-N/acre/day	0.005	0.021	0.098

No confidence limits can be placed on these estimates as the apparent density values at this sampling were obtained from a bulked sample from each area. In view of the large variability in the concentrations of N_2O measured, and the large fiducial interval of calculated losses resulting from random errors in the porosity terms (Section IV.3.3.2.), it is doubtful whether the differences in estimated losses between the two cropped treatments are significant.

Losses from the subsoil would have been smaller than in the previous year: the concentrations of nitrous oxide were lower and the duration of occurrence shorter.

Even with the initial irrigation applied before the crop was sown, the total 'rainfall' for the unwatered treatments was the lowest on record at the Waite Institute. The small but detectable losses of N_2O demonstrate the normal occurrence of denitrification in the Urrbrae fine sandy loam.

IV.3. PART II, SECOND SEASON (1968)

IV.3.1. Experimental

The experiment was continued in a similar manner to the previous year. The reservoirs in the A_1 horizon of the cropped areas were removed during the cultivation necessary for seeding, and replaced in new positions but at the same depth after the crop was sown. At the same time, thermistors were placed in the soil profile to allow measurement of soil temperatures.

IV.3.2. Materials and methods

The techniques and field operations were generally similar to those used in 1967, and are described in Appendix I. The main changes involved a 'split' application of the nitrate of soda and the seeding by hand of the crop and pasture to minimize damage to the gas sampling points. The same arrangement of treatments was used, and irrigation was not applied: this effectively increased the number of replicates to eight.

IV.3.2.1. Gas sampling and analysis

Over-heating of the gas chromatograph, and the subsequent repairs, prevented measurements over a 14 week period from February to April. During the repairs, the instrument was modified to allow a bridge current of 170 mA to be used instead of 100 mA. A three-fold increase in the sensitivity to N_2O was achieved, even though the volume of sample injected was reduced to 3 ml.

IV.3.2.2. Soil moisture content, apparent density and nitrate levels

Samples were collected from the 0-10 cm depth of every relevant plot immediately before, or after, a gas sampling. These were usually collected with a tube sampler, and either bulked and subsampled, or occasionally kept as individual cores. The sampled soil was immediately placed in a tin with a tight fitting lid, and dried in an oven (105 C) within 1-6 hr of collection for moisture content and bulk density determinations. These samples were also used for determinations of soil nitrate content (Appendix III).

IV.3.2.3. Soil temperature

On the 17th July, thermistors were installed at depths of 5, 10, 15, 30 and 60 cm on all treatments of Rep. 1 of the two cropped areas, and at 5, 10, 15 and 30 cm on the +N and +W treatments on Rep. 1 of the pasture area.

Bead-type thermistors with a nominal resistance of 50,000 ohms at 25 C and a negative coefficient of resistance of 4% per degree C were used. They were embedded in epoxy resin; the resultant block (approximately 4 cm by 1 cm diameter) provided protection from physical shock and breakdown of the insulation of the electrical connections with the P.V.C. twin-core bellwire leading to the surface.

The values for all treatments and rotations were pooled for each depth, as in almost all cases the maximum range of values was 0.5°C.

IV.3.3. Results and discussion

IV.3.3.1. Seasonal conditions

The extremely wet season of 1968 provided the greatest contrast between successive seasons since records began in 1925, as the total rainfalls of 12.82 in. and 32.28 in. in 1967 and 1968 were lowest and highest respectively recorded at the Waite Institute (Appendix X). In only 2 months (June and September) in 1968 was the rainfall less than average, whereas in 1967 the rainfall was above average in only 2 months (February and July).

Such a wet year produced many effects - the A_1 horizon was continually moist from April to November, deep drainage occurred below 165 cm in the profile, crops exhibited signs of nitrogen deficiency, and yet crop yields were the highest for many years.

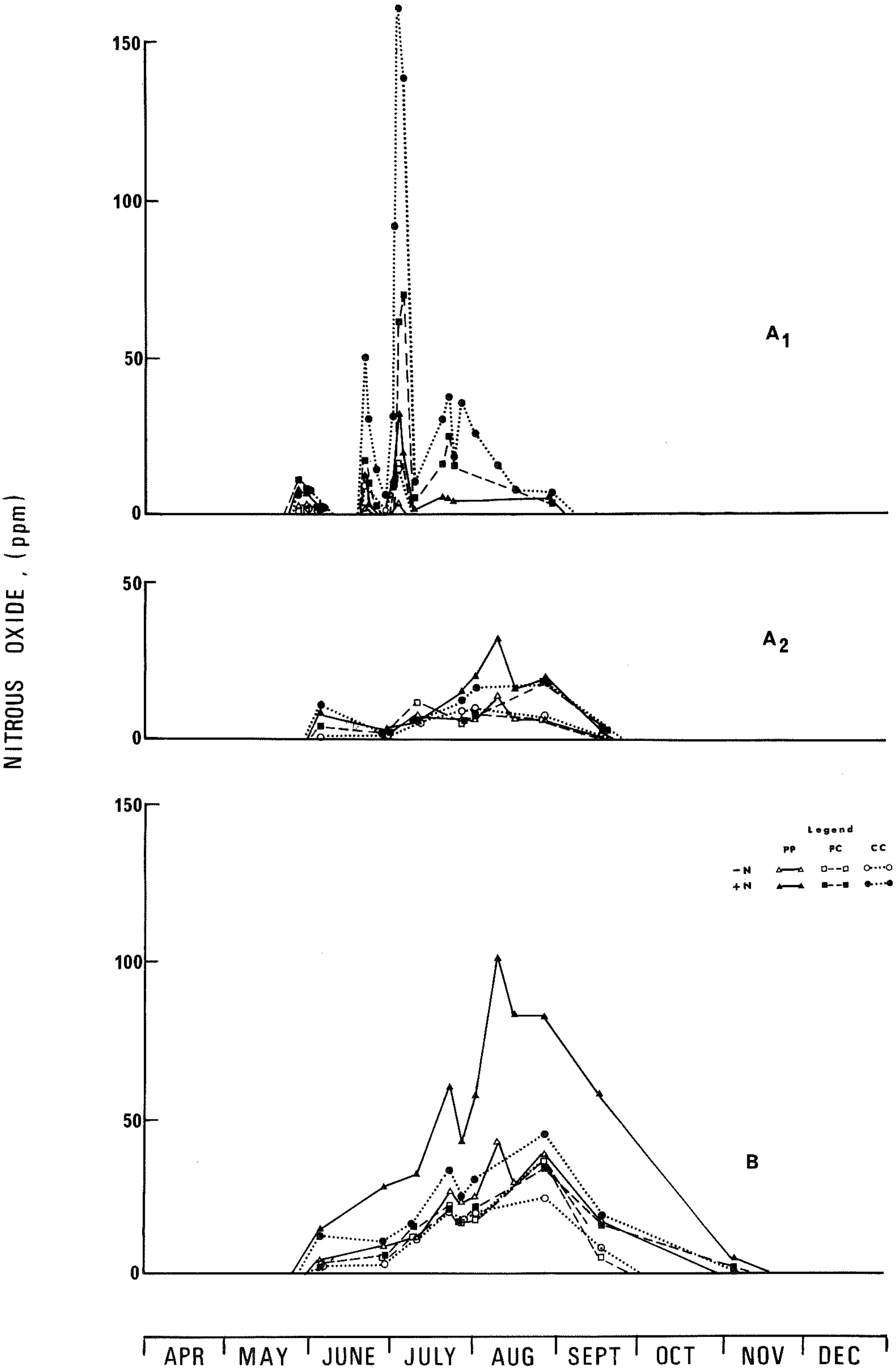
IV.3.3.2. Composition of the soil atmosphere

IV.3.3.2.1. Nitrous oxide

The detection of nitrous oxide in the soil profile over a period of 6 months and the highest average values yet recorded of 160 ppm (Fig. 24) reflect the long period during which the soil was exceptionally moist in the autumn, winter and spring of 1968 (Fig. 25, Appendix XIV). The general pattern of N_2O occurrence was in agreement with that observed in previous years, and measurement of soil temperature indicated that evolution occurred when the soil was at its lowest seasonal temperature (Fig. 25). Once again the maximum concentrations in the B horizon were observed later than those in the A horizon, which were fluctuating rather then ephemeral. Levels in the A_2 horizon were usually lower than those in either the A_1 or B horizon.

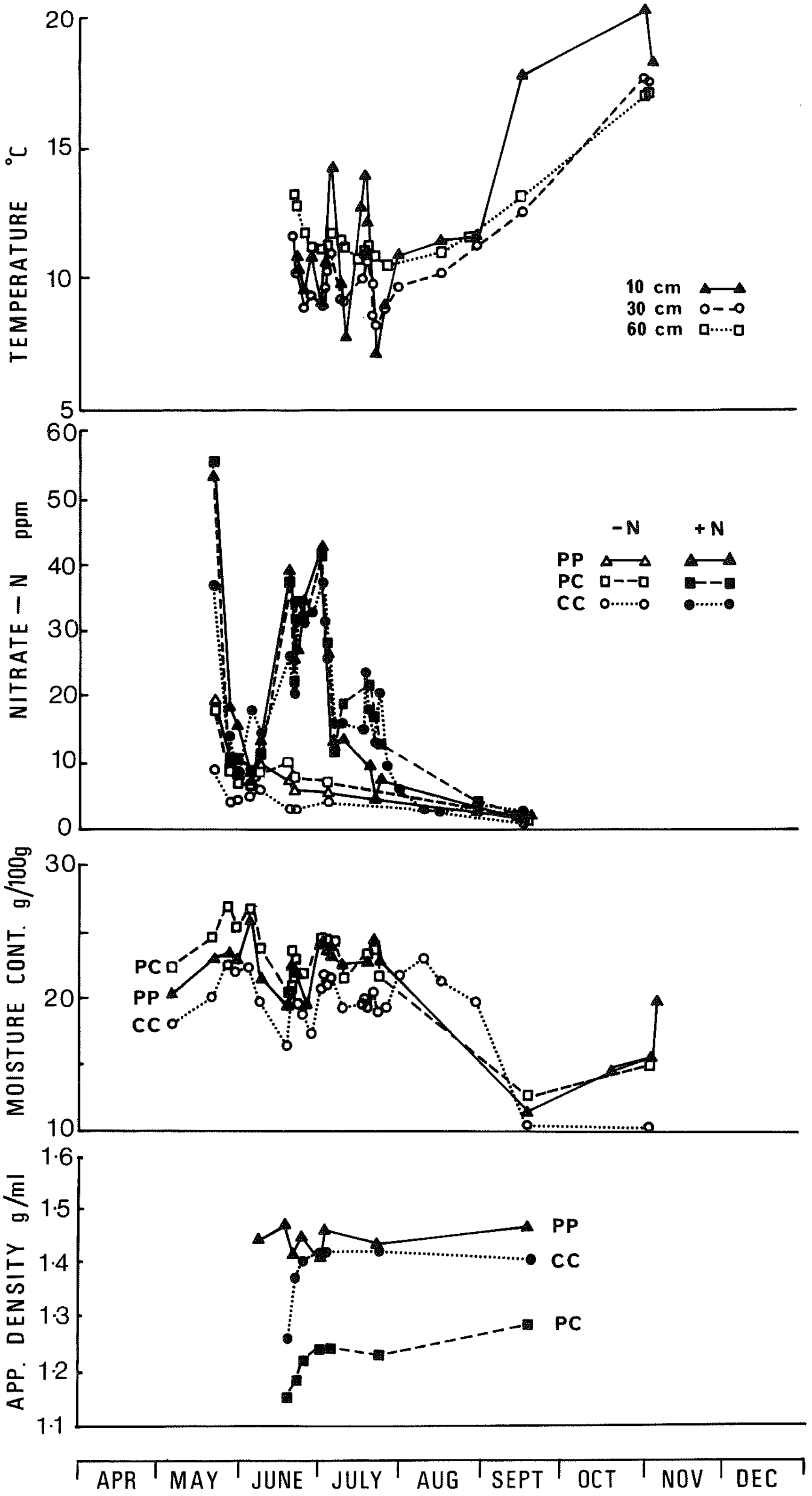
The cultural treatments and the application of nitrate fertilizer greatly influenced nitrous oxide levels, but the evolution

FIGURE 24. NITROUS OXIDE CONCENTRATIONS IN THE SOIL ATMOSPHERE, 1968.



NITROUS OXIDE , (ppm)
150
100
50
0
A1
50
0
A2
150
100
50
0
B
Legend
PP
PC
CC
−N
+N
APR
MAY
JUNE
JULY
AUG
SEPT
OCT
NOV
DEC

FIGURE 25. ANCILLARY SOIL DATA, 1968. TEMPERATURES AT 3 DEPTHS AND NITRATE-N CONTENTS, MOISTURE CONTENTS AND APPARENT DENSITIES OF THE 0-10 cm DEPTH.


TEMPERATURE °C
20
15
10
5
10 cm
30 cm
60 cm
NITRATE — N ppm
60
50
40
30
20
10
0
−N
+N
PP
PC
CC
MOISTURE CONT. g/100g
30
20
10
PC
PP
CC
APP. DENSITY g/ml
1·6
1·5
1·4
1·3
1·2
1·1
PP
CC
PC
APR
MAY
JUNE
JULY
AUG
SEPT
OCT
NOV
DEC

of nitrous oxide under all rotations and all treatments indicated that some losses of N_2O can be expected under most systems of agricultural management on the Urrbrae fine sandy loam, even on land under a permanent pasture rotation.

The treatment effects were most pronounced in the A_1 horizon: this was most obvious on the 4th and 6th July, when concentrations were increased 5-10 fold by the various treatments. These effects were significant: the nitrous oxide levels have been ranked using the Mann-Whitney U test as:

$$+N \text{ plots} \qquad CC \underset{**}{>} PC \underset{p<0.10}{>} PP$$

$$\text{and for } \underline{\text{all}} \text{ rotations} \qquad +N \underset{**}{>} -N$$

In the A_2 horizon, N_2O concentrations were also much greater where N fertilizer had been applied to the soil surface but differences due to rotation were not apparent.

In the B horizon, the treatments resulted in relatively small differences; N_2O concentrations in this horizon were significantly greater under the PP +N plots than under the PP -N plots or under those of any other rotation - with or without added nitrogen fertilizer ($P < 0.05$). Increases due to the nitrogen treatment were smaller for the CC area than under the PP area, and absent for the PC rotation.

Estimates of the relative rates of losses from the rotations require calculations from porosity data. However, the relative N_2O levels on the +N and -N treatments of each rotational area provide a comparison of the losses due to increased nitrate levels. The moisture contents and air filled porosity of the soil were not influenced by the NO_3^- levels during the winter periods, and so the calculated diffusion coefficients are similar for both treatments. Thus, nitrogen fertilizer increased losses of N_2O 3-10 fold in the A horizon and up to 2.5 fold in the B horizon.

Such a marked effect of soil nitrate levels on the rate of denitrification has not been noticed in incubation experiments (Wijler and Delwiche, 1954; Nommik, 1956; McGarity, 1961). Nitrate levels did have small effects on the shape of denitrification curves presented by Nommik and McGarity. Schwartzbeck, MacGregor and Schmidt (1961) have observed a tendency for higher denitrification rates at higher nitrate levels.

The discrepancy cannot be attributed to a complete lack of nitrate in the soil (Fig. 25) but may be due to differences in the experimental conditions: the previous laboratory experiments were conducted with extremely wet soils (0.90-1.0 saturation level) and at high soil nitrate contents (40-200 ppm). The levels of nitrate observed in the field (1-50 ppm NO_3-N) and the soil moisture contents (approximately field capacity or half saturation) were much lower than those of the previous workers: both effects would tend to reduce the rate of nitrate movement to micro-sites within a generally well aerated medium, particularly as denitrification generally occurs for short periods following rainfall.

The effect of soil nitrate levels on N_2O levels may also be a reflection of the effect of nitrogenous fertilizers on the plants. It has been shown that the presence of wheat plants on the Urrbrae fine sandy loam increases the denitrification rate: Woldendorp (1963) has suggested that the addition of nitrogen fertilizers will increase denitrification by the stimulation of denitrifiers as a result of the excretion of amino acids by the plant roots. However, it has been shown that the proportion of N_2:N_2O in denitrification products is greater at lower nitrate contents (Nommik, 1956). It is possible that the overall denitrification rate was similar on the +N and -N plots, and that the differences in N_2O lost are due to differences in the composition of the gases evolved by denitrification.

IV.3.3.2.2. Nitrogen and N_2/A ratio

Nitrogen contents of the soil air were almost always within 1% of that in air (Fig. 26) although large positive deviations occurred during August, when the soil was maintained at a very high moisture content by exceptionally wet weather. Subsequently, the concentrations were less than that in the atmosphere in late August.

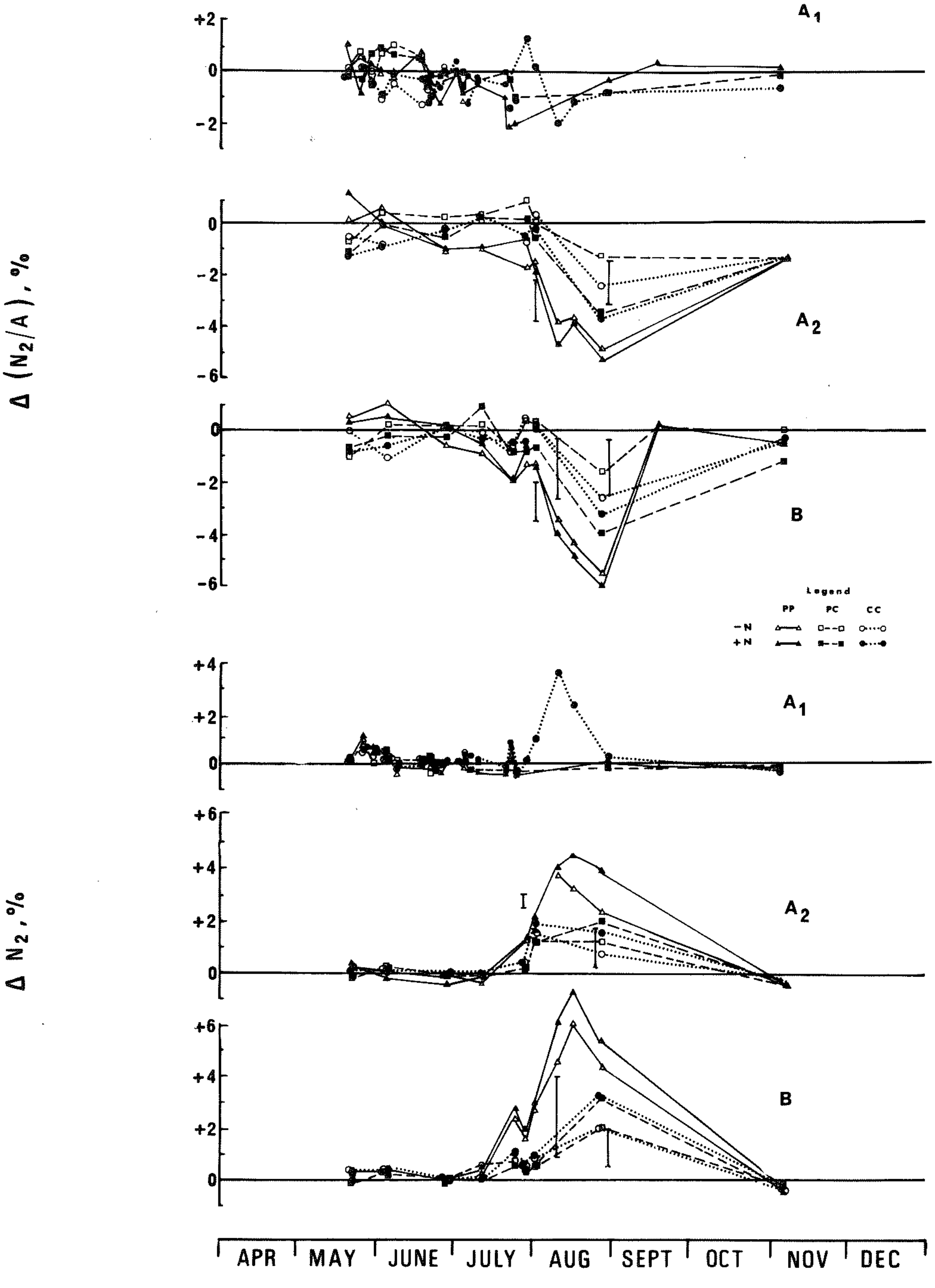
The maximum increases in the nitrogen contents of up to 7% were accompanied by decreases in the N_2/A ratio. Both these changes in concentration were significant.† There was no indication that

---

† All statistical analyses of N_2, A, O_2 and CO_2 concentrations were made on untransformed data, as trial analyses using angular transformations indicated that such transformations were not necessary.

FIGURE 26. N_2/A RATIOS AND NITROGEN CONCENTRATIONS IN THE SOIL ATMOSPHERE, PLOTTED AS DEVIATIONS (Δ) FROM ATMOSPHERIC VALUES, 1968.

Least significant differences at $P = 0.05$ are indicated by I.


A1
A2
B
Δ (N2/A), %
+2
0
-2
-4
-6
Legend
PP
PC
CC
−N
+N
A1
A2
B
Δ N2, %
+6
+4
+2
0
APR
MAY
JUNE
JULY
AUG
SEPT
OCT
NOV
DEC

nitrogen was evolved by denitrification, although only extremely large losses could have been detected. The deviations of nitrogen concentrations from atmospheric levels can be attributed to the mass flow resulting from changes in the oxygen and carbon dioxide content of the soil air, and decreases in N_2/A ratio can be attributed to the differences of N_2 and A diffusion rates.

IV.3.3.2.3. Oxygen and carbon dioxide

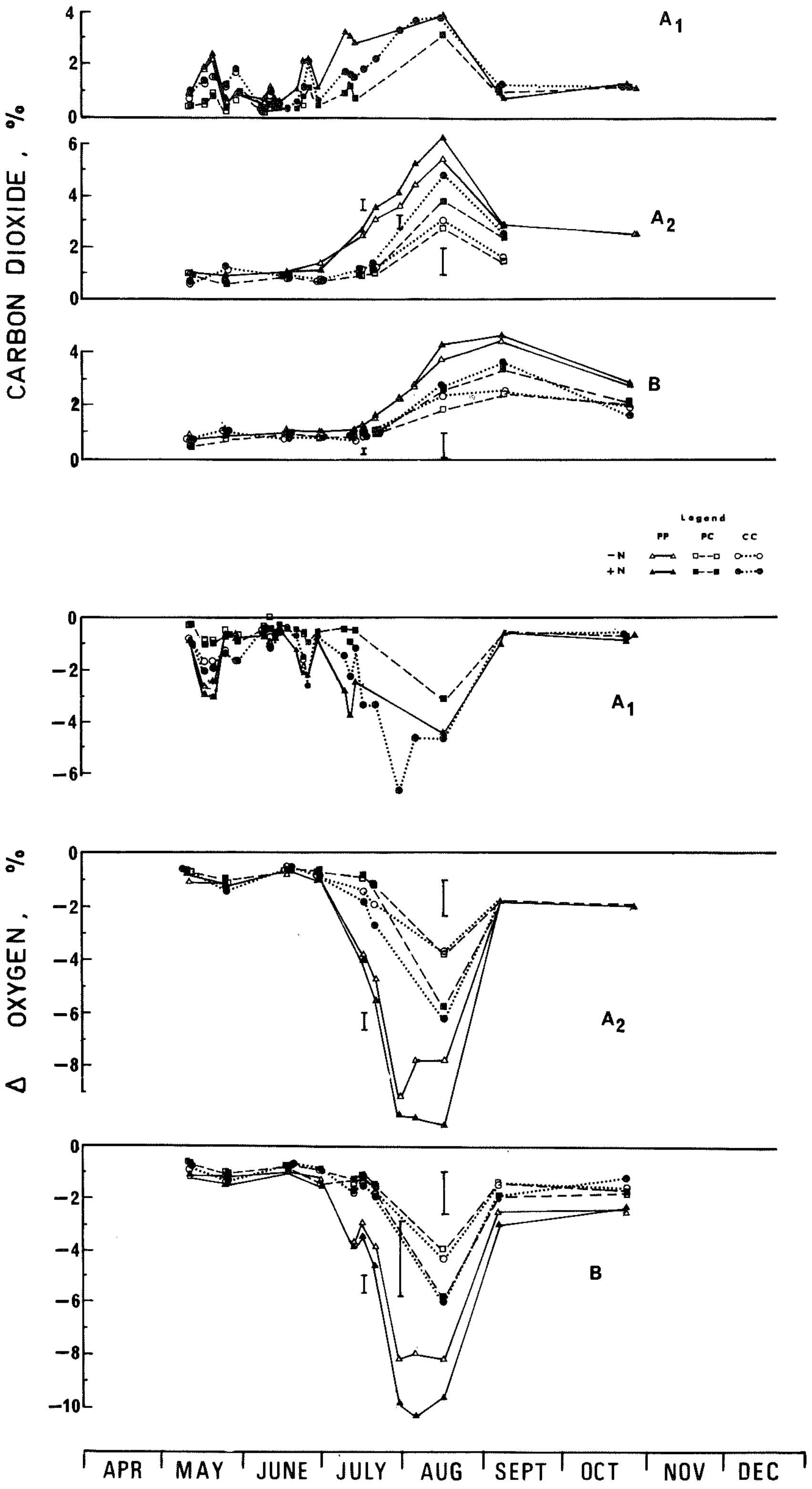
The temporal trends for O_2 and CO_2 (Fig. 27) were similar to those obtained in the previous year on the irrigated plots, although the magnitudes of the CO_2 levels and O_2 deficits were much greater than in the earlier measurements.

Oxygen deficits occurred mainly at times of high soil moisture content. Carbon dioxide levels were usually small in winter when temperatures were low, moisture contents high and the plant growth rate small: increases occurred at the same time as increases in soil temperature from mid-July onwards, although the concentrations decreased rapidly in the surface soil during the dry period in September (which was one of the two months in which the rainfall was less than the long term average). The peaks in the A_2 and B horizon exhibited a pronounced lag behind those in the A_1 horizon.

This pattern is attributed to the increase in soil temperatures with subsequent expulsion of CO_2 from the plant roots under the favourable growing conditions. It is not possible to determine whether the root plus microbial respiration rates or the solubility effects are more

FIGURE 27. OXYGEN AND CARBON DIOXIDE CONCENTRATIONS IN THE SOIL ATMOSPHERE, 1967: OXYGEN CONCENTRATIONS PLOTTED AS DEVIATIONS (Δ) FROM ATMOSPHERIC VALUES.

Least significant differences at $P = 0.05$ are indicated by I.


CARBON DIOXIDE , %
A1
A2
B
0
2
4
6
Legend
PP
PC
CC
−N
+N
Δ OXYGEN , %
0
−2
−4
−6
−8
−10
A1
A2
B
APR
MAY
JUNE
JULY
AUG
SEPT
OCT
NOV
DEC

important in producing these peaks in spring. Soil respiration must be important, as the large increases in the above ground herbage production due to the addition of nitrogenous fertilizers (Appendix XII) were also associated with increases of 10-40% in the levels of CO_2 in the A_2 and B horizons of the soil.

On no occasion did the soil atmosphere contain zero concentrations of oxygen. In one reservoir underneath a large phalaris plant on the pasture area, levels as low as 1.2% O_2 were recorded in early August. In all other reservoirs, the oxygen content was always greater than 5%, and mean values for the A_1, A_2 and B horizons were always greater than 14, 11, and 10% even under the wettest soil conditions (respectively).

The general conclusions concerning CO_2 and O_2 levels are therefore also similar to those made previously: the observed soil denitrification occurred in a soil in which the soil air was not anaerobic, and the non-equality of O_2 deficits and CO_2 concentrations resulted in a mass flow of soil air in the soil profile.

IV.3.3.3. Magnitude of losses

As significant increases in the N_2/A ratio could not be detected, it is not possible to make estimates of the magnitude of losses as N_2. However, the nitrous oxide concentrations of up to 160 ppm indicated that the losses as this gas were much larger than in previous years.

Measurements of soil moisture contents in the 0-10 cm depth were made at all gas samplings, and measurements of apparent densities (Fig. 25) and porosities obtained at seven samplings. The values for the 4th July provided estimates for the maximum rate of N_2O loss from the A horizon, and these have been compared with calculated maximum losses for the B horizon in Table 10.

For the cropped soils, these estimates indicate that losses were much greater in the A horizon than in the B horizon, even when due allowance is made for the less transient situation in the B horizon. Differences in the losses from the A and B horizons were less marked under pasture.

The losses in the A horizon were much greater under the wheat crop than under the pasture, and under the wheat crop the magnitudes of the losses were similar for the PC and CC areas.

It is not clear from the field data whether the greater loss of N from the cropped area compared with that under pasture was due to the effect of the wheat plant on denitrification, or to the cultivation associated with the cropping. Rovira and Greacen (1957) have indicated that each cultivation will have an effect on stimulating microbial activity by increasing the availability of previously inaccessible organic matter, and thus there would be created fresh potential micro-sites for denitrification. But the effects of each disturbance should have subsided within 3-4 days. Alternatively, Woldendorp (1963) has proposed that specific differences may occur between plants in their

TABLE 10

Estimated maximum diffusive losses of nitrous oxide†

	Treatment		
	PP +N	PC +N	CC +N
A_1 horizon: 4th July, 1968			
N_2O, ppm (at 7.5-10 cm)	32	61	161
Porosity (ml/ml);			
ε_T	0.444 (±0.019)	0.533 (±0.026)	0.446 (±.035)
ε_W	0.350	0.307	0.299
ε_A	0.094 (±0.031)	0.226 (±0.043)	0.147 (±.043)
Diffusive loss, lb N_2O-N/acre/day.	0.009 (0.002 -0.028)	0.25 (0.11 -0.49)	0.23 (0.06 -0.67)
B horizon: 9th August, 1969			
N_2O, ppm (at 60 cm)	100	43‡	55‡
Porosity (ml/ml):			
ε_T	0.40	0.40	0.40
ε_W	0.35 -0.30	0.35 -0.30	0.35 -0.30
ε_A	0.05 -0.10	0.05 -0.10	0.05 -0.10
Diffusive loss, (lb N_2O-N/acre/day)	0.0007-0.0075	0.0003-0.0034	0.0004-0.0041

† 5% fiducial intervals are enclosed by parentheses.

‡ Calculated from comparisons of N_2O concentrations for PP, PC and CC on 1st and 24th August, 1968.

stimulation of denitrification, and in this case the wheat plant may have a greater effect than the pasture plants (wimmera ryegrass and subterranean clover).

The absence of large differences in losses as N_2O between the two cropped areas is of interest. It is known that after several years of pasture, the levels of easily decomposable organic matter are increased, and that this rapidly mineralizes on opening the pasture; and so the levels of easily decomposable substances ("light fraction") are much lower under continuous wheat than under a newly opened pasture soil (Ford and Greenland, 1968). It was expected that the abundance of such organic substrates on the PC area would have provided the opportunity for greater gaseous losses. However, the old cropland soil is noted for its poor structure (e.g. Fig. 21). This was shown by the differences in apparent density values for the CC and PC areas (1.43 and 1.25 g/cc respectively), and it was noticeable that oxygen levels were much lower on the CC area (Fig. 27). In practice, it seems that these two factors compensate for each other, i.e. the better structure and aeration of the PC top soil offsets its higher content of labile light fraction. Current results of Stefanson and Greenland (unpublished data) also indicate that the losses as nitrous oxide from the PC and CC soils are similar when wheat plants are grown in these soils inside gas lysimeters.

While it was not possible to make an estimate of the amounts of N_2 gas evolved, the method of estimating the N_2/A ratio would have been sufficiently accurate for detecting losses as nitrogen in the B horizon. A 1% increase in the nitrogen content of the atmosphere due to evolved nitrogen could have been statistically significant: this would have corresponded to losses of 0.1-1.5 lb N_2-N/acre/day. However, the corresponding calculated loss for the A_1 horizon is 6-58 lb N_2-N/acre/day. A very accurate method is required to detect losses in this horizon.

IV.4. CONCLUSIONS

The important findings from this second field experiment have been the effects of the treatments on losses as nitrous oxide, and the demonstration of the effects of mass flow on the composition of the soil atmosphere.

After application of fertilizer nitrate losses as N_2O were 3-10 fold greater than where no fertilizer was applied. This effect was much larger in the A horizon than in the B horizon. The reason is not known but may have been due to higher soil nitrate levels per se, to the increased amounts of amino acid exudates from plant roots, or to increases in the proportion of N_2:N_2O in the gases evolved where soil nitrate contents were lower.

Losses as nitrous oxide were much greater on the cropped rotations than under the pasture, but losses under the wheat crop were of similar magnitude and were not greatly influenced by the large differences in the structure and organic matter status of the two areas. Such effects could indicate the importance of the plant species on denitrification rather than the range of soil properties within a soil type.

The losses as nitrous oxide were recorded under all rotations, all treatments and during the wettest and driest seasons on record at the Waite Institute. Thus denitrification may occur in Urrbrae fine sandy loam in every winter and under all systems of land utilization when the oxygen content of the large soil pores is only partly lowered by the slight restriction of aeration resulting from high soil moisture contents.

The rates of losses as N_2O were small, and there is a need for an assessment of the losses as N_2. Measurements of the N_2:A ratio demonstrated that this method was sufficiently accurate to detect losses in the B horizon, but the approach was not satisfactory as decreases in the N_2/A ratio indicated that the diffusion coefficient for argon is lower than that for nitrogen. Thus the basic assumption for using argon as a reference gas was not tenable, viz. that the physical behaviour of the two gases in the soil was similar. Nevertheless, an important side-issue arising from this study has been the establishment of the importance of mass flow of soil gases during measurements of the composition of the soil atmosphere.

CHAPTER V. DENITRIFICATION MECHANISMS IN THE URRBRAE FINE SANDY LOAM

V.1. INTRODUCTION

Throughout the previous discussions, it has been assumed that the denitrification observed was biological in origin as the concentrations of nitrous oxide showed a great dependence on the moisture content of the soil. However, there is very little evidence indicating the optimum moisture content for chemical mechanisms, although large losses have occurred at about field capacity, or half saturation capacity (Reuss and Smith, 1965; Bremner and Nelson, 1968). To establish the mechanism involved, it was decided to conduct an incubation experiment.

V.2. EXPERIMENTAL

Duplicate samples (150 g) of sterilized and unsterilized soil from the A (0-10 cm) and B (45-60 cm) horizons of the Urrbrae fine sandy loam were incubated with 200 ppm of added NO_3^--N or NO_2^--N. The moisture content was about 0.9 saturation level and argon was used to provide an inert, anaerobic atmosphere.

By comparing sterilized and unsterilized soils it was hoped to establish the relative importance of chemical or biological losses. Chemical losses are associated with the nitrite ion, and if a chemical mechanism is operating, then there should be a strong interaction between the effect of sterilization and the form of nitrogen (nitrite versus nitrate).

V.3. MATERIALS AND METHODS

V.3.1. Treatment of soils

Samples of 150 g of air dry soil were placed in 1 in. diameter polyethylene tubing and the ends knotted. Pairs of samples were placed inside larger tubing and the ends sealed. Samples for sterilization were placed inside a 1 gallon paint tin, the lid sealed, and a plastic envelope thermally sealed around the whole tin. The tin as such was sterilized with a 5 megarad dose of γ irradiation at the Australian Atomic Energy Research Establishment, Lucas Heights, New South Wales.

V.3.2. Incubation flasks

The incubation flasks consisted of 1 litre Erlenmeyer flasks equipped with a B29 'Quickfit' socket at the neck and a removable connector consisting of a B29 cone, high vacuum stopcock and a 'Quickfit' capillary ball joint.

V.3.3. Sterilization of flasks and equipment

Distilled water and 30 mg N as KNO_3 or Na NO_2 were added to the flasks to make up the required moisture content (41 and 36 g/100 g for Urrbrae A and B respectively). The flasks, connectors, vacuum grease, and accessories were autoclaved and transferred to a sterile room equipped with U.V. lamps. The tins of sterile soil were transferred to this room before opening, and sterilized soils were handled before those which were not sterilized.

V.3.4. Commencement of incubation

Sterile distilled water added to compensate for that lost during autoclaving: the amount required was determined by weighing the flasks before and after the sterilization. The soils were added, connectors greased, and the flasks filled with argon (C.I.G., A.R.) after 3 flushings. The levels of nitrogen (280-610 ppm, v/v) and oxygen (< 100 ppm) in the atmosphere of the flasks were determined at 'zero' time (< 4 hr after the addition of water and NO_2^- or NO_3^-), and the flasks placed in a constant temperature room (22 C).

V.3.5. Gas analysis

The atmosphere in the flask was analyzed by the usual gas chromatographic technique (Appendix VI), at intervals varying from 4 hr to 10 days. The gas sampling valve on the gas chromatograph was used with a 1.7 ml sampling loop. The stainless-steel inlet of the valve was cemented (with epoxy resin) to a small female ball joint for sampling the flasks. The dead volume inside the sampling system was 2.3 ml, and in the connections 0.6 ml. As the total volume removed from the flask during sampling was 4.6 ml, and up to 25 samplings were made, corrections were applied for the proportion of the atmosphere above the soil removed during samplings.

The argon peaks enabled corrections to be made, if necessary, for any variation in the volume sampled by the gas sampling valve: a variable leak in a PFTE ('Teflon') washer allowed small amounts of the helium carrier gas to leak into the evacuated sample loop just prior to sampling.

V.3.6. Mineral nitrogen

At the conclusion of the experiment soil NH_4^+-N, NO_2^--N and NO_3^--N levels were determined on one of the two duplicates (Appendix III).

V.4. RESULTS AND DISCUSSION

V.4.1. Gaseous evolutions

The amounts of nitrogen and carbon dioxide evolved as N_2, N_2O, (N_2 + N_2O), and CO_2 are shown in Fig. 28 and 29. Marked differences occurred between most treatments, both in the pattern of evolution, the amounts of N_2O and N_2, and in the total amounts of nitrogen and carbon dioxide evolved at the end of the experiment. The sterilized soils had always lost less nitrogen than those unsterilized, nitrite treatments caused a pronounced lag period in the unsterilized samples, and losses were much greater for the A horizon samples than for those from the B horizon. Appreciable amounts of CO_2 were produced in all sterilized flasks.

V.4.1.1. Urrbrae A plus nitrate

In the unsterilized soil, the sequence of products was in agreement with the sequence:

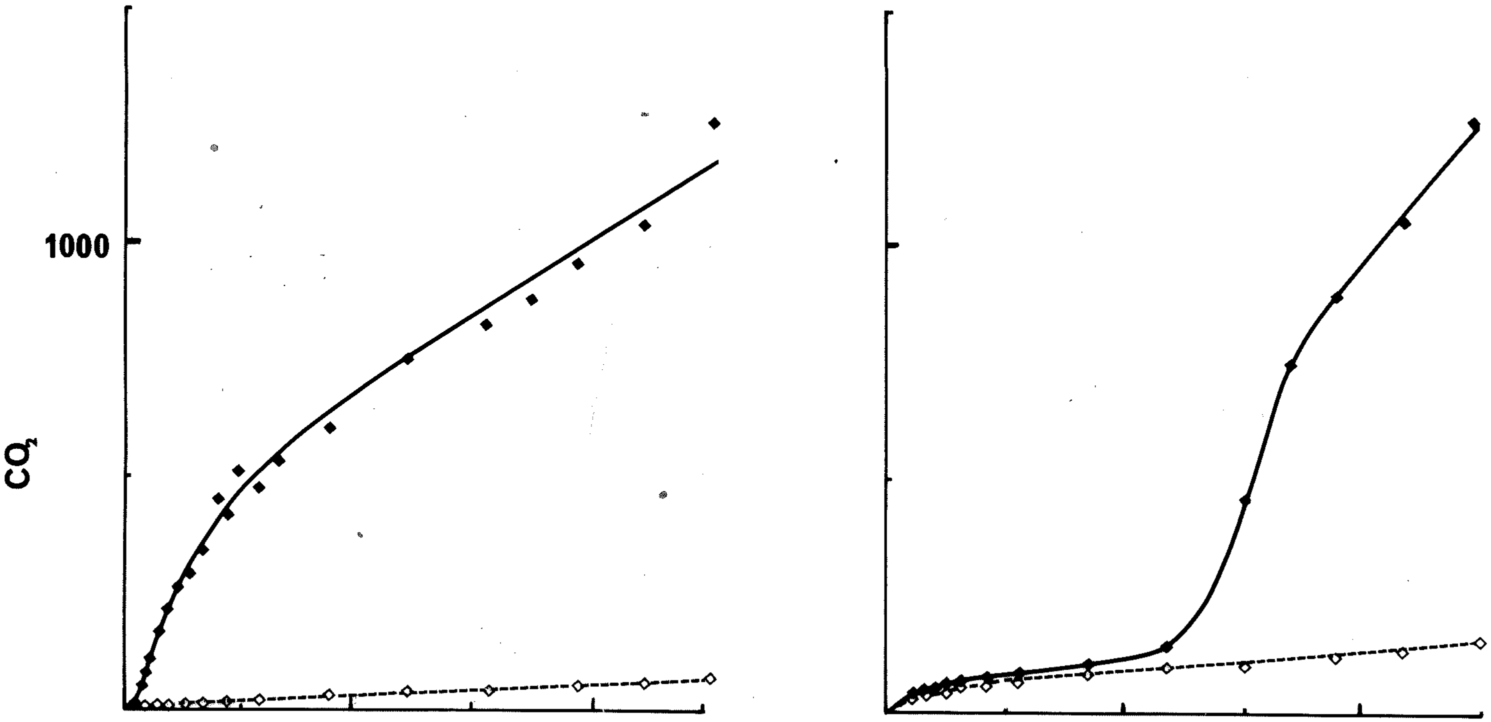
$$NO_3^- \ (\longrightarrow NO_2^-) \longrightarrow N_2O \longrightarrow N_2$$

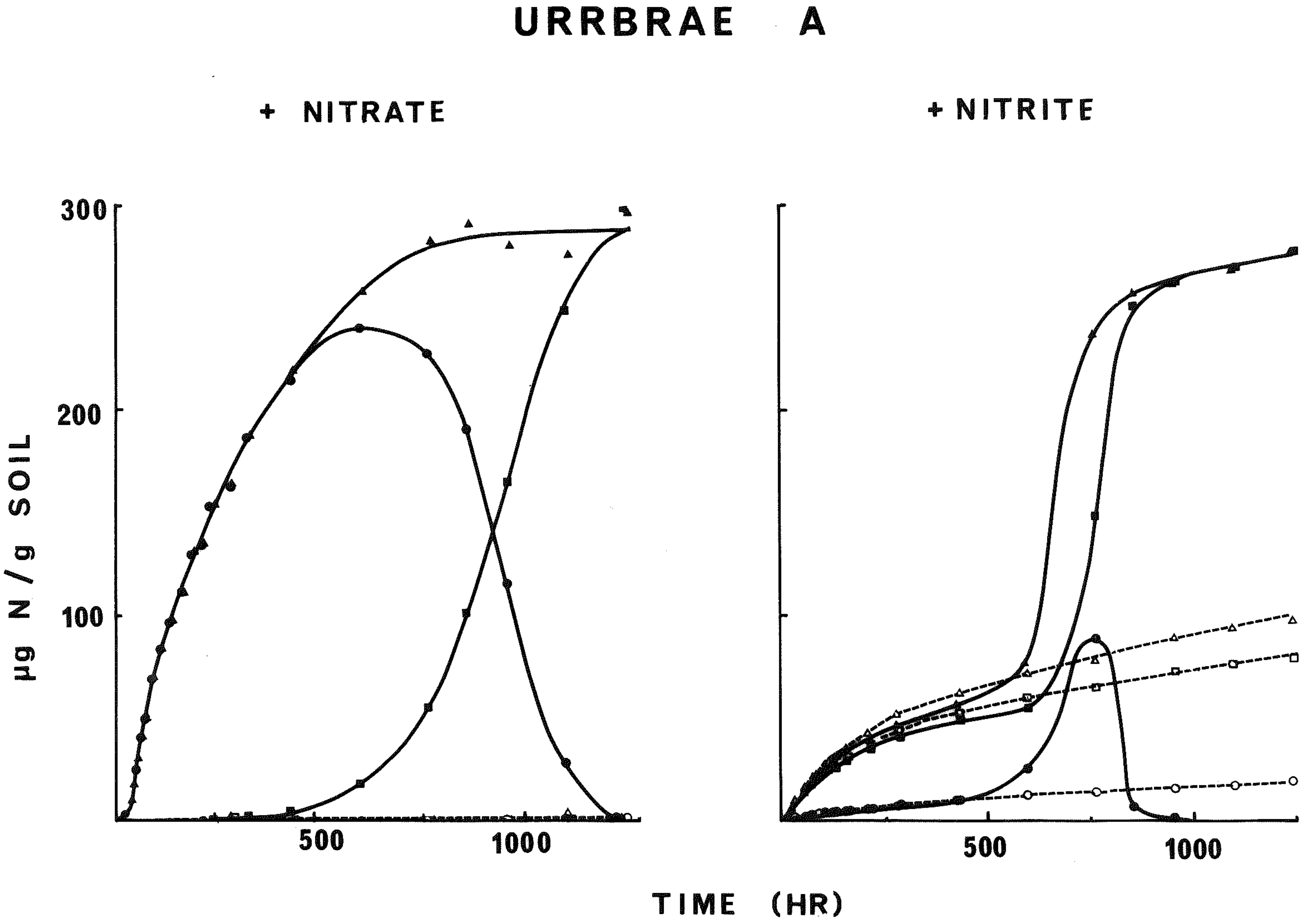
observed by many other workers (Verhoeven, 1952; Wijler and Delwiche,

FIGURE 28. **AMOUNTS OF NITROGEN, NITROUS OXIDE AND CARBON DIOXIDE (OVERLAY) EVOLVED FROM SAMPLES OF THE URRBRAE FINE SANDY LOAM A HORIZON.**

Legend

	Sterilized	Not sterilized
N_2O	○	○
N_2	□	■
$N_2O + N_2$	△	▲
CO_2	◇	◆


1000
CO$_2$


URRBRAE A
+ NITRATE
+ NITRITE
300
200
100
μg N / g SOIL
500
1000
500
1000
TIME (HR)

1954; Nommik, 1956; Cady and Martholomew, 1960: Cooper and Smith, 1963; Laskowski and Moraghan, 1967). The shape of the curve for total N loss as gas from the unsterilized soil is similar to that obtained by McGarity (1961) for A_1 horizon samples of the Urrbrae fine sandy loam. A short lag phase was followed by a short period of very rapid denitrification which then continued at a decreasing rate. No linear portion was detected although the data of both McGarity (1961) and Nommik (1956) indicate that this occurs only at low nitrate N contents. The initial nitrate content in this experiment was 258 ppm, there being 58 ppm in the soil before nitrate was added.

The shape of the curves for total N loss as gases (N_2O + N_2) from the unsterilized soil is in agreement with that for CO_2, which may be used as an index of respiration rate. After an initial lag, CO_2 production rapidly increased for a short time, then gradually decreased until an approximately linear rate occurred after about 500 hr. However, at 500 hr, most (75%) of the initial nitrate had been evolved as gases, and there was thus little opportunity for the rate of nitrogen evolution to become linear.

A continuous, very small evolution of N_2O occurred in the sterilized soils but the total loss was only 0.7 ppm soil nitrogen at the end of the 53 day incubation period. Thus, there is little doubt that losses from nitrate are biological in origin.

It is unlikely that the activity in the sterile soils was due to a very small microbial population as growth of this would have resulted in a sigmoid pattern of evolution: the rate of evolution of CO_2 and N_2O was nearly linear. It is generally agreed that while doses of 5 megarads are sufficient to kill most, if not all, microorganisms, such doses are not sufficient to denature all enzymes. While new enzymes cannot be formed, those surviving are capable of considerable activity (McLaren, Luse and Skujins, 1962; Paterson, 1962).

The low rate of nitrous oxide evolution was approximately linear with time indicating a chemical reaction, although nitrite was not detected in the initial soil. The production of N_2O by an enzyme system seems very unlikely, as from an initial nitrate source at least 4 different enzymes would be required to survive the irradiation treatment.

V.4.1.2. Urrbrae A plus nitrite

There was a large gaseous loss of nitrogen in all soils but the total losses, as N_2 and N_2O, from the samples with nitrite added, were less from sterilized soils than those unsterilized (Fig. 28).

The chemical loss (sterilized soils) exhibited a large rate of loss shortly after commencement of the incubation, with a decreased rate after about 100 hr, and an approximately linear rate over 500 to 1000 hr. The total amount lost after 53 days was 45% of the initial 200 ppm of NO_3-N added. Carbon dioxide production followed a similar pattern. The losses occurred both as N_2O and N_2 with N_2O being about

20% of total production of the two gases. Other workers have indicated that much more nitrogen than nitrous oxide is evolved by chemodenitrification (Reuss and Smith, 1965; Bremner and Nelson, 1968; Greenland, unpublished data).

In the unsterilized soils the pattern of gaseous evolution indicates an initial loss by a chemical mechanism to about 300 hr, then the onset of a very vigorous biological loss at 500 hr. The amounts of N_2O evolved were much smaller than with NO_3^- added, and the maximum rate of CO_2 production and denitrification (at about 600-750 hr) were much greater than in the unsterilized + nitrate flasks. Biological denitrification (even of the 38 ppm NO_3^--N also present in the soil) was inhibited for the first 500 hr by nitrite, and the CO_2 values show a similar lag in respiration rates. Similar, but not so pronounced temporary inhibition effects, have been previously noted by Nommik (1956). Jansson and Clark (1952) and Bremner and Shaw (1958b) have also noticed a pronounced inhibition in acid soils, and a greater tolerance to NO_2^- at alkaline reactions. This is in agreement with earlier microbiological work (Meiklejohn, 1940). The reason for the lag in this experiment would seem to lie in the reduction of nitrite levels by a chemical mechanism until the stage was reached where the threshhold toxicity level is passed.

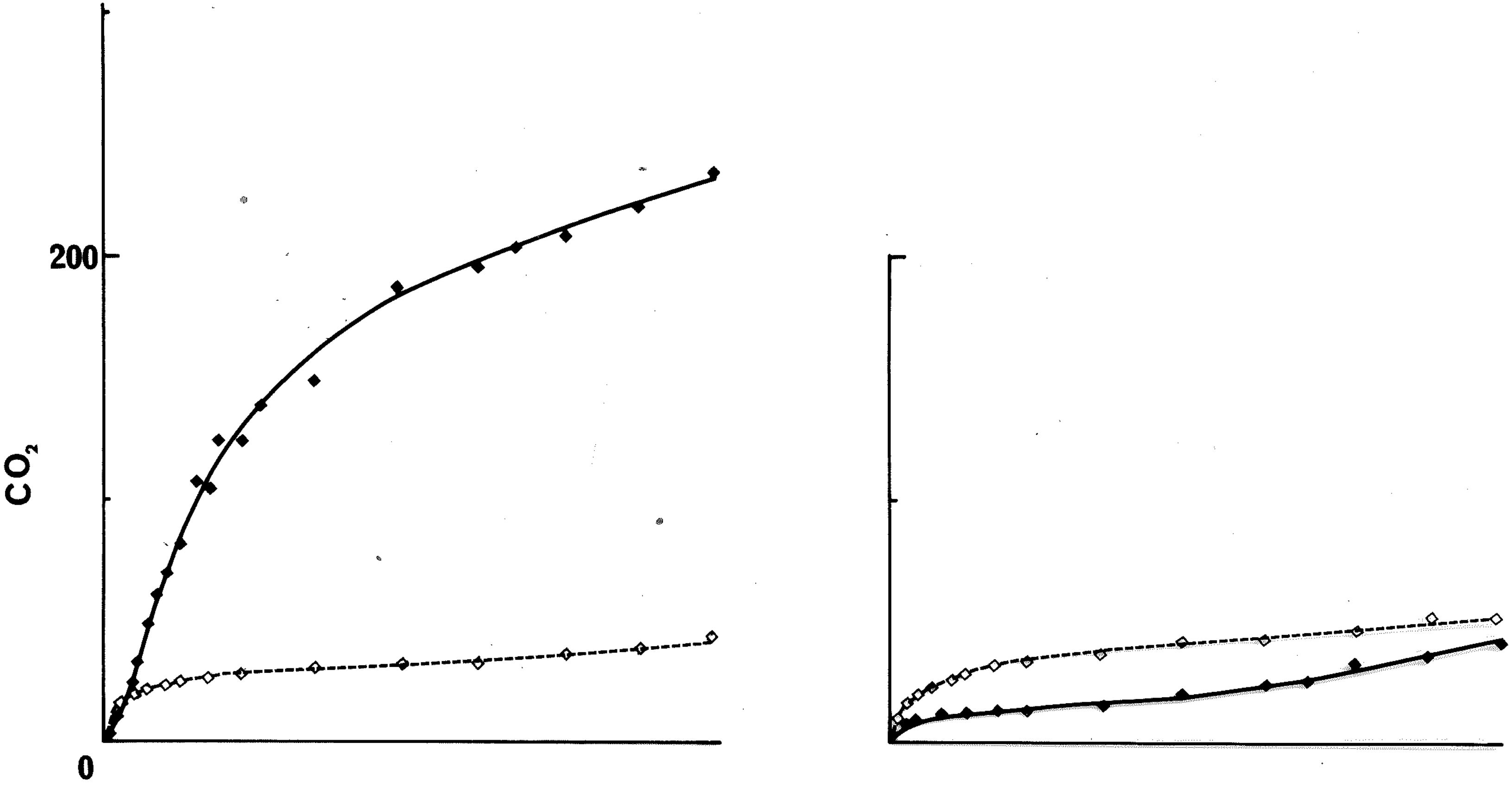
V.4.1.3. Urrbrae B plus nitrate

The evolution curves for Urrbrae B (Fig. 29) show some similarities with those for the Urrbrae A soil plus nitrate and some

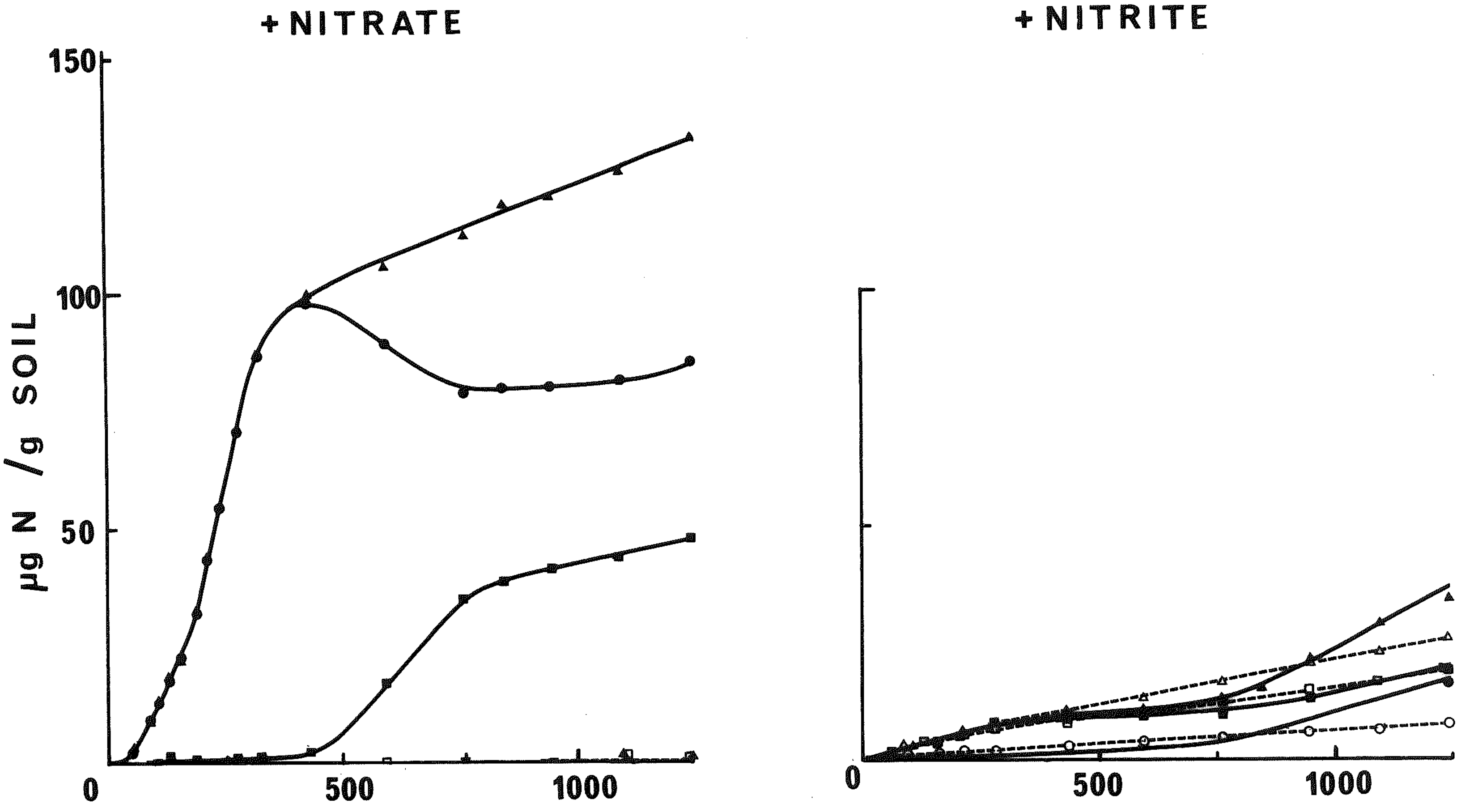
FIGURE 29. AMOUNTS OF NITROGEN, NITROUS OXIDE AND CARBON DIOXIDE (OVERLAY) EVOLVED FROM SAMPLES OF THE URRBRAE FINE SANDY LOAM B HORIZON.

Legend

	Sterilized	Not sterilized
N_2O	○	●
N_2	□	■
$N_2O + N_2$	△	▲
CO_2	◇	◆


200
CO$_2$
0

URRBRAE B



important deviations. The overall pattern of denitrification is similar except that no denitrification could be detected in the sterilized samples, although once again CO_2 was produced, but evolution of this became almost negligible after 500 hr. The rate of loss was lower than for the A horizon, and the reduction of N_2O to N_2 was not complete. The reason for this cessation in N_2O dissimilation, when 50% of the original nitrate was still present in the soil, is not known.

The lesser overall rate of denitrification can be attributed to the lower amounts of available organic substrates in the B horizon. McGarity (1961) showed that the addition of glucose could both decrease the lag period and markedly increase the maximum rate of denitrification.

V.4.1.4. Urrbrae B plus nitrite

A slow evolution of nitrous oxide and nitrogen was evident. The rate of evolution was much less than in the A horizon. No obvious difference occurred between the sterilized and unsterilized soils, although there was a tendency for evolution in the unsterilized soils to increase towards the end of the experiment. The almost complete inhibition of biological denitrification was not expected, in view of the known greater tolerance of denitrifiers to nitrite at an alkaline pH.

The relative proportions of N_2O and N_2 of about 1:4 and 1:2 from the sterilized Urrbrae A and Urrbrae B samples respectively are of considerable interest, as previous accounts of losses by nitrite reactions have shown the ratio to be as low as 1:100 (Bremner and Nelson, 1968), 1:20 (Greenland and Gasser, unpublished data), and 1:10 (Reuss and Smith, 1965). The rates of chemical loss were in agreement with the suggested nitrosation mechanism (Bremner and Nelson, 1968; Stevenson and Swaby, 1964), in that losses were higher in the A horizon than in the B horizon samples, the latter being of higher pH and lower organic matter levels. The shape of the curves also indicated a reaction dependent on nitrite concentration. Stevenson and Swaby (1964) observed large differences in the amounts and proportions of N_2, N_2O, CO_2 and CH_3ONO produced in the reaction of nitrous oxide and various phenolic compounds. The different ratios of $N_2O:N_2$ reported by the various workers may therefore reflect different compositions of the soil organic matter. The higher CO_2 production from nitrite treated than from nitrate treated soils, could also be attributed to a nitrosation reaction.

V.4.2. Mineral nitrogen at the conclusion of incubation

The values of mineral N and gaseous N at the conclusion of the experiment (53 days incubation) are shown in Table 11. Complete disappearance of the nitrite and nitrate initially added occurred in only the unsterilized Urrbrae treatments. This is in agreement with the amounts of N_2 and N_2O lost by these soils.

A balance of the labile nitrogenous products measured (gaseous + inorganic forms) has been attempted, and in all except two treatments an increase has occurred in the amounts of such nitrogen present.

TABLE 11

Mineral nitrogen in Urrbrae A and Urrbrae B soils, and total losses as N_2 and N_2O from the soils after 53 days incubation period. (All values expressed as ppm N on an OD soil basis).

	†Nitrogen as						
	NH_4^+	NO_2^-	NO_3^-	N_{TM}	N_{TG}	$N_{T_{G+M}}$	Δ
URRBRAE A							
‡Original soil	21	0	53	74	-	74	-
Sterilized + NO_2^-	40	61	59	160	97	257	-17
Sterilized + NO_3^-	37	0	248	285	2	287	+13
Not sterilized + NO_2^-	86	0	0	86	276	362	+86
Not sterilized + NO_3^-	75	0	0	75	286	361	+85
URRBRAE B							
‡Original soil	2	0	0	2	0	2	-
Sterilized + NO_2^-	6	102	0	108	26	134	-68
Sterilized + NO_3^-	4	0	202	206	0	206	+4
Not sterilized + NO_2^-	4	181	0	185	34	219	+17
Not sterilized + NO_3^-	2	2	101	105	128	233	+31

†N_{TM} - total mineral N

N_{TG} - total gaseous N $[(N_2O + N_2) - N]$

Δ - Change in balance of labile N compounds [initial + 200 ppm N - $N_{T_{G+M}}$]

‡ - Not sterilized.

Comparison of the NH_4 values indicates that anaerobic mineralization of nitrogen from organic matter has occurred in all soils. Previous work has indicated that the only product of such mineralization is NH_4^+ (Harmsen and Kolenbrander, 1965) so that there was probably very little, if any, extra nitrate or nitrite provided for denitrification reactions. Increases in NH_4^+ were greatest in the Urrbrae A unsterilized samples: the increases in the sterilized soils can be attributed to the irradiation effects as they were of a similar magnitude to the increases observed by Rovira and Bowen (1969) for a red-brown earth soil from Parafield, South Australia.

The two soils for which decreases in labile constituents occurred, were the sterilized + nitrite treatments for both Urrbrae A and Urrbrae B. It would seem that some of the nitrogen was present as NO and/or CH_3ONO in the atmosphere of the incubation flask; additionally nitrite fixation may have occurred. The formation of NO by the decomposition of nitrous oxide in acidic media is well known (Broadbent and Clark, 1965), and it has been recently suggested that fixation of nitrite is possible (Bremner and Nelson, 1968). Both nitrite fixation and decomposition are favoured by an acid pH and high soil organic matter levels. These reactions provide a satisfactory explanation for the small deficit in the Urrbrae A sample, but not for the large one in the Urrbrae B where the pH was tending to alkaline and the organic matter levels were low (Fig. 19).

The data in Table 11 suggests the formation of NO from NO_2^- in the Urrbrae A. For the sterile Urrbrae A + nitrite treatment, equal amounts of nitrate and nitrite (60 ppm) were present. The nitrate could have resulted only from the oxidation of NH_4^+, which is unlikely in an anaerobic situation, or from the decomposition of nitrous acid. This has been shown to occur by Reuss and Smith (1965) and Bremner and Nelson (1968). The proposed sequence of events is:

$$4\ HNO_2 \longrightarrow 2\ NO + 2\ NO_2 + 2\ H_2O$$

$$2\ NO_2 + H_2O \longrightarrow HNO_3 + HNO_2$$

or the nett reaction $$3\ HNO_2 \longrightarrow HNO_3 + 2\ NO + H_2O$$ (Reuss and Smith, 1965).

Bremner and Nelson (1968) consider this reaction to be of importance in closed incubation vessels, such as those used in this experiment, but not in open systems.

A mass spectrum of the atmospheres above the soils was obtained at 66 days for the Urrbrae A treatments (Table 12). Appreciable amounts of NO were present in the sterilized + nitrite flasks. If very small amounts were produced in the other flasks, these could not be detected due to other contributions to the m/e peak 30 of the spectrum, in particular that from nitrous oxide. The values for the peaks at m/e 31, 60 and 61 indicated that appreciable amounts of methyl nitrite were not present.

The evolution of NO and formation of NO_3^- provide additional information regarding the observed lag, due to nitrite inhibition, observed in the microbial dissimilation of nitrite treated samples. The amounts of nitrite lost by NO and NO_2, with conversion of the NO_2

TABLE 12

Mass spectral patterns of incubation flask atmospheres for the Urrbrae A samples, 66 days after the commencement of incubation[†].

m/e ratio	Relative intensities of peaks[‡]					
	Sterilized		Not sterilized		N_2O	CO_2
	NO_2^-	NO_3^-	NO_2^-	NO_3^-		
28	100	50.1	33.1	32.9	15.6	10.6
29	1.5	1.9	0.3	0.3	0.1	-
30	54.7	3.2	0.3	0.2	18.1	0.1
31	0.2	0.1	-	-	0.1	-
32	2.4	2.3	1.1	1.0	-	0.4
44	100	100	100	100	100p	100p
45	1.2	1.3	1.2	1.2	0.7	1.1
46	0.3	0.4	0.4	0.4	0.2	0.4

[†] An A.E.I. MS-2 mass spectrometer was used.

[‡] All peaks relative to m/e = 44; p = parent peak.

to nitrate, and that lost by the observed chemical reaction, would both have materially reduced the nitrite level in the incubation flask below the threshhold critical value.

V.5. CONCLUSIONS

The incubation results clearly indicate that losses of nitrogen can occur by either biological or chemical mechanisms in both the A and B horizon samples of the Urrbrae fine sandy loam. However, the relationship of these results to those obtained in the field have to be interpreted in terms of the conditions imposed on the samples during incubation.

A flooded soil, high nitrate levels of about 200 ppm, and an atmosphere of an inert gas are the usual conditions selected to give optimal rates of dissimilation. While biological losses from the A horizon samples attained a maximum of about 1 ppm N/hr, the estimate of the greatest losses under field conditions was 0.2 lb/acre/day, or if this is considered over an acre 10 cm of soil, 0.2 ppm of N/day, or about 1/120 the rate of loss under the conditions of the incubation.

Little is yet known of the factors affecting losses by chemical reactions, although the rate of loss is dependent upon the nitrite concentrations in the soil (Bremner and Nelson, 1968). Myers (1966) has shown that levels in a solodized solonetz were almost always much less than 1 ppm, and it has generally been observed that nitrite levels in acid and neutral soils are very low although there is very little published data available.

Nitrification rates in the Urrbrae series are low (<1 ppm NO_3^--N/day). Cole (unpublished data) has not been able to demonstrate 'side-tracking' of nitrification in an incubation experiment using soil from the PP area, and losses as N_2O in the field experiment were dependent on soil nitrate level: there seems to be little doubt that the losses under field conditions resulted from biological denitrification rather than a chemical reaction.

The incubation data provided evidence against both mechanisms operating concurrently, i.e. chemical losses of nitrite accumulating during biological dissimilation of nitrate. Chemical losses occurred mainly as N_2 gas with an initially rapid, but thereafter decreasing rate. No such pattern of N_2 loss of nitrogen conformed with the normally expected sequence of $NO_3^- (\longrightarrow NO_2^-) \longrightarrow N_2O \longrightarrow N_2$. Comparing these results with previously reported data, the rate of denitrification was not great and some nitrite must have been present, but for the unsterilized (+ NO_3^-) Urrbrae A this must have been at a very low level by the time nitrogen was produced, as at this stage over 70% of the final (N_2 + N_2O) had appeared as N_2O. Presumably some nitrite was present but no evidence of N_2 evolution typical of the chemical mechanism was shown; it can be concluded that there was no occurrence of bio-chemo-denitrification. It would appear that micro-organisms compete strongly for such nitrate, and additionally the pH rise occurring during biological denitrification (Bremner and Shaw, 1958a; Laskowski and Moraghan, 1967) would render the site of activity less favourable for chemical losses. However, it might be expected that the nitrite ion could have diffused to sites inaccessible to micro-organisms.

The shape of the biological denitrification curves indicates the importance of environmental conditions on the losses in field situations. It might be expected that denitrification could occur rapidly in soils which were suddenly wet by heavy rain after a long dry period: the combination of a flush of microbial activity, high soil temperatures and high soil moisture content should provide optimum conditions for biological denitrification. However, at high temperatures the soil would dry quickly in the surface, and the presence of a lag period in the micro-organisms could mean the disappearance of optimum environmental conditions before microbial activity could reach the maximum rate.

CHAPTER VI. GENERAL DISCUSSION AND CONCLUSIONS

VI.1. THE OCCURRENCE OF DENITRIFICATION IN AN 'AEROBIC' SOIL

The experimental findings have confirmed previous predictions from laboratory and lysimeter studies that biological denitrification may occur at anaerobic micro-sites in a soil normally regarded as aerobic; where this reference to aeration applies to the coarse soil pores. Previous predictions also have been confirmed that denitrification may occur in the B horizons of some soils due to restricted aeration for considerable periods (McGarity, 1961), but that such losses are much less than those from the surface horizons (Broadbent and Clark, 1965).

Greenwood (1963, 1968) has discussed the importance of both organic matter and moisture content in the formation of anaerobic micro-sites. The temporal distribution patterns of nitrous oxide in the soil atmosphere at a particular depth were of similar shape for all treatments, indicating that a single common factor must have been responsible for the initiation of denitrification in the Urrbrae fine sandy loam: this presumably was the soil water content. The lack of evidence indicating the importance of temperature reflects the importance of the relationships between temperature, soil moisture and evapotranspiration in this climate: in regions with humid summers, it might be expected that the previously observed effects of temperature (Nommik, 1956; Bremner and Shaw, 1958b) would increase the rates of losses.

However, some treatments had large effects on the rates of losses as N_2O when the micro-environmental conditions allowed denitrification to proceed. The occurrence of the greatest losses in the A horizon shortly after sowing the wheat crop on pasture can be attributed to the coincidence of high soil moisture and nitrate contents, the presence of living plant roots, and cultivation of the soil. It is not possible to define the relative importance of these factors, but it might be expected that the soil cultivation effects would last only 3-4 days as Rovira and Greacen (1957) showed that these were only important for 24 hrs at higher temperatures. It is now known that both the soil nitrate content and the presence of plants are important factors.

There was a lag in the time maximum accumulation of nitrous oxide with depth in the soil profile, an effect of nitrogenous fertilizer on N_2O concentrations in the B horizon, and only slow increases in the amounts of N_2O in the B horizon. Good evidence exists for the importance of the downward leaching of organic materials in the soil profile on transient, high denitrification rates in B horizons of some solodized solonetz soils (Myers, 1966; McGarity and Myers, 1968). The observed pattern in the B horizon of a buildup to a pronounced peak and subsequent decline is in agreement with the leaching of a diffuse 'slug' of nitrate and/or organic matter substrates downwards, with the peak level occurring at the time when the soil profile is subject to the most restricted aeration of the winter due to the highest soil moisture contents.

VI.2. SIGNIFICANCE OF THE LOSSES BY DENITRIFICATION

Losses as N_2O occurred under all rotational areas and treatments. As the three year period 1966-68 included the wettest and driest years recorded in the previous 40 years at the Waite Institute, it can be concluded that denitrification normally occurs in the Urrbrae fine sandy loam during the winter of every year and under almost all systems of management.

However, estimates of the losses have indicated that even in 1968, the maximum rates of loss were quite small - 0.23 lb N/acre/day (range 0.06-0.67) on the cropped soil (CC) which had been fertilized with nitrogen. Approximate integration has yielded an estimate of 3 lb N/acre for the total seasonal losses using the mean values for porosity, and from the previous fiducial interval based on the porosity data the upper limit for losses would be less than 10 lb N/acre. This is smaller than the generally quoted value for gaseous losses of 10-30% of the added nitrogen fertilizer (Broadbent and Clark, 1965). No estimate of the losses as N_2 gas, or other gaseous forms of nitrogen, was possible. Losses were much smaller where nitrate fertilizer had not been applied, but these occurred during the cold winter months when plants were young, the growth of plants slow, and a shortage of nitrogen most critical. Even the pastures on the moderately high N status (for the Urrbrae series) PP rotation exhibited large responses to added nitrogen in a drought year (Fig. 30) as well as in wet years (Appendix XII).

Figure 30. Southern portion of the experimental area, photographed facing west on 6th October, 1967.

The nearer strip of pasture is PP Replicate 3, and the treatments applied to each plot -

+N +(N+W) (nil) +W

The nearer strip of wheat crop is PP Replicate 3, and the treatments applied to each plot -

+W (nil) +(N+W) +N

It is difficult to assess the importance of such losses, but an estimate can be made assuming a response of 0.13 bushels of grain per pound of fertilizer nitrogen applied. The average response to 23 lb NH_4^+-N fertilizer/acre obtained in Department of Agriculture trials in this State over the 1956-61 period was 2.7 bushels of wheat/acre (French, Matheson and Clark, 1968) and the response to 100 lb/acre NO_3^--N in this experiment was 13 bushels/acre in 1968 (Table 13). A maximum loss of 10 lb N_2O-N/acre during the extremely wet conditions in 1968 would have resulted in a loss of only 1.3 bushels per acre from the +N treatments. Similarly the loss from the unfertilized crop areas would have been less than 0.5 bushels/acre.

The use of nitrogenous fertilizers in South Australia is not widespread, although this is generally attributed to economic seasons rather than a lack of expected responses. As the losses are caused by a biological mechanism, continuation of the present practice of using ammonium forms rather than nitrate forms of fertilizer should keep the losses small.

It has been indicated that the denitrification losses as N_2O are small. But nitrate levels in the 0-10 cm soil depth decreased rapidly during the wet winter months in 1968, and the difference in total N uptake by the above ground portions of the plants due to N fertilization was only 25 lb N/acre (Table 13). Then in 1968, even if the losses as N_2O were as high as 10 lb N/acre, there was still 75 lb NO_3^--N/acre unaccounted for. The depressing effects of heavy rain

TABLE 13

†Some yields of grain and nitrogen contents from the cropped areas, Experiment II, 1967-68.

				L.S.D.		
		-N	+N	0.05	0.01	0.001
GRAIN YIELDS, bushels/acre						
1967	PC	35.0	36.4	4.3	5.9	8.1
	CC	24.4	27.6			
1968	PC	42.3	56.1	5.0	6.9	9.4
	CC	24.4	37.2			
NITROGEN CONTENT (Grain + Straw), lb N/acre						
1967	PC	70	96	10	14	19
	CC	35	63			
1968	PC	74	99	8	12	16
	CC	38	60			

† See Appendix XII.2 for full details.

falling shortly before and after seeding on the final grain yield of wheat grown on the Urrbrae series (Millington, 1961; Piper and de Vries, 1964) has been attributed to the deep leaching of nitrate into the subsoil (Piper and de Vries, 1964) although surface sealing and compaction of the seedbed, with resultant restricted aeration are known to be important when the soils are maintained under close rotations (Millington, 1960, 1961). It is usually considered that leaching results in greater losses than denitrification (Woldendorp, 1963; Allison, 1966), and at present this must be considered as the more important mechanism. However, there is an urgent need for measurement of the losses as N_2, and until these are obtained, the relative importance of nitrogen movements and the various transformations in the soil profile must remain unresolved.

IV.3. FURTHER RESEARCH

The initial objectives in this study have been fulfilled: denitrification products have been detected and measured in the atmosphere of an apparently well aerated soil, there is good evidence suggesting that a biological mechanism is involved and the magnitudes of losses have been estimated.

The system of permanently installed sampling points in the soil profile was very satisfactory for demonstrating the presence of denitrification and for investigating the nature of the losses. But the mean nitrous oxide levels had a very high variance, and estimates of the magnitudes of losses as N_2O encompassed a range of at least an order of magnitude resulting from random errors in the porosity measurements. There are many other sources of error. A small amount of compaction during bulk density measurements could introduce large systematic errors, due to underestimates of the air-filled porosity. The effect of mass flow was shown to influence N_2 levels: this would also have an effect on the diffusive loss from the soil. Finally, the calculated diffusion coefficients may not be satisfactory at low air-filled porosities (Shearer, Millington and Quirk, 1966).

Additionally, the measurement of losses as N_2 is not possible by the use of N_2/A ratios, and an alternate method is required. As the present work indicated that the major losses emanate from the surface soil, the laborious aspects of deep placement of reservoirs can be eliminated: a system of trapping products emerging from the soil surface and the use of N^{15} labelled fertilizers has much merit, despite the cost of the latter. Enlarging the cross-sectional area of soil sampled should reduce the spatial heterogeneity observed in the nitrous oxide data. Concurrent work is also required in situations where the environment can be controlled. The present use of gas lysimeters has shown the advantages of this system.

However, some caution may be necessary in utilizing N^{15} in field measurements of small losses of nitrogen. The diffusivity of N^{15} in the soil air will be smaller than that of N^{14}, and the measurements of N_2/A ratios have shown that such effects can be important.

The soil used in this study was well-drained and well-aerated in the macropores: even so small denitrification losses occurred.

There are many other agricultural soils which exhibit signs of localized or general poor aeration in the soil profile, either in the form of temporary perched water-tables or mottling in the soil profile. In such soils biological dissimilation of nitrate must occur readily, and losses in subsoil horizons may be more important than in better aerated soils: the work of Myers (1966) and McGarity and Myers (1968) has indicated that such subsoils may possess a very large transient denitrification activity. Alternatively, the structural arrangement of well-aerated soils merits attention: soils with dense aggregates or localized high concentrations of easily decomposed organic matter may be more prone to denitrification losses.

It was demonstrated that chemical losses could occur in the Urrbrae soils, but the importance of these was rejected primarily on the basis of low nitrite levels. However, in urine patches from grazing animals it is likely that the resultant high pH will favour the accumulation of nitrite. While at this pH chemical reactions may proceed only slowly, downward leaching of the nitrite to less alkaline regions could result in rapid chemical losses. These reactions may be of considerable importance in our present agriculture where the nitrogen economy is based on the restoring of fertility to cropped soils by the use of legume based (grazed) pastures.

Finally, the importance of anaerobic micro-environments in a well-drained, well-aerated soil has been emphasized, but little is known about these areas of localized activity. Soil structure, organic matter disposition, plant roots and types of plants all appear to be important. Hauck (1968) has drawn attention to the need for a more complete characterization of micro-sites, and while some workers (e.g. Greenwood, 1963, 1968) have shown some of the difficulties involved, this approach is needed to enable a fuller understanding of the agricultural importance of denitrification.

APPENDIX I

AGRONOMIC PRACTICES AND CHARACTERISTICS OF EXPERIMENTAL SITES

I.1. EXPERIMENT I (1966)

Location

The experimental site was located on the northern boundary of paddock W24 of the Education Block, Waite Agricultural Research Institute (W.A.R.I.). The dimensions were approximately 2 chains by 1.5 chains: the total area was about 0.3 of an acre. The site was only very gently sloping (1-2%) to south-west, and the altitude 250 feet above mean sea level.

Past history

The experimental site had been used in the past on a crop-pasture rotation, and prior to the establishment of this experiment it had been under pasture for the past 7 years. The last crop (1958) was a combination of peas and oats. Fertilizers applied during this period were 90-112 lb superphosphate per acre per annum.

Agronomic practices (1966)

June 9 : Initial plowing and cultivation.
28 : Harrowed.
29-30 : Installation of neutron moisture meter (NMM) access tubes.

June 30 : Rotary hoed (10 cm depth).
Sodium nitrate handspread: rate = 625 lb/acre (= 100 lb N/acre).
'Seewari' wheat sown at 60 lb/acre with superphosphate (112 lb/acre), using a conventional rigid-time seed-drill.

July 13 : Emergence of wheat crop.

14 : Soil gas reservoirs installed.

15 : Commenced measurements of soil gas composition.

Aug. 20-25 : Permanent irrigation system of 24 'Pope Rotoframe' sprinklers installed.

31 : Irrigation (0.50 in.)

Sept. 1 : Irrigation (0.50 in.)

2 : Irrigation (0.50 in.)

3 : Irrigation (0.50 in.)

APPENDIX I (cont.)

AGRONOMIC PRACTICES AND CHARACTERISTICS OF EXPERIMENTAL SITES

I.2. EXPERIMENT II (1967/68)

Location

The experimental site was located on the southern boundary of paddock W10 of the W.A.R.I. and straddled the boundary between two former subsections of this paddock: Section B - the eastern (pasture section of area 0.80 acres, and Section A - the western (cultivated) section of 0.58 acres. The complete area used in the experiment was 120 ft x 120 ft or about 0.33 acre. The site was gently sloping (slope approximately 4%), aspect west, and the altitude 323 ft above sea level.

Past history

The two areas had been completely under pasture and crop for the previous seven years. Over the previous 20 years the cropped area had been cropped or fallowed continuously, and the pasture area had been cropped or fallowed in 6 years. The cultivation of the pasture area was an attempt to reduce the weed population, which becomes a problem in the annual pastures (Subterranean clover - Wimmera ryegrass), which usually degenerate within 2-3 years of re-establishment. Further details of previous histories are supplied in Table 14.

TABLE 14

Previous history of paddock W-10

Year	Section A (old cultivated land)	Section B (old pasture land)
1948		C 96 pasture experiment
1949		Cultivation
1950		Fallow
1951		Safflower
1952		Flax
1953	C 95 rotation	Pasture
1954	wheat-barley-fallow	Pasture
1955		Pasture
1956		Pasture
1957		Pasture
1958		Fallow
1959		Fallow
1960		+ Sown pasture WRG-SC
1961	Fallow	Pasture
1962	Wheat	Pasture (weedy)
1963	Oats	Ploughed, pasture resown
1964	Oats	Pasture
1965	Fallow	Pasture
1966	Oats	Pasture (weedy)

+ WRG: Wimmera ryegrass (*Lolium rigidum*, Gaud.)
SC: Subterranean clover (*Trifolium subterraneum*, L.)

Agronomic practices

In general, all initial cultivations were made in a north-south direction to avoid erosion and traffic over the pasture plots.

First season (1967)

June 2 : PP and PC initially cultivated with rigid tine cultivator.

20 : Irrigation (0.60 in.) over whole area (PP + PC + CC) to facilitate further seedbed preparations on PC and CC.

23 : Cultivation (PC and CC).

27 : Irrigation (0.45 in.) over whole area.

28 : Cultivation (PC and CC).

June 28 to July 3 : Installation of NMM access tubes, soil sampling.

5 : PP sprayed with 'Diquat' and 'Paraquat' (20 oz each/acre), with Agral LN as wetting agent.

6 : Cultivation (PC and CC).
Handspread application nitrate of soda (615 lb/acre = 100 lb N/acre) to all +N plots, and basal dressing of superphosphate (224 lb/acre).
'NAPO' wheat sown (50 lb/acre) with rigid tine drill: centre tine of drill removed to avoid NMM tubes, this row sown by hand. PC and CC areas lightly harrowed.

7 : PP sown with subterranean clover (*Trifolium subterranean*, L) and Wimmera ryegrass (*Lolium rigidum*, Gaud.) at 30 and 15 lb/acre using conventional seed drill. Seed was deposited in a small furrow 1/4 in. wide, 1/2 in. deep and covered by raking with set of inverted harrows.

July 7-11 : Gas reservoirs installed (techniques similar to 1966).

18 : First gas sampling.

Aug. 1-10 : Irrigation equipment installed. 'Pope Rotoframe' sprinklers were used, two sprinklers connected to each water meter.

16 : PC and CC sprayed with 'Buctril' (1 pt/acre) to control wireweed.

Sept. 7 : Irrigation to all +W plots (0.50 in. in 35 min). Runoff from CC plots.

12 : Irrigation (0.42 in.).

24 : Irrigation (0.23 in.).

29 : Irrigation (0.20 in.))
)
30 : Irrigation (1.00 in.)) All plots irrigated at rate of 0.20 in. each hour
)
Oct. 1 : Irrigation (1.00 in.))

10 : Pasture harvested. Whole plot area cut with autoscythe, green herbage weighed and two 500 g subsamples weighed in forced draught oven.

22 : Wilting of wheat crop, even on (+W) plots. Irrigated (0.50 in.) to PP, PC and CC).

Dec. 2 : Wheat harvested. Four areas, each 4 rows wide and 2 ft. long, cut from each plot. Total above ground herbage weighed, wheat thrashed in stationary thresher, and wheat and straw samples kept for analysis.

<u>Second season</u> (1968)

May 11 : PP sprayed with 'Diquat' and 'Paraquat' (1 pt each/acre): subterranean clover (Bacchus Marsh, Clare, Geraldton var.) and wimmera ryegrass handspread over plots at 70 and 16 lb/acre respectively and soil surface lightly raked.

13 : Superphosphate (224 lb/acre) plus MoO_3 (2 oz/acre) spread over all plots.
Nitrate of soda (50 lb N/acre) spread over all (+N) plots.

13 : PC and CC rotary hoed, uncultivated areas close to gas reservoir leads sprayed with 'Diquat' and 'Paraquat'.

13- Pasture seed eaten by pigeons.

30 : PP plots resprayed with 'Diquat' and 'Paraquat' and pasture resown. Seed buried in 3/8 in. deep furrows, 7 in. apart.

June 16 : Gas reservoirs removed from 10 cm depth, PC and CC.

17 : PC and CC rotary hoed, first in N-S direction, second time in E-W direction. Between first and second cultivations, the second application of nitrate of soda (50 lb N/acre) was handspread over all plots, and areas close to the permanent installations (PC and CC only) were cultivated by hand. 'NAPO' wheat sown by hand at 14 in. spacings (normal spacing = 7 in.).

18 : Gas reservoirs replaced on PC and CC at 10 cm depth (in new position).

19 : Thermistors installed.

Aug. 7 : 'Diquat' and 'Paraquat' sprayed between rows of crop (PC and CC) to kill ryegrass.

Oct. 10 : Pasture harvested: technique similar to 1967.

Dec. 17 : Wheat harvested: technique similar to 1967, except that only 2 quadrats (each 2 rows x 6 ft) were cut per plot.

APPENDIX II

SOIL DESCRIPTIONS

Both Experiment I and Experiment II were situated on the Urrbrae fine sandy loam (Litchfield, 1951), a red-brown earth (Piper, 1938; Stephens, 1953; Stace et al., 1968), developed on alluvium from slates and shales of the nearby Mount Lofty Ranges. The soil at Experiment I had been mapped as Type A-1 of the Urrbrae series and that at Experiment II as Type A-1 and Type A-2: the latter is a gravelly phase (Litchfield, 1951).

II.1. EXPERIMENT I (1966)

The A horizon consisted of about 15-20 cm of a reddish brown fine sandy loam. The A_2 horizon was either very weakly developed or absent; more probably, the whole A horizon can be considered an A_p layer, as the top of the B horizon is not prismatic, as is usual with the Urrbrae series, but is of granular structure. It would seem that a very deep cultivation may have taken place at some time in the past. The B_1 horizon (15-20 cm to about 30 cm) is of a red-brown granular clay loam. The B_2 horizon, which commences with a reasonably abrupt boundary at about 30 cm, is a red-brown heavy clay, with prismatic structure in the upper portion, gradually becoming a reddish-brown massive clay below 60 cm. At about 75 cm flecks of lime appear, and gradually increase with depth so that at 90 cm, the soil consists of a brown clay containing numerous fine particles as well as porous clumps and occasional nodules of lime. Some characteristics of the soil have been presented in Table 1.

II.2. EXPERIMENT II (1967/68)

The main difference between this soil and that of Experiment I lay in the deeper A horizon (11-14 in.), the presence of a definite although a weakly developed A_2 horizon, the more gradual boundary with the B horizon, and the presence of a prismatic structure in the upper portion of the B horizon. Flecks of lime began to appear at 30 in. with a definite lime enriched horizon occurring at 36 in. Some characteristics of the soil have been shown in Fig. 19.

APPENDIX III

MEASUREMENT OF SOIL INORGANIC NITROGEN

Soil inorganic nitrogen measurements

Samples were analyzed for inorganic nitrogen by a 1 hr shaking with water or normal Na_2SO_4; and either semi-micro distillation with MgO, Devarda's alloy and sulfamic acid for the determination of NH_4^+, NO_3^-, NO_2^- (Bremner, 1965b) or using the 'Orion nitrate electrode' for NO_3^- (Anon., 1968; Myers and Paul, 1968; Bremner, Bundy and Argarwal, 1968).

Variations

Experiment	Extracting solution	Soil:Soln. g ml	Method	Forms of N measured
Experiment I (1966)	N Na_2SO_4	100:250 (OD)	Distn.	NH_4^+ NO_3^-
Experiment II (1967)	N Na_2SO_4	100:250 (Moist)	Distn.	NH_4^+ NO_3^-
Experiment II (1968)	H_2O	100:250 (OD)	Distn. Elect.	NO_3^-
Incubation experiment	N Na_2SO_4	150:750	Distn.	NH_4^+, NO_3^-, NO_2^-

APPENDIX IV

MEASUREMENT OF SOIL WATER CONTENT BY THE NEUTRON MOISTURE METER (NMM) METHOD

Estimation of soil moisture content was made using a neutron moisture meter (Model Number 8402 : Nuclear Enterprises Ltd., Edinburgh, United Kingdom), comprising a portable scaler (NE 5011) and moisture probe (NE 5592) with a 45 mc Americium/Beryllium source.

Access tubes were made of polypropylene (Field Experiment I and the 'normalization' water drum), or of polyvinylchloride (Field Experiment II).

Counts were usually measured at the 15, 45, and 75 cm depths below the soil surface with less frequent measurements (Field Experiment II) at the 105, 135, and 165 cm depth. Duplicate 60 second counts (Field Experiment I and Field Experiment II - 1967) or 30 second counts (Field Experiment II - 1968) were made, and the individual counts bulked. At least 5 replicates were used for each normalization.

Count ratios were calculated for all soil readings, where:

$$\text{Count ratio} = \frac{C - B}{N - B}$$

C = cts/min in soil
N = cts/min in water
B = cts/min in air

Volumetric water contents were determined from a calibration curve, obtained from field calibration on 4 individual holes to a depth of 6 ft:

$$\ln(CR) = -0.1869 + 0.9094 \ln(MC) \qquad r^2 = 0.97$$

where CR = count ratio
MC = moisture content (v/v)

APPENDIX V

COMMENTS ON CLIMATIC DATA

All climatic data have been extracted from the records of the meteorological station at the Waite Institute. The meteorological station is located on the gently rising foothills of the scarp of the Mt. Lofty Ranges and is at an altitude of about 373 feet above m.s.l. The meteorological station records have been adopted to enable comparisons with previous records.

Both experimental sites were located further away from the scarp, and were at lower altitudes on the gently sloping land of the Adelaide Plains than the meteorological station. Experiments II and I were situated about 0.25 and 0.80 miles respectively, from the meteorological station, and at altitudes of 138 and 50 ft. lower.

Comparisons of the total rainfall in 1967 and 1968 at a site close to Experiment I with that at the meteorological station indicates that the average annual rainfall at the sites of Experiment I and II is about 2.5 and 0.5 in. lower than that at the meteorological station.

Monthly rainfall and evaporation data are provided in Appendix X.

APPENDIX VI

OPERATING CONDITIONS FOR 'SHIMADZU GC-IC' GAS CHROMATOGRAPH

	1966	1967	1968	Incubation experiment
Helium carrier gas at				
Inlet pressure (Kg/cm^2)	1.7	3.0	2.7	2.7
Exit flow rate (ml/min)	100	78	85	85
Sample				
- size (ml)	5	5	3	1.7
- introduction	Injection port	By-pass injector	By-pass injector	Gas sample loop
Thermal Conductivity Detector:				
Bridge: Voltage	100	100	100	100
Current (mA)	85-100	100	170	170
Temperature (C)	220	125	125	125
Column Packing				
HT	Composite†	MS 5A 'flour' (<270 mesh)/Chromosorb		
MT		MS 5A (32-60 mesh)		
LT		MS 13X (32-60 mesh), washed		
Column length (cm) x 3 mm i.d.				
HT	150	225	150	150
MT		38	55	55
LT		75	75	75
Column Temperature (C)				
HT	‡ 60-220	144	144	144
MT		24	36.5	36.5
LT		-98	-98	-98

† 1966 analyses were made on a composite column packed with MS 5A materials similar to those used in 1967/8 HT and MT columns: ($A + O_2 + N_2$) - N_2O - CO_2 separations were made at 220 C with bridge current of 100 mA, and ($A + O_2$) - N_2 separations were made at 60 C with bridge current of 85 mA.

APPENDIX VII

CALCULATION OF COMPOSITION OF SOIL ATMOSPHERE SAMPLES

1. Calibration curves

Calibration curves relating volume of gas at 15 C and 760 mm Hg pressure to peak height were obtained for (A + O_2), N_2, N_2O, CO_2, A and O_2. Those for N_2O and CO_2 were determined by introducing 1.7 and 4.7 ml aliquots of prepared gas mixtures into the gas chromatograph using a gas sampling valve; and those for (A + O_2), N_2, A and O_2 were obtained by injections of 1, 2, 3, 4, 5 and 6 ml air before and after several gas samplings, and mean calibration curves obtained.

For all components good linear relationships were obtained when the data was plotted on a log-log scale [$r^2 = 0.998$ N = 6 for (A + O_2), N_2, A and O_2] and N_2O calibration curves were also linear ($r^2 = 0.990$ N = 48) on a linear scale (0-2.0% N_2O in 5 ml). The values of slopes and constants used over all samplings are shown in Table 15.

2. Variable nitrogen calibration curves

Nitrogen calibration curves of freshly activated columns differed from those obtained after analyzing a sampling, and this was due to differences in intercepts rather than slopes (Table 16). No such effects were observed with (A + O_2) (Table 16), A or O_2. From

TABLE 15

Slopes and intercepts for gas calibration curves: ln (peak height) = ln a + b ln (μl gas)‡

	1967		1968					
			I		II		III	
	b	ln a	b	ln a	b	ln a	b	ln a
$(A + O_2)$	1.033	-2.8668	1.006	-2.2792	0.963	-1.9902	0.917	-1.6595
†N_2O	1.000	1.3755	1.000	2.898	1.000	2.898	1.000	2.898
CO_2	1.101	0.5002	1.119	1.904	1.119	1.904	1.119	1.904
A	1.007	0.1253	0.994	1.1252	1.029	1.1156	1.013	0.5015
O_2	0.8539	-1.5996	0.8924	-1.5665	0.8744	-1.4082	0.8510	-1.2525
N_2	0.7859	††	0.7846	††	0.7538	††	0.6500	††

† Actual calibration curve used was the linear relationship y = bx.

‡ Volume gas at 760 mm Hg pressure and 25 C.

†† Value calculated from the two air samples before and after a group of soil atmosphere samples.

TABLE 16

Slopes, intercepts, and standard errors of calibration regressions for $(A + O_2)$ and N_2. Equations were of the form: $\ln y = \ln a + b (\ln x)$, where y = peak height and x = ml of gas at 760 mm P and 25 C

Calibration Number	†Column status	Slope (b)	SE (b)	Constant (ln a)	SE (ln a)
		ARGON + OXYGEN			
1967	b	1.032	0.013	4.261	0.009
	a	1.034	0.015	4.277	0.015
1968 - I	b	1.012	0.013	4.674	0.014
	a	1.000	0.010	4.666	0.011
1968 - II	b	0.963	0.022	4.650	0.021
	a	0.963	0.014	4.673	0.014
1968 - III	b	0.917	0.004	4.675	0.004
	a				
		NITROGEN			
1967	b	0.792	0.009	3.200	0.009
	a	0.780	0.013	3.274	0.013
1968 - I	b	0.785	0.009	3.666	0.005
	a	0.785	0.006	3.685	0.004
1968 - II	b	0.751	0.007	3.660	0.005
	a	0.757	0.005	3.713	0.004
1968 - III	b	0.660	0.014	3.923	0.009
	a	0.640	0.014	3.951	0.010

† Before (b) or after (a) analysis of a field gas sampling.

this data, a nitrogen calibration curve was calculated for each group of 8 or 12 samples. For the two air samples the volume of $(A + O_2)$ was determined from the peak height, then the volume of N_2 calculated from:

$$\mu l\ N_2 = \mu l\ (A + O_2) \times \frac{78.08}{21.884}$$

The value of <u>ln a</u> was calculated by substituting the actual values of $\mu l\ N_2$, b and peak height in the equation:

$$\ln\ (\text{peak height}) = \ln a + b \ln\ (\mu l\ N_2)$$

and this value was used for this group of samples and the two air samples.

3. Calculation of μl, A, O_2, $(A + O_2)$

(1) Correction of $(A + O_2)$ μl for different relative response factors for A, O_2 in $(A + O_2)$ peak height.

The relative response factors for A and O_2 in the $(A + O_2)$ peak height were 83.2 and 68.2. To correct for this the apparent volume of $(A + O_2)$ read from the calibration curve was multiplied by the correction factor (C.F.) to give $\mu l\ (A + O_2)_{\text{true}}$:

$$\text{C.F.} = \frac{(A + O_2) \times 68.84}{68.2\ (O_2) + 83.2\ (A)}$$

where (O_2), (A), $(A + O_2)$ are the approximate μl of these gases determined from the O_2, A calibration curves and peaks (next subsection).

(2) Determination of µl, A, O_2.

Although the peak heights of A, O_2 commonly exhibited up to a 10% variation due to column conditions, both peaks behaved similarly and the ratio of A:O_2 (µl) could be regarded as independent of column conditions so long as the resolution was satisfactory.

From the calibration curves of A and O_2, the 'approximate' amounts of A, O_2 were determined. The true amounts were calculated from:

$$A_{true} = \frac{(A + O_2)_{true}}{(A + O_2)_{approx.}} \times A_{approx.}$$

4. Loss of N_2O and CO_2 in syringes

The loss of N_2O and CO_2 from the air space of the polystyrene syringes was determined by sampling a standard mixture with syringes, and analysing such samples after elapsed times of 1-70 hours. The loss of component gases has been adequately described by an equation (Fig. 31) in which it is assumed that all losses are due to adsorption by the polystyrene syringe:

$$\frac{X_t}{X_o} = \frac{k_2 + k_1 e^{-(k_1 + k_2)t}}{k_1 + k_2}$$

where X_1 = N_2O, CO_2 after t hours

X_o = N_2O, CO_2 at sampling

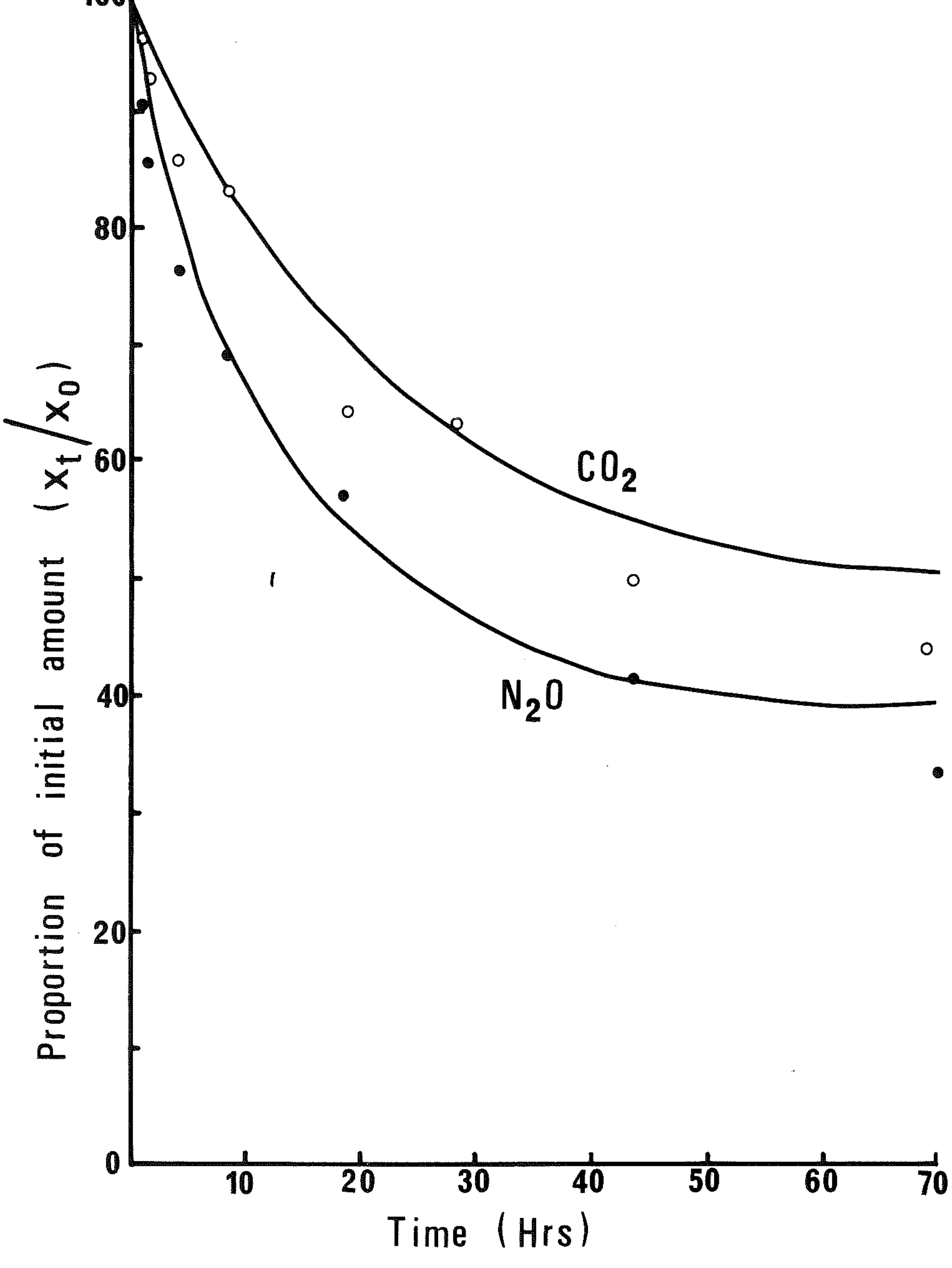
FIGURE 31. LOSSES OF N_2O and CO_2 FROM THE AIR SPACE OF POLYSTYRENE SYRINGES. THE EQUATIONS OF THE FITTED CURVES ARE:

$$CO_2 : \qquad \frac{X_t}{X_o} = \frac{0.0216 + 0.0235\, e^{-0.451t}}{0.0451}$$

$$N_2O : \qquad \frac{X_t}{X_o} = \frac{0.0290 + 0.0460\, e^{-0.075t}}{0.075}$$

where X_t = volume of N_2O, CO_2 in air space of syringe after t hours

and X_o = initial volume of N_2O, CO_2 in space in syringe.



100
80
60
40
20
0
Proportion of initial amount (x_t/x_0)
CO$_2$
N$_2$O
10
20
30
40
50
60
70
Time (Hrs)

and k_1 = rate loss N_2O, CO_2 from air space (sorption)

k_2 = rate gain N_2O, CO_2 by air space (desorption)

The following values for k_1 and k_2 were used for all soil atmosphere samples, but not for the air samples:

	k_1	k_2
N_2O	0.046	0.029
CO_2	0.0235	0.0216

5. Variable volume of injected samples

The variable volume was compensated for by the usual technique of summation, whereby all calibration curves were based on volumes of gas (at 15 C, 760 mm pressure) and the percentage composition calculated from the volumes of the individual components and the total volume of components.

6. Calculation of results

All field gas samplings were analysed by a computer program designed for this purpose. Statistical treatment involved two stages: firstly the calculation (and punching onto computer cards) of the percent composition of the air and soil atmosphere samples using a CDC 6400 computer (program - Appendix VIII) and then calculation of means and confidence limits on a CDC 3200 computer using the Waite-Wellesbourne General Statistical Program ('GENSTAT').

APPENDIX VIII.1

SIMPLIFIED FLOW DIAGRAM OF COMPUTER PROGRAM FOR SOIL GAS ANALYSES

READ PROGRAM

READ 2 AIR SAMPLES

Calculate µl $(A + O_2)$ FROM $(A + O_2)$ PEAK HEIGHT

Calculate µl N_2 (from µl $A + O_2$)

Calculate (ln a) for group 8-12 soil air samples (for nitrogen)

CALCULATE composition of two air samples using similar process to that for soil air samples but without N_2O calculations or correction for loss CO_2 from syringe.

CALCULATE COMPOSITION OF SOIL AIR SAMPLES and two air samples

Read peak height $A + O_2$, N_2, N_2O, CO_2, A, O_2, time since sampled.

Calculate approximate volumes from calibration curves

Correct $(A + O_2)$ for $A:O_2$ ratio

Calculate correct volumes A, O_2 from $\dfrac{(A + O_2)\text{ approx.}}{(A + O_2)\text{ true}}$

Correct N_2O, CO_2 volumes for time lapse

Summation and punching calculated percentages onto cards

Read into CDC 3200 computer

Use 'GENSTAT'

Calculate means, confidence limits

PRINT

APPENDIX VIII.2.

COMPUTER PROGRAM FOR SOIL GAS ANALYSIS

```
                PROGRAM BURGAS (INPUT,OUTPUT,PUNCH)
000003          DIMENSION TITLE(8),B(6).C(6),A1(5).A2(5).CAL(5).X(6)
000003          READ 101.P.Q,R.S
          C     READ NUMBER OF SAMPLINGS AND CALIBRATION VALUES
000017        7 READ 105.NS
000025      105 FORMAT (I6.10F6.0)
000025          READ 101.B(1),C(1),(B(I),C(I).I=3.6).B(2)
000047      101 FORMAT (11F6.0)
          C     A+O2=1,N2=2,N2O=3,CO2=4,A=5,O2=6
000047          IF(NS.LE.0) GO TO 9
          C     READ SAMPLING IDENTIFICATION, NO OF RUNS
000051          DO 8 L=1.NS
000052          READ 102.NGAS.NP
000061      102 FORMAT (A6,I6)
000061          IX=1
000062          PRINT 103,NGAS
000070      103 FORMAT (1H1,10X*GAS SAMPLING NUMBER*A6)
000070          DO 1 J=1,NP
000072          PRINT 104
000075      104 FORMAT(70X*PERCENTAGE COMPOSITION*/15X*REP*4X*PLOT*3X*DEPTH*4X*NIT
               $R*3X*WATER*4X*TIME*5X*N2/A*8X*N2*7X*N2O*7X*CO2*9X*A*8X*O2*/)
          C     FOR EACH SAMPLING READ NUMBER OF SAMPLES AND VALUES FOR AIR
          C     SAMPLES
000075          READ 105.N.(A1(I),I=1,5).(A2(I),I=1.5)
000117          DO 2 I=1.5
000121          A1(I)=ALOG(A1(I))
000124          A2(I)=ALOG(A2(I))
000131          CAL(I)=A1(I)+A2(I)
000135         -CAL(I)=CAL(I)/2.0
000137        2 CONTINUE
000141          APLUSO=(CAL(1)-C(1))/B(1)
000144          GASN=APLUSO+ALOG(78.08)-ALOG(21.884)
000153          C(2)=CAL(2)-B(2)*GASN
000156          DO 12 I=1.2
000160          A1(I)=(A1(I)-C(I))/B(I)
000163          A2(I)=(A2(I)-C(I))/B(I)
000166          A1(I)=EXP(A1(I))
000171          A2(I)=EXP(A2(I))
000177       12 CONTINUE
000201          DO 13 I=3.5
000203          A1(I)=(A1(I)-C(I+1))/B(I+1)
000206          A2(I)=(A2(I)-C(I+1))/B(I+1)
000211          A1(I)=EXP(A1(I))
000214          A2(I)=EXP(A2(I))
000222       13 CONTINUE
000224          APPROX=A1(5)+A1(4)
000226          RATIO=68.2*A1(5)+83.2*A1(4)
000231          RATIO=68.84*APPROX/RATIO
000234          A1(1)=A1(1)*RATIO
000236          A1(4)=A1(4)*A1(1)/APPROX
000237          A1(5)=A1(5)*A1(1)/APPROX
000240          APPROX=A2(5)+A2(4)
000242          RATIO=68.2*A2(5)+83.2*A2(4)
000245          RATIO=68.84*APPROX/RATIO
000250          A2(1)=A2(1)*RATIO
000252          A2(4)=A2(4)*A2(1)/APPROX
000253          A2(5)=A2(5)*A2(1)/APPROX
```

```
000254         A1(1)=(A1(1)+A2(1))/2.0
000256         TOT=0
000257         DO 14 I=2,5
000261         A1(I)=(A1(I)+A2(I))/2.0
000264         TOT=TOT+A1(I)
000266      14 CONTINUE
000270         A1(1)=A1(2)/A1(4)
000272         DO 15 I=2,5
000273         A1(1)=100.0*A1(I)/TOT
000276      15 CONTINUE
000277         PRINT 130,(A1(1),I=1,5)
000311     130 FORMAT(47X*AIR SAMPLES*2F10.4,10X,3F10.4)
000311         DO 3 K=1,N
000313         READ 106,IR,IP,ID,IN,IW,IT,(X(I),I=1,6)
000340     106 FORMAT (6I3,6F6.0)
         C     CALCULATE MICROLITRES OF GAS FROM PEAK HEIGHTS X(I)
000340         DO 4 I=1,6
000342         IF(I.EQ.3) 10,11
000346      10 X(I)=X(I)/B(I)
000351         GO TO 4
000351      11 X(I)=(ALOG(X(I))-C(I))/B(I)
000360         X(I)=EXP(X(I))
000365       4 CONTINUE
000367         APPROX=X(5)+X(6)
000371         RATIO=68.2*X(6)+83.2*X(5)
000374         RATIO=68.84*APPROX/RATIO
000377         X(1)=X(1)*RATIO
000401         X(5)=X(5)*X(1)/APPROX
000402         X(6)=X(6)*X(1)/APPROX
000403         RATIO=X(2)/X(5)
         C     CORRECT X(3) AND X(4), (N2O AND CO2) FOR TIME ELAPSED
000405         PK=P+Q
000407         X(3)=PK*X(3)/(Q+P*EXP(-PK*IT))
000417         PK=R+S
000421         X(4)=PK*X(4)/(S+R*EXP(-PK*IT))
000431         TOT=0.0
000432         DO 5 I=2,6
000434         TOT=TOT+X(1)
000436       5 CONTINUE
000440         DO 6 I=2,6
000441         X(I)=X(I)*100.0/TOT
000444       6 CONTINUE
000446         PRINT 107,IR,IP,ID,IN,IW,IT,RATIO,(X(I),I=2,6),IX
000477         PUNCH 140,IR,IP,ID,IN,IW,IT,RATIO,(X(I),I=2,6),IX,NGAS
000533     140 FORMAT(6I3,6F8.4,I6,*M *,A6)
000533         IX=IX+1
000535     107 FORMAT(10X6I8,6F10.4,I8)
000535       3 CONTINUE
000537       1 CONTINUE
000542       8 CONTINUE
000544         GO TO 7
000545       9 STOP
000547         END
```

APPENDIX IX

†MEANS AND SIGNIFICANCE LEVELS OF LOGARITHMIC TRANSFORMATIONS (ln x) OF SOIL NO_3^--N AND NH_4^+-N CONTENTS (ppm), 1966.

Sampling date		July			August		September		Oct.	L.S.D.		
		11th	21st	29th	5th	18th	1st	29th	31st	P = 0.05	0.01	0.001
ln (NITRATE-N)												
Soil depth (cm)	0-30	3.77	3.65	3.29	3.50	3.57	3.20	2.60	2.83	0.39	0.53	0.70
	30-60	2.17	2.36	2.72	2.82	2.96	2.94	2.91	3.28	0.28	0.36	0.47
	60-90	0.59	1.36	1.52	1.68	1.81	1.89	1.65	2.17	0.54	0.73	0.97
ln (AMMONIUM-N)												
Soil depth (cm)	0-30	2.24	1.52	1.41	1.59	1.16	1.13	2.19	0.78	0.49	0.67	0.89
	30-60	-1.06	-1.61	-1.26	-0.78	-0.01	-0.43	1.81	-0.95	0.90	1.20	1.62
	60-90	-0.71	-0.71	-1.15	-0.60	-0.53	-0.13	-0.83	-0.69	0.58	0.80	1.06

† Measurements for December 8th were not included, due to zero values for NO_3^- in surface samples.

APPENDIX X

†RAINFALL AND EVAPORATION DATA

Monthly averages (1966/67/68) and mean monthly averages (1925-68) of daily evaporation (0.01 in./day)

MONTH	Year 1966	 1967	 1968	MEAN
January	28.1	25.5	30.2	28.1
February	24.9	27.0	32.0	25.3
March	20.2	20.6	19.6	20.8
April	13.7	15.4	13.3	13.4
May	8.1	11.3	5.6	8.3
June	5.8	6.3	5.6	5.9
July	5.1	5.9	4.5	5.5
August	7.2	7.4	5.6	7.3
September	10.7	11.8	11.1	10.9
October	13.6	18.7	14.2	15.1
November	22.4	24.8	17.9	20.4
December	20.2	26.6	24.6	24.9
TOTAL	15.0	16.8	15.3	15.5

†Source of data: Waite Agricultural Research Institute Meteorological Station records.

APPENDIX X (cont.)

Actual monthly rainfall (1966/ 7/68) and mean monthly rainfall (1925-68) (0.01 in.).

MONTH	Year 1966	1967	1968	MEAN
January	15	65	159	88
February	97	150	183	103
March	138	7	195	81
April	48	36	262	208
May	325	150	565	323
June	391	50	253	297
July	430	335	309	329
August	212	225	441	286
September	254	139	117	232
October	158	59	348	212
November	109	8	228	156
December	356	58	168	118
TOTAL	2533	1282	3228	24.33

APPENDIX XI

†TOTAL POROSITY (ε_T) VALUES AND ‡STANDARD ERRORS: EXPERIMENT II, 1967 (ml/ml).

Depth (cm)	Rotation		
	PP	PC	CC
0- 10	0.449 ± 0.021	0.567 ± 0.020	0.553 ± 0.037
10- 20	0.429 ± 0.008	0.423 ± 0.034	0.408 ± 0.062
20- 30	0.400 ± 0.011	0.383 ± 0.015	0.379 ± 0.048
30- 45	0.324 ± 0.036	0.474 ± 0.033	0.427 ± 0.038
45- 60	0.439 ± 0.034	0.458 ± 0.030	0.424 ± 0.021
60- 75	0.456 ± 0.032	0.472 ± 0.016	0.479 ± 0.023
75- 90	0.435 ± 0.035	0.448 ± 0.008	0.440 ± 0.031
90-120	0.335 ± 0.011	0.362 ± 0.053	0.362 ± 0.033
120-150	0.410 ± 0.015	0.382 ± 0.044	0.421 ± 0.068
150-180	0.418 ± 0.060	0.389 ± 0.020	0.432 ± 0.070

† Data obtained during soil sampling, 28th June-3rd July, 1967.

‡ Standard error = $s/\sqrt{n}$, n = 4

APPENDIX XII.1

YIELDS AND NITROGEN CONTENTS OF HERBAGE - FIELD EXPERIMENT II (1967/68) : PASTURE

	Treatment				L.S.D.			Main Effects			
	-	N	W	N + W	0.05	0.01	0.001	-N	+N	-W	+W
Herbage Yield (lb OD material/acre)											
1967	1650	2042	2647	3241	436	626	922	2149	2642*	1846	2944**
1968	4741	6131	4662	6476	475	682	1000	4702	6304***	5436	5569
Nitrogen Content (%, OD basis)											
1967	2.41	2.88	2.31	2.86	0.26	0.37	0.55	2.36	2.87***	2.64	2.58
1968	1.85	1.65	1.87	1.63	0.11	0.15	0.22	1.86	1.64***	1.75	1.75
Nitrogen Uptake (lb/acre)											
1967	40	59	61	92	13	19	27	51	76***	49	77***
1968	88	101	88	104	10	15	21	88	103***	95	96

APPENDIX XII.2

YIELDS AND NITROGEN CONTENTS - EXPERIMENT II (1967/68) : WHEAT CROP

	Rotation	Treatment				L.S.D.			Main Effects			
		-	N	W	N + W	0.05	0.01	0.001	-N	+N	-W	+W
† GRAIN YIELD (lb/acre)												
1967	PC	1858	1858	2347	2514	366	502	684	2102	2186	1858	2430
	CC	1310	1477	1619	1839				1464	1658	1394	1729
	Mean	1584	1668	1982	2176	259	355	484	1783	1922	1626	2080***
1968	PC	2877	3520	2206	3203	425	583	794	2541	3362	3199	2705
	CC	1549	2243	1372	2219				1460	2231	1896	1795
	Mean	2213	2882	1789	2711	301	412	561	2001	2796***	2547	2250**
† STRAW YIELD (lb/acre)												
1967	PC	3842	4511	5012	5935	521	714	973	4427	5229	4177	5473
	CC	2448	3349	2998	4494				2723	3922	2898	3746
	Mean	3145	3930	4005	5214	370	505	688	3575	4572***	3537	4610***
1968	PC	4409	5765	3667	5263	588	805	1097	4038	5514	5087	4465
	CC	2650	4638	2343	4560				2496	4599	3644	3452
	Mean	3530	5201	3005	4912	416	569	776	3267	5057***	4365	3958**

	Rotation	Treatment				L.S.D.			Main Effects			
		-	N	W	N + W	0.05	0.01	0.001	-N	+N	-W	+W
TOTAL YIELD (Grain + Straw lb/acre)												
1967	PC	5670	6369	7359	8449	846	1159	1580	6530	7409	6034	7904
	CC	3758	4827	4617	6333				4187	5580	4292	5475
	Mean	4729	5598	5988	7391	598	820	1117	5358	6494***	5163	6689***
1968	PC	7286	9285	5873	8466	987	1352	1843	6579	8876	8286	7169
	CC	4199	6881	3714	6779				3956	6830	5540	5247
	Mean	5742	8083	4793	7623	698	956	1303	5268	7853***	6913	6208**
GRAIN/(Grain + Straw)												
1967	PC	0.325	0.291	0.318	0.306	0.029	0.040	0.055	0.322	0.298	0.308	0.312
	CC	0.348	0.305	0.348	0.288				0.348	0.296	0.326	0.318
	Mean	0.336	0.298	0.333	0.297	0.021	0.028	0.039	0.335	0.297***	0.317	0.315
1968	PC	0.395	0.379	0.375	0.378	0.025	0.034	0.046	0.385	0.378	0.387	0.376
	CC	0.367	0.327	0.369	0.327				0.368	0.327	0.347	0.348
	Mean	0.381	0.353	0.372	0.352	0.017	0.024	0.033	0.377	0.353***	0.367	0.362

Rotation		Treatment				L.S.D.			Main Effects			
		-	N	W	N + W	0.05	0.01	0.001	-N	+N	-W	+W
‡ GRAIN N, %												
1967	PC	2.81	3.22	2.68	2.95	0.17	0.24	0.32	2.74	3.08	3.01	2.81
	CC	2.17	2.88	2.22	2.97				2.19	2.93	2.53	2.59
	Mean	2.49	3.05	2.45	2.96	0.12	0.17	0.23	2.45	3.00***	2.77	2.70
1968	PC	2.64	2.71	2.70	2.72	0.13	0.18	0.25	2.67	2.71	2.67	2.71
	CC	2.35	2.44	2.42	2.36				2.38	2.40	2.39	2.39
	Mean	2.49	2.57	2.56	2.54	0.09	0.13	0.18	2.52	2.56	2.53	2.55
‡ STRAW N, %												
1967	PC	0.48	0.83	0.40	0.70	0.13	0.18	0.25	0.44	0.77	0.66	0.55
	CC	0.24	0.56	0.23	0.51				0.23	0.54	0.40	0.37
	Mean	0.36	0.70	0.31	0.61	0.09	0.13	0.18	0.34	0.65***	0.53	0.46*
1968	PC	0.32	0.32	0.40	0.37	0.07	0.09	0.13	0.36	0.35	0.321	0.384
	CC	0.32	0.30	0.28	0.30				0.30	0.30	0.31	0.29
	Mean	0.32	0.31	0.34	0.34	0.05	0.07	0.09	0.33	0.32	0.32	0.34

	Rotation	Treatment				L.S.D.			Main Effects			
		-	N	W	N + W	0.05	0.01	0.001	-N	+N	-W	+W
GRAIN N CONTENT (lb/acre)												
1967	PC	47	54	57	67	10	14	19	52	60	51	62
	CC	26	38	32	49				29	44	32	41
	Mean	36	46	45	58	7	10	13	41	52***	41	51***
1968	PC	68	85	54	78	10	14	19	61	81	77	66
	CC	32	49	30	47				31	48	41	38
	Mean	50	67	42	62	7	10	13	46	65***	59	52**
STRAW N CONTENT (lb/acre)												
1967	PC	17	34	18	38	6	9	12	18	36	25	28
	CC	5	17	6	21				6	19	11	13
	Mean	11	26	12	29	5	6	8	12	27***	18	21
1968	PC	13	17	13	18	3	4	6	13	17	15	15
	CC	8	12	6	12				7	12	10	9
	Mean	10	15	10	15	2	3	4	10	15***	12	12

Rotation		Treatment				L.S.D.			Main Effects			
		-	N	W	N + W	0.05	0.01	0.001	-N	+N	-W	+W
TOTAL N UPTAKE (lb/acre)												
1967	PC	64	88	75	105	14	19	26	70	96	76	90
	CC	31	55	38	71				35	63	43	55
	Mean	47	72	57	88	10	14	19	52	80***	60	72
1968	PC	81	102	68	95	12	16	22	74	99	91	82
	CC	40	61	36	59				38	60	51	47
	Mean	60	82	52	77	8	12	16	56	79***	71	64*

† Moisture contents: grain 10.7% (1967) and 11.9% (1968)
straw 10.0% (1967) and 11.0% (1968)

‡ Oven dry basis (70 C)

APPENDIX XIII

SUCCESSIVE SAMPLING FROM SOIL GAS RESERVOIRS† 2nd OCTOBER, 1966,: ‡N_2O and CO_2 CONTENTS OF 1st, 3rd, 6th and 9th SAMPLES

Sample origin		Volume removed from reservoir including 5 ml sample for analysis			
Replicate	Depth (cm)	5	15	30	45
	NITROUS OXIDE (ppm)				
2	15	6	6	6	3
	30	17	23	n.d.	17
	60	6	9	12	6
	90	0	3	3	3
8	30	9	9	3	3
	60	12	9	3	3
	CARBON DIOXIDE (%)				
2	15	1.12	1.39	1.46	1.59
	30	0.11	0.16	n.d.	0.25
	60	0.70	0.80	0.85	0.32
	90	0.39	0.43	0.59	0.50
8	30	0.56	0.49	0.54	0.30
	60	0.08	0.07	0.04	0.05

† Internal volume of reservoir = 30 ml.

‡ N_2O and CO_2 values <u>not</u> corrected for syringe losses.

n.d. = not determined.

APPENDIX XIV

PROFILE DISTRIBUTION OF SOIL MOISTURE, EXPERIMENT II, 1967/68.

Least significant differences at P = 0.05 and 0.01 are shown by]

(a) 1967

Key

	PP	PC	CC
-W	△——△	□---□	○...○
+W	▲——▲	■---■	●...●

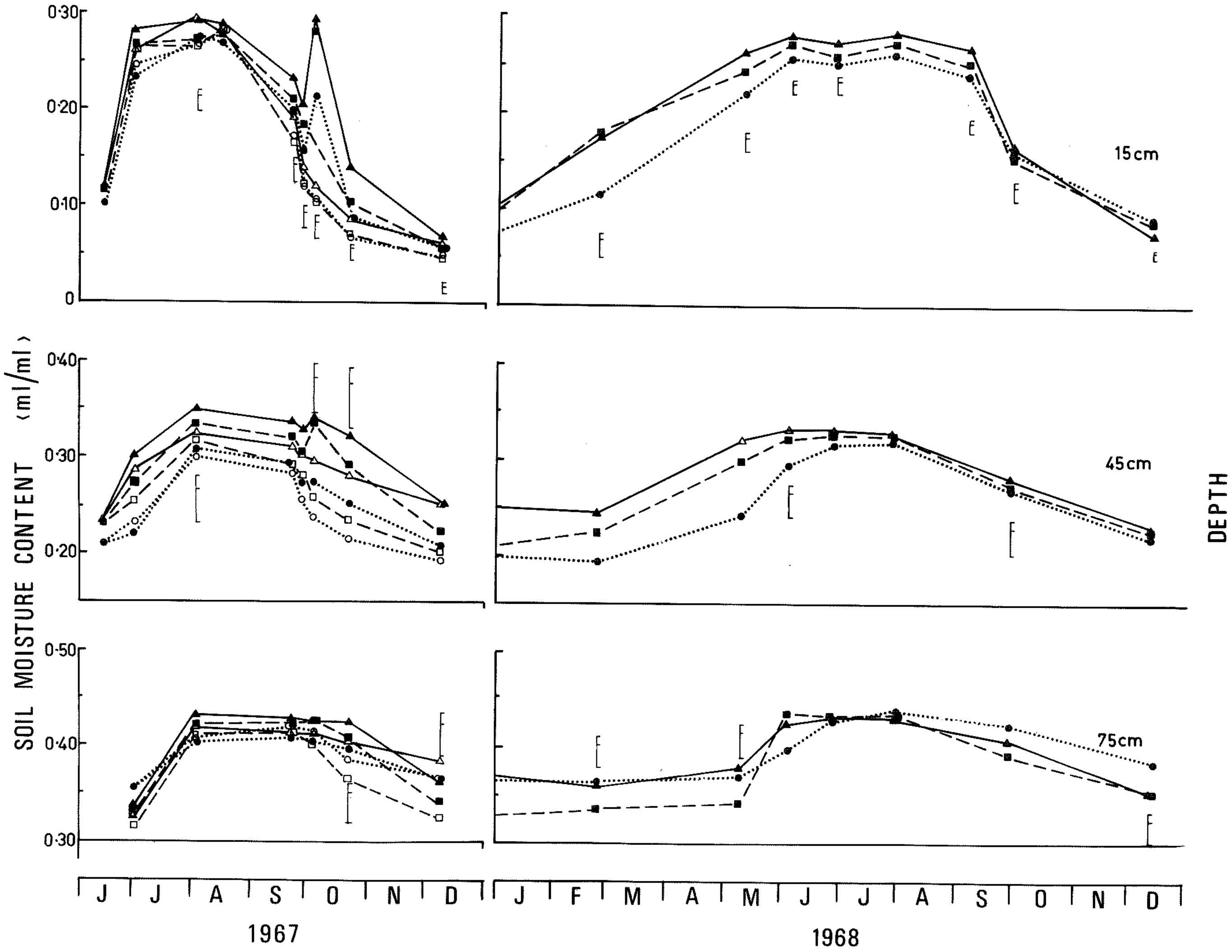
(Values for nitrogen treatments have been pooled).

(b) 1968

Key

PP	PC	CC
▲——▲	■---■	●...●

(Values for nitrogen and water treatments have been pooled).


0·30
0·20
0·10
0
15 cm
SOIL MOISTURE CONTENT (ml/ml)
0·40
0·30
0·20
45 cm
DEPTH
0·50
0·40
0·30
75 cm
J
J
A
S
O
N
D
1967
J
F
M
A
M
J
J
A
S
O
N
D
1968

APPENDIX XV

MINERAL NITROGEN LEVELS (ppm) IN THE SOIL PROFILE OF EXPERIMENT II, SAMPLED 28th JUNE-3rd JULY, 1967.

Depth (cm)	$NH_4^+ - N$			$NO_3^- - N$		
	PP	PC	CC	PP	PC	CC
0- 10	27	26	9	12	6	4
10- 20	5	8	3	28	29	13
20- 30	2	2	2	8	10	8
30- 45	1	1	1	7	5	2
45- 60	1	1	0	2	2	2
60- 75	1	1	0	1	1	1
75- 90	1	1	0	1	0	2
90-120	0	0	0	0	0	0
120-150	0	0	0	0	0	0
150-180	0	0	0	0	0	0

ADDENDUM

DENITRIFICATION MECHANISMS

Chemo-denitrification in neutral to alkaline soils

Emphasis has been placed on the losses from acidic soils, as it is well established that there is a relationship between the rate of loss of nitrogen by chemo-denitrification and the degree of soil acidity. The mechanisms of losses from neutral and alkaline soils are not well understood, although measurements of losses (by gas analysis) have indicated that the rates of losses are small (Reuss and Smith, 1965; Meek and MacKenzie, 1965).

Slow losses by the nitrosation and nitrous acid self-decomposition reactions are possible in neutral soils, as small but significant concentrations of nitrous acid can occur in neutral solutions: the proportion of nitrite existing as undissociated HNO_2 is ca. 0.02, 0.2, 1.7 and 14% at pH 7, 6, 5, and 4 respectively (using the value for the dissociation constant of 6×10^{-4} from Reuss and Smith, 1965). Additionally, the soil solution is usually at a lower pH than that indicated by pH measurements made on a 1:5 or 1:2½ soil:water system (see Jackson, 1958; White, 1969). The concentration of HNO_2 will depend on the nitrite concentration, and so increases in the concentration of the salts in the soil solution during drying must be a contributing factor to the increased rates of losses during dessication of the soil (Nelson and Bremner, 1969).

It is of interest that the mechanism of formation and decomposition of ammonium nitrite in soils appears to be of importance only when alkaline soils containing high concentrations of ammonium and nitrite ions are dessicated (Wahhab and Uddin, 1954; Bremner and Nelson, 1968). This result is in apparent contradiction with the reaction in solutions in closed systems where the losses are markedly dependent on acid conditions (Smith and Clark, 1960): the anomaly can be attributed to the faster rates of the van Slyke, HNO_2 self-decomposition, and nitrosation reactions in open incubation systems in acid to neutral soils, and that these reactions do not compete with the very slow ammonium nitrite reaction at high pHs.

Bio-denitrification: the 'anaerobic micro-site' hypothesis

The 'anaerobic micro-site' hypothesis, originally proposed by Jansson and Clark (1952), provides a satisfactory mechanism for the occurrence of denitrification in an 'aerobic' soil. One aspect of the theory is that, for continuous denitrification in a well aerated soil, the formation of nitrate (or nitrite) in an aerobic portion of the soil is assumed (e.g., the outer portion of an aggregate); the nitrate then diffuses to another portion of the soil (e.g., the inner portion of an aggregate), which is anaerobic due to the much slower rate of diffusion of oxygen in water than in air. The feasibility of this mechanism depends on the relative diffusion rates of oxygen and nitrate, yet little critical analysis of this nature has been made previously.

Using the simple model of a water-filled pore, the maximum content of O_2 in the water in equilibrium with the air is ca. 10 ppm (w/v), and it is not unreasonable to assume a value of 10 ppm NO_3-N close to the air-water interface. Thus, the concentration gradient would be similar for both species, and as the diffusion coefficients in pure solution are similar (ca. 2×10^{-5} cm^2/sec, from Greenwood and Goodman, 1967, and Romkens and Bruce, 1964), then the rates of diffusion would be similar. The biological reduction of nitrate would occur only when the requirement for oxygen was greater than the supply by diffusion. In a situation where the demand was very large and greatly exceeded the supply, the maximum rates of utilization of nitrate and oxygen would be limited by their diffusivities.

In a soil aggregate in which all the pores were filled with water, nitrate and oxygen would have comparable diffusivities also. But the presence of some air-filled pores would result in an increase in the diffusivity of oxygen and a decrease in that of nitrate. As the amount of air-filled pore space increases, there must be predicted a decrease in the maximum rate of nitrate reduction due solely to a reduced rate of transport of nitrate (all other factors assumed to be constant), although of course the number of anaerobic sites should decrease with the increased oxygen supply.

However, as indicated on p. 14, the basic difficulty in attempting treatments of this nature is that of describing the heterogeneity of the soil system. The presence of discrete pieces of decomposing organic materials, or of abnormally long water-filled pores, is not allowed for in the mathematical treatments for which 'average' diffusivities and oxygen requirements for the soil mass are assumed. Only a very thin film of water may be necessary for the depletion of oxygen close to a very rapidly decomposing organic substrate, and it is important that Ford and Greenland (1968) have estimated that mineralization of the N in their 'light fraction' contributes significantly to the nitrate formation in soils from cropland. This situation of intense microbial activity around pieces of 'light fraction' would seem a very suitable one for the occurrence of nitrification and denitrification in close proximity to each other.

LOSSES OF NITROGEN FROM THE SOIL

Estimation of total and diffusive losses

For the 1966 data, the total loss of nitrous oxide from the soil was estimated (p. 31) from the equation:

$$\Delta M = -J + S$$

The diffusive flux, J, was calculated from the simple diffusion equation:

$J = D\frac{dc}{dx}$ where c = the average concentration of N_2O in the soil air at the source (g/ml)

x = distance of the source from the soil surface (cm)

and D = the diffusivity of N_2O in the soil (p. 32)*

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*The value used for D_o, the diffusion coefficient of N_2O in air, was 0.16 cm^2/sec.

In the preliminary paper by Burford and Millington (1968), the diffusive losses were calculated from the steepest gradient. For this it was convenient to assume that the source was at the 30 cm depth, and the apparent bi-modal N_2O distribution in the soil was ignored as the concentrations in the A_2 horizon could not be shown to be statistically smaller than those in the A_1 and B horizons (at $P = 0.05$). However, the profile distributions of N_2O in the 1968 experiment clearly demonstrated that there were two major sources of N_2O: one in the A_1 horizon and one in the B horizon. The calculation of source strengths is very difficult in this situation, and so to provide estimates of the order of magnitude of losses, calculations of the diffusive losses only were used for the majority of the estimates.

The atmosphere as the main 'sink'

In calculating the losses, it has been assumed that the atmosphere is the main 'sink' for N_2O. The main basis for this was the small amount of air-filled pore space in the B horizon at field capacity:

Soil depth (cm)	Total* porosity (ml/ml)	Water-filled* porosity (ml/ml)	Calculated air-filled porosity (ml/ml)
15	-	0.28	-
15-30	0.40	0.29**	0.10
30-45	0.45	0.32**	0.13
45	-	0.33	-
45-60	0.44	0.36**	0.08
60-75	0.47	0.40**	0.07
75	-	0.43	-
75-90	0.44	0.43**	0.01

*Source of data: total porosity, Appendix XI.
water-filled porosity, Appendix XIV except assumed values (**).

When the soil is at field capacity, there is an increasing gradient of air-filled pore space from the 90 cm depth to the surface, and so an increasing volume of pore space available for diffusive loss. As the soil was at field capacity to a depth of 90 cm by July 29, 1966 (Fig. 5), appreciable amounts of N_2O should not have been diffusing downwards as there was only a negligible pore space available for diffusion at the 75-90 cm depth at this stage. The temporal distributions (Fig. 4) are in accord with this: the rate of decrease of the N_2O concentrations in the soil atmosphere from July 28 onwards was fastest in the surface horizons and slower with increasing soil depth, indicating major diffusive loss upwards (from 60 cm). The lower 'tail' (60-90 cm) of the profile distribution curves (Fig. 3) should have increased substantially after July 28 if there had been appreciable downward diffusion. Only a slight increase in the N_2O concentration was obvious at the 90 cm depth, indicating that the extent of such movement was relatively small. It seems obvious that, in the soil between the 60-90 cm depths, the denitrifying activity was decreasing rapidly with depth and that this was the main reason for the large decline in N_2O concentrations between 60 and 90 cm.

It must be stated that the total porosity values in the above table were obtained when the subsoil was relatively dry in June-July, 1967. Wetting of the subsoil is known to cause swelling and so result in different porosity values, but it seems reasonable to assume that the porosity gradient to the surface will be present under most conditions. As it is difficult to obtain accurate air-filled porosity values in the range 0.05-0.10 ml/ml and to sample these soils when wet, the only course available for the diffusion calculations was to adopt the limits of 0.05-0.10 ml/ml for the B horizon as suggested by Millington (pers. comm.).

It was also possible that a 'sink' for N_2O exists at the 90 cm depth due to further denitrification ($N_2O \longrightarrow N_2$), especially as the small air-filled pore space would be suitable for the occurrence of very anaerobic conditions. However, the oxygen content was always higher than at the 60 cm depth (Fig. 7) indicating that the microbial activity was lower. Additionally, such denitrification should result in a decrease in the soil nitrate concentrations as it has been indicated that NO_3^- is a preferred substrate to N_2O (e.g. Nommik, 1956), but nitrate concentrations did not decrease.

As there is very little evidence to indicate a 'sink' at the 90 cm depth in the soil, it has been assumed in all calculations that the atmosphere is the main 'sink'.

Losses as nitrogen gas

As the N_2/A technique was not successful in the attempt to assess the losses (if any) as N_2, the total gaseous losses from the soil could not be estimated. This raises important implications for future research. The need for measurements of denitrification products in field situations has been realized for some time, and in this thesis it has been shown that N_2O can be measured using techniques which are reasonably convenient and inexpensive. However, measurement of the losses as N_2 have always presented the formidable difficulty of detecting small increases above the 'background' of 78% in the soil atmosphere. It would seem that research using N^{15} would be needed to provide the highly sensitive technique necessary to detect losses in the A_1 horizon. Yet, there are difficulties involved in the analysis of gas samples containing several nitrogenous gases if these are enriched in N^{15} (Cheng and Bremner, 1965; Hauck, 1968), N^{15} is expensive to use on a field scale, and the results from this thesis indicate that there may be difficulties in interpretation due to differing diffusion rates of N^{14} and N^{15}.

Although other approaches have been attempted on the Urrbrae fine sandy loam (Millington, unpublished data; Stefanson and Greenland, 1970), the estimation of losses as N_2 (or total losses) in the field is still not possible, and so the losses as N_2 in this thesis are not known.

Millington examined the leaching of Cl^- and NO_3^- down the soil profile, and concluded that a sudden large loss of NO_3^-, not accounted for by changes in Cl^-, was good evidence of massive denitrification. The estimated magnitudes of losses as N_2O in this thesis are not in agreement with Millington's conclusion, yet because of the difficulty in measuring the evolution of gaseous nitrogen, no definite conclusion can be drawn. However, a tendency for N immobilization in the 0-30cm depth and the leaching from the 0-30 cm depth to the 30-60 cm depth during the wet July in 1966 could be taken as indications that massive losses due to denitrification did not occur. It is possible that the losses observed by Millington were due to immobilization rather than to conversion to gaseous forms.

In the gas lysimeter experiments of Stefanson and Greenland (1970), in which the soil samples of the Urrbrae fine sandy loam were obtained from the site of Experiment II, the ratio of N_2/N_2O in the evolved gases was about 1:2, that is, there was more N_2O than N_2 evolved. While their evidence indicates that large losses as N_2 were not occurring, the natural field structure of the soil was not preserved in Stefanson and Greenland's experiments, and it is known that this is important (Greenwood and Goodman, 1967).

As the N_2O concentrations can be relatively easily measured in field soils, it has been suggested that a knowledge of the expected N_2/N_2O ratio of the evolved gases from incubated soil might be useful in estimating the losses in the field as N_2. But, it is even more difficult to infer satisfactorily the N_2/N_2O ratio of the evolved gases under field conditions by extrapolation from previous laboratory experiments than by extrapolation from gas lysimeter experiments. The laboratory incubations have usually involved highly artificial conditions, often those which are optimal for denitrification. Many experiments have started with a certain amount of NO_3^-, and the denitrification products measured after various periods of elapsed time. Nitrogen will usually be the only product, if the incubation time is sufficiently large (e.g. Fig. 28). But from the rates of evolution of N_2 and N_2O, it can be concluded that a low ratio of N_2/N_2O is favored by:

Variable	Value for variable		
	low N_2/N_2O	versus	high N_2/N_2O
- low soil moisture content	field capacity	versus	above saturation
- low temperature	5-10 C	versus	up to 65C
- high pO_2	ca. 20%	versus	often nil
- low levels of easily available organic substrates	nil	versus	+ glucose or straw
- low pH	pH 4	versus	ca. pH 8
- high NO_3^--N	ca. 600 ppm	versus	ca. 40 ppm

Nommik's (1956) classic paper is the main source for these suggestions, although supplementary data is available in other papers, such as Wijler and Delwiche (1954), Cady and Bartholomew (1960), Schwartzbeck et al. (1961), and Cooper and Smith (1963).

The only possible, but speculative, conclusion is that the marginal conditions under which denitrification occurred in the field would tend to produce a mixture with a low N_2/N_2O ratio. This is supported by the results of the incubation experiment (where N_2 was not evolved until 70% of the initial nitrate had been converted to N_2O), and by the ratio of 1:2 obtained by Stefanson and Greenland (1970). But this suggestion must remain speculative until more is known about the 'anaerobic micro-sites'.

In this discussion, a completely biological source of N_2 and N_2O has been assumed. If there was a contribution from chemo-denitrification, then it is important to note that the ratio of N_2/N_2O was much lower for the Urrbrae soils than those reported in the few other sources of data.

MOVEMENT OF GASES DOWN THE PROFILE

Nitrous oxide

It is generally understood that the A horizon has a much higher potential denitrifying capacity than the B horizon due to its much higher content of available organic substrates. As leaching of nitrate occurred, it is possible that the N_2O found in the B horizon was actually evolved in the A horizon but diffused down or was transported down in the soil water. But such movement should have been apparent from the distributions of N_2O in the soil profile. In particular, it should have resulted in high N_2O concentrations in the A_2 horizon.

In most cases, the levels of N_2O in the A_2 horizon were much lower than those in the B horizon. This is best shown by the 1968 data (Fig. 24) where the denitrification activity in the A_1 horizon resulted in very high N_2O levels in the A_1 horizon (up to 160 ppm), but did not seem to result in increases in the A_2 or B horizon. The concentrations in the B horizon appear to be originating quite independently from those in the A_1 horizon, and the only significant increases in the A_2 horizon occurred in mid-August: this result may be attributed to the diffusion of N_2O from the B horizon to the surface.

It would be expected that CO_2 would move down the profile as well as N_2O, because they have similar solubilities. In Fig. 27, CO_2 concentrations were as high as 2.5% in the A_1 horizon in May, and yet the concentrations in the A_2 and B horizons remained sensibly constant at <1% until early July, and the increases at that time are clearly due to the respiration in the soil, since the CO_2 increases were accompanied (and preceded) by large decreases in the O_2 concentration. Similarly, Fig. 8 indicates that there is very little association between the levels of CO_2 and N_2O. Although the change in CO_2 values was often not large, some effects would have been obvious if significant transport in solution had occurred.

The possibility that appreciable movement downwards occurred but was masked by an increased absorption of N_2O in the soil water due to lower temperatures in the deeper soil layers does not seem probable as the temperature gradients in the profile were small (see Fig. 25 and Addendum p. 14).

Estimates have indicated that the rate of loss of N_2O is much larger from the A_1 horizon than from lower in the profile. It must be concluded that after each evolution of N_2O in the A_1 horizon (following a rain), that the N_2O is much more rapidly lost by diffusion to the atmosphere than by downward leaching.

Solubilities of N_2 and A in water and the N_2/A ratio of the soil atmosphere

It has been shown that the much greater absorption of CO_2 in the soil water than that for O_2, A, and N_2 has an important effect on the composition of the soil atmosphere. It is appropriate that the relative solubilities of N_2 and A in water be discussed in terms of the possible effects on the concentrations of N_2 and A in the soil atmosphere.

If a sample of atmospheric gas is equilibrated with water, in a closed system and the water is initially free of dissolved gases, then a greater amount of A and O_2 will be dissolved in the water at equilibrium, i.e., the water will be enriched in A and O_2 and the atmosphere will contain a relatively higher concentration of N_2 (see Table 5 for solubility coefficients). Thus, the atmosphere could exhibit higher N_2/A ratios if a mechanism of this nature was important. However, the (soil) water is in equilibrium even before it (as rain) hits the soil. Thus, movement of water down the profile should not result in any changes, except if there were any large temperature gradients, when on warming the gas evolved from the water would be enriched in A compared to N_2. But this mechanism requires temperature changes: a decrease in the N_2/A ratio could be caused by an increase in the temperature of the B horizon, with an expulsion of a low N_2/A gas from the soil water into the soil air. Assuming that $\varepsilon_A = 0.10$ ml/ml and $\varepsilon_W = 0.30$ ml/ml and a temperature increase of 10 C, then it can be shown that an immediate decrease of ca. 1.5% in the N_2/A ratio would be expected. However, this is not only much smaller than the decreases in the N_2/A ratio in August, 1968, but the temperature at the 60 cm depth was less than 1 C greater than that at the 10 cm depth (Fig. 25).

Other effects of a similar nature can be postulated, but it is difficult to conceive that they could have produced the large decrease in the N_2/A ratio as the temperature gradients down the profile were small and variable. For example, if there was a uniform gradient of increasing temperature with depth and air moving down the profile due to mass transfer was enriched in A and O_2 and depleted in N_2, the maximum effect could only be as in the above example (change in N_2/A = -1.5% for a 10 C gradient), as the air and water phases are in equilibrium. The gradient was usually less than 3 C/50 cm.

It must be concluded that, from the data available, a solubility mechanism is unlikely to have been responsible for the observed decreases in the N_2/A ratio.

EXPERIMENTAL DETAILS

Gas data for all field experiments

All gas measurements have been reported on the basis of concentrations (v/v) in the gas phase, unless specifically stated otherwise.

Spatial arrangement of the soil gas reservoirs, Experiment I (1966)

The six sampling positions in each group were arranged in 2 files of 3 positions with a 5 ft distance separating the files and the positions in each file. The groups were regularly spaced over the experimental area.

Calculation of the composition of soil gas samples, Experiment I (1966)

The ratio of $N_2/(A + O_2)$ was determined by first estimating the volumes of N_2 and $(A + O_2)$ from the individual calibration graphs for N_2 and $(A + O_2)$, and then calculating the ratio $N_2/(A + O_2)$. This ratio was then used to calculate the N_2 and $(A + O_2)$ concentrations in the samples, on the assumption that $[100 - (\% CO_2 + \% N_2O)]$ was the volume occupied by $(N_2 + O_2 + A)$.

The $(N_2 + O_2 + A)$ peak was not a reliable measure of the total volume of these gases, as the column was 'overloaded' by the 5 ml volume of sample injected for the N_2O and CO_2 determinations.

Soil sampling, Experiment I (1966)

At each sampling for inorganic nitrogen content, 10 random cores were obtained over the experimental area (with the area closer than about 5 ft from the gas sampling units being excluded). The sampling dates are shown in Appendix IX (p. A.21).

Discussion of N_2/A ratio results, Experiment II (1967)

The main point in the discussion of the N_2/A decreases in 1967 was that during the winter of 1967, moderate but non-significant increases of 2% in the nitrogen content of the atmosphere in the B horizon were accompanied by decreases, also non-significant, in the N_2/A ratio. The significant changes in the N_2 content of the atmosphere in the A_2 horizon were not accompanied by any noticeable change in the N_2/A ratio. However, immediately following the irrigation on Sept. 29 - Oct. 2, increases in the N_2 concentration and decreases in the N_2/A ratio (significant at $P = 0.05$) for both the A_2 and B horizon were observed. As the decreases in the latter were not expected, a mechanism was proposed.

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