THE FORMATION AND DECOMPOSITION OF NITRITE IN GAMMA-IRRADIATED SOIL

by

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ABSTRACT

The formation and subsequent decomposition of nitrite after gamma irradiation of soils has been studied over a wide dose range, up to 12 Mrad. According to the dose received, either biological or chemical (radiolytic) reduction of nitrate can be responsible for accumulation of nitrite, and both types of action later lead to reduction of nitrite to oxides of nitrogen.

Biological reduction of nitrate to nitrite, nitrous oxide, nitrogen dioxide and nitric oxide is predominant in calcareous soils treated in the region of 0.75 Mrad at medium levels of moisture; the formation of nitrite and oxides of nitrogen can be prevented by autoclaving. $^{15}NO_3$ was used to confirm that reduction to nitrite occurred, and the extent to which nitrite accumulated was shown to be greatly influenced by moisture, temperature, aggregate size and conditions of storage before use, all factors known to affect microbial activity. It was concluded that reduction is carried out by enzyme systems of denitrifying bacteria which are merely prevented from proliferating by 0.75 Mrad. There was good agreement between the ability of an irradiated soil to accumulate nitrite at medium moisture, and its capacity when unirradiated to biologically reduce nitrate under the ideal conditions of waterlogging and high concentrations of available carbon and nitrate, which is termed "denitrifying potential".

Radiolytic reduction of nitrate at medium soil moisture becomes increasingly evident between 4 and 12 Mrad, the yield of nitrite being restricted by the concentration of nitrate present; reduction is not prevented by autoclaving. Experiments on recovery of $^{15}NO_2$ indicated that a decline in nitrite level during incubation occurs by fixation to organic matter, and by chemical decomposition leading to volatilisation.

The current use of gamma radiation in various fields of soil research has been reviewed, and some suggestions made for further experimental application.

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1 INTRODUCTION

1-1 Properties of gamma radiation

Gamma-rays and X-rays are electromagnetic radiations of identical properties with wavelengths in the range 10^{-11} to 10^{-6} cm, the separate terms being retained to denote their respective origin. Gamma-rays are emitted by nuclei of atoms which are decaying to more stable states, while the term "X-rays" is restricted to the radiation resulting from the displacement of an internal electron in an atomic orbit by bombardment with a beam of high energy electrons.

The radioactive isotope cobalt-60 can be produced for approximately 6/- per curie by neutron activation of cobalt-59 in a nuclear power reactor, and the specific activity required for irradiation work is usually between 2 and 10 curies per gramme. Cobalt-60 has a half-life of 5.2 years, and is a valuable source of pure high-energy gamma-rays of 1.1 and 1.3 MeV. When a photon of this energy passes through matter it ejects orbital electrons (Compton recoil electrons) from the constituent atoms, causing dense formation of positive ions, and continues its path at lower frequency to produce further ionisations. If the energy transferred to the orbital electron is insufficient to cause further ionisation, there is excitation of the molecule by displacement of electrons from one shell to another of higher energy. Finally, the electron is captured by a molecule to yield a negative ion. The positive and negative ion pairs, or free radical ions, exist for less than 10^{-10} seconds after irradiation owing to reaction either between themselves or with ordinary molecules. The Compton recoil electrons can also produce further ionisations, usually close to the site of the initial ionisation. X- and gamma-radiations do not react with the atomic nucleus unless the energy exceeds 1.7 MeV, therefore

the irradiated material is not rendered radioactive.

The average energy deposited per unit of track length is referred to as linear energy transfer (LET), and amounts to 0.42 KeV / micron path length for cobalt-60 radiation passing through water: the penetration of these rays is considerable, and it would require 11 inches of water to absorb 50% of the energy. The unit of energy absorption is the rad (radiation absorbed dose) which corresponds to the deposition of 100 ergs / g of irradiated material; a dose of 2.5 Megarads (Mrad), which is frequently used to sterilise materials, would deposit 6 calories / g in water, thus increasing the temperature by 6°C.

Several types of installation have been constructed in the United Kingdom to enable treatment of materials with cobalt-60 emissions. They range from the very large industrial unit such as the Package Irradiation Plant at Wantage Laboratory, which contains approximately 350,000 curies, to the small laboratory installation or "Mark IV Hotspot" containing up to 5,000 curies of cobalt-60 (Jefferson and Rogers, 1964). The latter type of unit has proved to be extremely useful for soil irradiation work, and is capable of delivering 0.6 - 1 Mrad / hr to samples placed in an irradiation chamber of 300 cu. cm.

1-2 General effects of radiation on soil

An interest in the effect of radiation on soil was aroused at the beginning of this Century by reports that plant growth could be stimulated when radioactive substances were mixed with soil (Stocklasa, 1913a, 1913b), and also from statements that irradiation of seeds and plants could lead to increased growth (Promsy and Drevon, 1912; Miège

and Coupé, 1914). In fact, the latter effect is controversial to this day.

Apart from experiments on irradiation of Azotobacter in pure culture, which led to conflicting claims of stimulation (Kayser, 1921) and inhibition (Whelden et al. 1941), detailed information on the soil response to radiation only became available after 1959. One of the main observations was that growth of plants could be greatly increased when the soil had been previously irradiated, either at doses towards the sterilising region of 2.5 - 5 Mrad, or at lower levels (Bowen and Cawse, 1962; Bovard and Grauby, 1963; Cummins and McCreery, 1964; Jenkinson et al. 1968). These investigations, together with the majority of the work subsequently described, have been made with acute radiation exposures as opposed to chronic (prolonged) treatments which have received little attention in soil research.

In one instance, a significant increase in dry matter production was obtained from lettuce (Lactuca sativa) grown in soil treated with only 0.01 Mrad (Bowen and Cawse, 1964a), and analysis of plant tissue from soil treated with 0.02 - 2 Mrad revealed that the total nitrogen removed had been increased by a factor of 2 - 4 relative to the control. Thus it appeared that considerable changes in soil nitrogen metabolism were brought about by radiation, most probably initiated by death and lysis of the radiation susceptible organisms: rapid mineralisation of organic-N to ammonium- and nitrate-N then provided highly available nitrogen for the plants (Bowen and Cawse, 1964a).

Up to this point, the possibility that increased availability of other elements could also contribute to stimulation of growth was not excluded, and Eno and Popenoe (1964) had found extra phosphorus as well as nitrogen after irradiation of a peat soil. Measurement of

13 elements present in soil solution after 5 Mrad showed that carbon, nitrogen and phosphorus concentrations had risen greatly (Bowen and Cawse, 1964b), but after 0.02 Mrad, which was known to stimulate plant growth, there was only a significant increase in nitrate (Cawse, 1967a). It therefore appeared that with low doses at least, increased availability of nitrogen was the major factor responsible for increasing the yield of plants, and experiments were designed to fully investigate the response of nitrogen mineralisation to radiation.

Several investigators had detected accumulation of ammonium in soils following irradiation, but not nitrate (Stotzky and Mortensen, 1959; Bernard and Geller, 1962; Eno and Popenoe, 1964; Splittstoesser and Massey, 1967). This led to the conclusion that nitrifying organisms were severely inhibited by 0.1 Mrad (Bernard and Geller, 1962), whereas the results at Wantage Laboratory indicated that oxidation of ammonium usually attained a maximum rate after 0.2 - 0.8 Mrad which was far in excess of the nitrification rate in the control. When the dose was raised from 0.8 to 3 Mrad, nitrification became inhibited with a corresponding rise in ammonium (Cawse, 1968): perfusion of irradiated soil with ammonium sulphate showed that although proliferation of nitrifying bacteria could be prevented by 0.2 Mrad, the organisms were still capable of oxidising ammonium to nitrate, and this behaviour accounted for the observed increase in nitrate in non-perfused soil. When 10 soils were treated with 0.25 - 2.5 Mrad, the rise in nitrate was most pronounced in the types which possessed an active nitrifying population before irradiation (Cawse and White, 1969a); soils with pH < 5.0, known to be unfavourable towards Nitrosomonas and Nitrobacter, gave little nitrate after irradiation. Any pre-treatment of soil by air-drying was avoided for these experiments to prevent

damage to the normal population of nitrifying bacteria, and samples were always treated in a fresh condition.

During the nitrification studies it was noticed that soils with pH > 7.0 tended to accumulate nitrite when treated with 0.6 - 2.5 Mrad, being most predominant at the lower dose region (Cawse and Crawford, 1967; Cawse and White, 1969b). Either reduction of nitrate or a radiation block to oxidation of nitrite by Nitrobacter could have been responsible, and preliminary tests suggested that the former mechanism was taking place. Such behaviour had not been reported elsewhere, and a detailed study of this occurrence constitutes a major part of the present thesis.

Measurement of soil respiration following irradiation has resulted in widely different observations. There are some reports of insignificant variation from the control rate up to 0.1 Mrad (Stanovick et al. 1961) or up to 0.25 Mrad (Stotzky and Mortensen, 1959), while other authors describe large increases in evolution of carbon dioxide after 0.2 Mrad (Popenoe and Eno, 1962), and also after 2.5 Mrad (Coleman and Macfadyen, 1966; Jenkinson and Powlson, 1970). Peterson (1962a) found that sterilisation of soil by radiation did not prevent respiration, and suggested that respiratory enzymes can remain active within non-viable cells; sterilisation only implies the impairment of cell division, often termed "inactivation".

Two extremely variable factors which could lead to differences in respiratory activity of irradiated soils are the initial density of the respective microbial populations, and their survival and re-colonisation after treatment. The degree of sterility obtained by a reasonably high dose such as 5 Mrad will depend on the initial microbial density because survival is an exponential process:

$$N/N^{o} = \exp(-KD)$$

where N is the number surviving dose D, and N^{o} is the initial population. The constant K is specific for each type of organism and will vary according to its radiosensitivity (McLaren, 1969): for example, the K value is definitely influenced by the soil moisture content at the time of irradiation (Kashkina and Abaturov, 1967; Jackson et al. 1967).

Information on the survival of a wide range of soil invertebrates irradiated in their natural environment was only recently available when Edwards (1967) reported that 0.2 Mrad was sufficient to lead to the death of all animals by 42 days after irradiation. The microfauna is thus the most radiosensitive group of soil organisms, and is followed by fungi, bacteria and enzymes in general order of susceptibility. While the selective property of radiation may be useful for agricultural research (see Appendix), there is at present no possibility of using it to eliminate pests and pathogens in the field. However, a patent for "Disinfection of Soils" was filed in 1968 by Conservatome Recherches, Paris (Patent no. 1,133,866. The Patent Office, 25, Southampton Buildings, London W.C.2.) describing a field irradiation machine, but it is extremely doubtful if the high dose rate and shielding requirements could ever be met to utilise cobalt-60. Another section of the patent refers to the use of electron generators to produce gamma-rays at ground level, and this system should certainly reduce the weight of the radiation shield.

Apart from the reports mentioned in this introduction on the overall or general response of soil to various levels of radiation, there has been more than equal effort devoted to the separate response of fungi, bacteria, and enzymes, and also to the behaviour of inert

constituents such as clay and humus. These findings will now be discussed, together with some information on the actual sites where radiation causes disturbance.

1-3 Specific effects of radiation on soil constituents

1-3.1 Inert constituents

When clay minerals with their exchange sites saturated with ammonium were treated with 2 Mrad, the amounts of KCl-extractable ammonium remained unaltered (Bowen and Cawse, 1964a), and the cation exchange capacity of bentonite and kaolinite clays was even unchanged by such massive doses as 193 - 1000 Mrad (Ojima et al. 1963).

Estimates of the original radiation dose received by roof tiles from the Hiroshima atomic bomb zone have been obtained by measurement of their thermoluminescence 19 years later (Higashimura et al. 1963). This encouraged Nishita and Hamilton (1968) to investigate the thermoluminescence of 9 soils when heated to temperatures within the range 150 - 375 °C after 0.001 Mrad, with special reference to the use of soil as a radiation dosimeter. The soils showed a considerable variation in thermoluminescence, but it was most marked in the calcareous types. When clay minerals and inorganic salts were irradiated they showed a similar variation, and gave glow peaks which coincided with those obtained from soils; in particular, irradiated calcium oxide, calcium carbonate and sodium carbonate gave extensive thermoluminescence which was relatively greater than that exhibited by a similar weight of clay mineral such as illite. Soils do show "natural" thermoluminescence which could be produced by cosmic radiation and emissions from radioactive elements, and this must be deducted from the artificially induced gamma contribution.

Later, Nishita and Hamilton (1969) stated that although ionisation in the sample is the most important effect of gamma radiation, there may also be production of ion vacancies and interstitial ions as defects in the crystal lattice which could affect the trap-depth of an electron.

Humus is not seriously degraded by a dose of 4 Mrad (Bowen and Cawse, 1964b), and Gibson (1959) found that peat required 10 Mrad before destruction of cellulose and an increase in reducing sugars was observed. It is known from the work of Smith and Mixer (1959) that the presence of lignin during treatment of cellulose with 7 - 20 Mrad reduced the number of scissions of cellulose chains, and it is probable that lignin in humus similarly protects other components against damage by radiation.

When radiation passes through the soil water, or water in actively metabolising cells of soil organisms, excited and ionised water molecules will be formed along its path. These ions can react with another molecule of water to give free radicals, which are neutral atoms with an unpaired electron in the outer orbit:

$$H_2O^+ \xrightarrow{H_2O} H^+ + OH^\circ$$

and $$H_2O^- \xrightarrow{H_2O} OH^- + H^\circ$$

superscript$^\circ$ = no charge

If oxygen is dissolved in the water it reacts with these radicals to form peroxyl radicals, which then combine to yield hydrogen peroxide:

$$H^\circ + O_2 \longrightarrow HO_2^\circ$$

$$HO_2^\circ + HO_2^\circ \longrightarrow H_2O_2 + O_2$$

and in the absence of oxygen the following reactions will take place:

$$H^{\circ} + OH^{\circ} \longrightarrow H_2O$$

$$H^{\circ} + H^{\circ} \longrightarrow H_2$$

$$OH^{\circ} + OH^{\circ} \longrightarrow H_2O_2$$

but the net yield of hydrogen peroxide may be lowered by reaction with other radicals such as H° :

$$H^{\circ} + H_2O_2 \longrightarrow OH^{\circ} + H_2O$$

Although these reactions in water are virtually instantaneous and the intermediate free radicals exist for only about 10^{-3} seconds (Ghormley, 1956), they do attack neighbouring molecules: thus the effect of radiation is exercised indirectly as well as by a direct hit to the molecule.

The presence of soluble organic matter in the soil water would favour the formation of organic peroxides which have a longer lifetime than free radicals, and high yields of such peroxides have been obtained following irradiation of amino acid solutions with 0.05 Mrad (Okada, 1958). However, when a test was made for the presence of combined organic and hydrogen peroxides in soil solutions extracted immediately after 2 Mrad, it indicated that <0.01 p.p.m. of peroxides was present (Cawse, 1967b), even with a highly organic soil from Icknield series.

Inorganic ions in the soil solution could be oxidised by OH° radicals or reduced by H° radicals, and the oxidation of ferrous iron to ferric in pure solution was first observed by Fricke and Morse (1927); it is now widely used for radiation dosimetry. Reduction of nitrate, which was reported on irradiation of pure sodium nitrate solutions (Mahlman and Schweitzer, 1958), was very much in evidence in soil solutions in the present work at doses from 4 - 12 Mrad. Investigation of oxidation and reduction pathways initiated by radiolytic action

has of necessity been confined mainly to pure systems owing to the complexity of the reactions, and the vast range of substances which are liable to be attacked by free radicals in irradiated soil has understandably discouraged any examination of the predominant effects.

Kertesz and Parsons (1963) have shown that exposure of air to 1 Mrad can yield 18 p.p.m. by volume of ozone, and they suggest that this gas may have contributed to microbial inhibition observed in irradiated foodstuffs, particularly as 1 - 3 p.p.m. of ozone has a strong germicidal effect and is recommended for improved cold storage of foods. The fate of ozone produced in the soil air is not known, but it may well be a contributory factor towards damage of soil organisms and enzyme systems by radiation.

1-3.2 Cellular constituents and microorganisms

Irradiation of pure carbohydrates, lipids and proteins to assess changes induced at the molecular level has shown that they are altered in various ways which may be responsible for radiobiological damage, since these compounds form the major part of organisms.

With regard to carbohydrates, an interesting effect is shown by hexose sugars: when glucose, fructose and sucrose are treated with 2 Mrad they form glyoxal $(CHO)_2$, (Gottlieb and Markakis, 1968), and the cytotoxicity of irradiated glucose solutions has been attributed to this product (Berry et al. 1965). On the other hand, Schubert et al. (1967) have found that hydroxyalkyl peroxides are formed by the interaction of radiolytic hydrogen peroxide with carbonyl compounds produced in the radiolysis of sucrose, and they state that these peroxides are more toxic to bacteria than glyoxal at a given dose of radiation applied to sucrose solutions.

Irradiation of pure lipids has shown that they undergo direct bond cleavage at any points in the chain to form alkyl free radicals; these can then re-combine to form members of the alkane series, up to n-nonane in the case of irradiated methyl oleate (Merritt, 1966). Lipid peroxides may also be formed, and Wills and Wilkinson (1967) demonstrated their occurrence after subcellular fractions, particularly microsomes, were exposed to 0.002 - 0.1 Mrad; it was thought that phospholipids may have been under attack, and as they are essential constituents of intra- and extra-cellular membranes, such damage would probably account for reports of enzyme leakage from irradiated cells.

Proteins subjected to radiation can show breakage of hydrogen or disulphide bonds between the peptide chains, leading to unfolding of the molecule and a change in its reactivity by exposure of amino acid groups (Alexander et al. 1959). In addition, amino acids are prone to attack by OH° and HO_2° radicals resulting in deamination (Kuzin, 1962). Recent work with barley root cells indicates that the protein moiety of phytochrome is particularly sensitive to radiation, damage being detectable after only 170 rad (Tanada, 1969).

Irradiation of nucleic acids and nucleoproteins in excess of 0.01 Mrad results in breakage of strands in the DNA helix, deamination and formation of hydroperoxides of thymine and uracil (Scholes and Weiss, 1960); the reactivity of the protein fraction of nucleoprotein with radiation-produced radicals has a protective effect on the DNA component (Scholes and Weiss, 1962). Rupture and restitution of chromosomes following irradiation has been discussed by Casarett (1968), and is pronounced after 200 rad.

The activity of enzymes in irradiated soil has been studied

by several workers. Skujins et al. (1962) found that phosphatase was considerably radioresistant, and required 19 Mrad to reduce its activity to 37% of the initial level. Roberge and Knowles (1968) reported that urease in a black spruce humus was almost completely inactivated by 4 - 5 Mrad, but on the other hand, McLaren et al. (1957) described stimulation of urease activity after 5 Mrad was applied to a clay loam; this effect was confirmed in a later study (Skujins and McLaren, 1969) and was also found in five other soils after 4 Mrad. The authors attributed the extra activity to better accessibility of substrate to an intracellular urease component, following the prevention of cell proliferation and damage to membranes by radiation. Only one soil was found in which 4 Mrad severely checked urease activity, and the majority required 8 Mrad to decrease activity below that present in unirradiated soil. Skujins and McLaren (1969) proposed that this difference in sensitivity of soil urease to radiation arises from the existence of extracellular as well as intracellular enzyme: soils that fail to show stimulation of urease activity on irradiation have a predominantly extracellular accumulation of enzyme, and the gradual inhibition of it with increasing radiation dose is not more than compensated by a rise in activity of intracellular urease following damage to cell membranes.

Fungi in fresh soil were decreased in number by 0.0025 Mrad, and were severely reduced by 0.25 Mrad (Kashkina and Abaturov, 1967); Penicillium and Trichoderma spp. proved to be most sensitive and Cladosporium spp. most resistant, in agreement with Johnson and Osborne (1964). Jackson et al. (1967) eliminated fungi in two clay soils with 0.8 Mrad, but detected differences in survival curves which probably indicated variability in radiosensitivity of the populations.

The level of radiation at which the bacterial flora begins to be inhibited has been quoted as 0.001 Mrad (Popenoe and Eno, 1962), 0.0025 Mrad (Kashkina and Abaturov, 1967) and 0.25 Mrad (Stotzky and Mortensen, 1959). Such wide differences in radiosensitivity may reflect the influence of soil properties and variability of bacterial species within a population. For example, Monib and Zayed (1963) reported Azotobacter spp. to be more sensitive to radiation than Clostridium spp., in agreement with the generally accepted view that organisms which sporulate readily show better survival owing to the radioresistance of these structures. Furthermore, Wood and Casida (1967) isolated a rare group of Bacillus spp. surviving in irradiated soil, and Vela and Wyss (1965) found that soil cultures of Azotobacter were far more resistant to gamma rays than laboratory reared populations.

The response of nitrifying organisms to radiation has already been discussed in connection with plant growth in irradiated soil, apart from the subject of their recovery. Perfusion experiments (Cawse, 1968) revealed that 25 - 30 days after treatment of soil with 0.2 Mrad the nitrifying bacteria suddenly proliferated and oxidised ammonium rapidly, and pure culture work by Vela (1969) has shown rapid recovery at 16 days after 0.25 Mrad. Recovery of the total bacterial flora in glucose amended soil was noticed at 15 days after 0.8 Mrad (Monib and Zayed, 1963), and although the population did not exceed that initially present, there was a 150-fold increase over the numbers detected immediately after irradiation. These reports of recovery suggest that treatment of soil with radiation does not produce any persistent cytotoxic factor, and some successful experiments on re-colonisation of gamma-sterilised soil with nitrifying bacteria are reported in this thesis. Exceptionally however, some soils may behave

differently, and Merzari and Broeshart (1968) failed to establish nitrifiers and other organisms in irradiated soil (see Appendix).

Soil actinomycetes are intermediate in radiosensitivity between fungi and bacteria (Kashkina and Abaturov, 1967). The dose required to eliminate various algae extends over a very wide range, and whereas green algae in soil were severely damaged above 0.06 Mrad, the blue-green types survived 2 Mrad (Popenoe and Eno, 1962).

1-4 Object and development of this study

The object of the present work was to investigate the mechanisms involved in the formation and subsequent removal of nitrite in irradiated soil, which was very evident in calcareous types treated with doses in the region of 0.75 Mrad (Cawse and White, 1969b).

Irradiation techniques have frequently been applied in various spheres of soil research (see Appendix) and are likely to be used to a greater extent in the future (Muller, 1968; McLaren, 1969). To facilitate this application, a complete understanding of soil response to radiation is needed: for example, the undetected presence of nitrite and its loss by certain mechanisms would present a problem in some experiments, and is further discussed towards the end of the present section. Furthermore, it was suggested by previous work that formation of nitrite after irradiation with 0.6 - 0.8 Mrad occurred by the biological reduction of nitrate (Cawse and Crawford, 1967); this process is particularly interesting because it represents the first major step in the true denitrification pathway to gaseous nitrogen, which results in severe loss of nitrogenous fertiliser. The accumulation of nitrite after irradiation occurred under conditions

of medium moisture, where encouragement of microbial nitrate reduction by oxygen deficiency was not expected; such behaviour was interesting because while reduction of nitrate has often been thought to occur under apparently aerobic conditions it is difficult to investigate, since nitrite is probably being formed and decomposed instantaneously in anaerobic microzones at the centre of aggregates (Hauck, 1968).

Irradiation of certain soils in the region of 0.75 Mrad thus appeared to selectively encourage formation of nitrite, and to begin the thesis it was decided to search for more substantial evidence to indicate that biological reduction of nitrate was the cause; positive indications were obtained in three ways. Firstly, by the addition of ^{15}N-labelled nitrate to soils before irradiation, with subsequent recovery of tracer in the nitrite pool after incubation (Section 3). Secondly, by studying to what extent the formation of nitrite after irradiation could be influenced by soil factors such as moisture, temperature, aggregate size, storage conditions before irradiation, presence of calcium carbonate and autoclaving, which are all known to influence microbial activity, reduction of nitrate and stability of nitrite in unirradiated soil (Section 4). Thirdly, by demonstrating that there was a good relationship between the ability of 15 soils to produce nitrite at medium moisture and their capacity to reduce nitrate by microbial action when waterlogged, the latter being termed "denitrifying potential" (Section 5).

From the above experiments it was concluded that the majority of nitrite originated from reduction of nitrate by biological means, with smaller amounts formed by oxidation of ammonium. A subsidiary experiment was designed to investigate the possibility that irradiation at the 0.75 Mrad region produced nitrite in some soils but not

others because soil properties somehow rendered nitrifying organisms more sensitive to radiation than denitrifying ones; thus, greater damage to nitrification could permit nitrate reduction to become dominant. However, pure cultures of nitrifying bacteria established in sterile soils failed to show differences in radiosensitivity over the range 0.05 - 1 Mrad (Section 6).

The eventual decline in nitrite concentration in soils treated with 0.75 Mrad was studied by measurement of the oxides of nitrogen released (Sections 7 and 8), and also by addition of ^{15}N-labelled nitrite (Section 9): oxidation back to nitrate as well as volatilisation of nitrite was detected, and large amounts of nitrous oxide and nitric oxide appeared. Gas chromatography of the soil atmosphere showed that the further reduction of nitrite was taking place at oxygen levels little different from the atmospheric concentration, and because autoclaving before irradiation prevented the reduction, a biological mechanism was indicated in preference to the chemical decomposition pathway discussed by Bremner and Nelson (1968).

Although it had been established earlier that accumulation of nitrite in irradiated soil could be partly inhibited by increasing the dose to 2.5 Mrad (Cawse and Crawford, 1967), the amount of radiation which gave complete inhibition was unknown. When experiments were undertaken to determine this point, it was revealed that nitrite formation could not be eliminated by extension of the dose to 12 Mrad; in fact, the concentration rose steadily to reach approximately 100 p.p.m. nitrite-N immediately after 12 Mrad, both in soils which had or had not shown biological accumulation of nitrite after 0.75 Mrad. Further studies were made on irradiation of nitrate solutions, with reference to reports of radiolytic reduction of nitrate to nitrite

which have appeared in the field of radiation chemistry. Evidence for radiolytic action in soil was strong because the nitrite increase was instantaneous, could not be prevented by autoclaving before irradiation (unlike microbial formation of nitrite after 0.75 Mrad), and depended on the nitrate concentration in soil at the time of irradiation (Section 10).

Examination of the persistence of nitrite formed in soils treated with 12 Mrad at medium moisture level revealed that the concentrations had fallen by approximately 75% after 21 days, and this feature was interesting in relation to reports of nitrite fixation to organic matter by Nelson and Bremner (1969), and nitrite volatilisation in autoclaved soils (Bremner and Nelson, 1968). By addition of ^{15}N-labelled nitrite to a soil previously given 12 Mrad it was proved that fixation to organic matter and volatilisation were responsible for nitrite loss with time (Section 11). Under these conditions, nitrous oxide was not detected as a reduction product, unlike the situation where nitrite was being further reduced after 0.75 Mrad.

During the course of experimental work, two reports appeared in the literature which stated that there was loss of nitrate following irradiation of Hawaiian soils. In the first paper (Singh et al. 1969) the authors concluded that certain chemical reactions may be responsible for the loss of nitrate after 3 Mrad, and in the second one (Singh and Kanehiro, 1970) they stated that increasing the dose to 5 Mrad intensified the loss. Such observations are very consistent with the findings in the present thesis on radiolytic reduction of nitrate by high gamma doses, with subsequent removal of nitrite by fixation and volatilisation processes.

The final pattern of nitrite behaviour which emerged after treatment of soil over a wide dose range up to 12 Mrad is well shown by the situation at 7 days after irradiation, and is presented schematically in Figure 1-4. Owing to the distinctly separate effects of radiation on the behaviour of soil nitrite according to dose applied, the level of radiation used in various experiments has been specified in all main titles of the Experimental Section to avoid confusion.

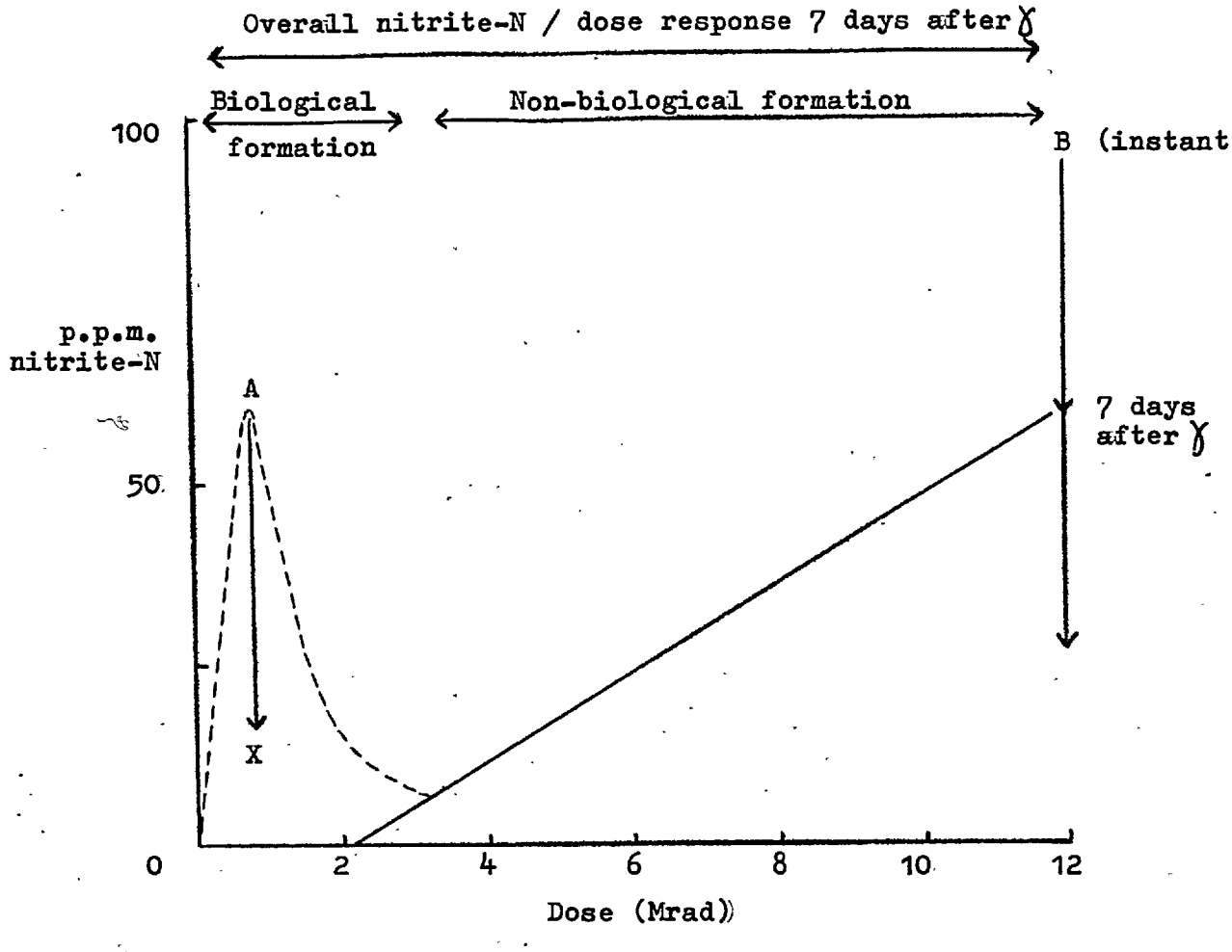


Figure 1-4 **Pattern of nitrite behaviour in irradiated soil at medium moisture level**

A - Not present immediately after irradiation, but gradually rises in calcareous soils to reach a maximum at various times up to 7 days; rise is prevented by autoclaving.

A to X - Declines after 7 days by volatilisation, organic matter fixation and microbial oxidation back to nitrate. After 21 days (X), 30 - 70% of nitrite remains, depending on soil.

B - Present immediately after irradiation in all soils; not eliminated by autoclaving.

B to Y - Declines immediately after irradiation by volatilisation and organic matter fixation, and approximately 25% of nitrite remains after 21 days (Y).

2 MATERIALS AND METHODS

2-1 Description and location of soil types used

Fifteen soils from recognised series were used during the course of the work, and were taken from the top 6" at arable, grassland and woodland sites throughout Berkshire. Their exact location and major properties are shown in Table 2-1. Sampling from the field was carried out at least every 12 months, and often every 6 months: freshly dug soils were stored outside in perforated polythene bowls, but were not allowed to dry out. To avoid severe freezing and difficulty of sampling during the Winter, the containers were removed to an unheated glasshouse. Exceptionally, a small plot of Broad soil measuring 1 m^2 X 15 cm depth was kept entirely outside for 3 years to investigate the influence of fallowing and cropping on formation of nitrite after 0.8 Mrad.

For experimental use the soils were passed in fresh condition through a 2 mm sieve, except for aggregate size studies and work on perfusion of sterile soil inoculated with nitrifying bacteria. For perfusion, soils were air-dried at 20 - 25°C and the 0.6 - 2 mm fraction was used.

Distilled water was added to raise the soil moisture to the level demanded by each experiment, and was normally followed by a period of equilibration lasting 2 days before experimental use. If field samples were excessively wet they were dried in a current of air; complete air-drying was avoided to minimise damage to the microflora which might affect the response obtained from radiation, but in the few experiments where air-drying was essential it is mentioned in the text.

Table 2-1 Description of soil series taken from arable (A), grassland (G) and deciduous woodland (W) sites

No.	Soil Series	pH in: H_2O	pH in: $CaCl_2$	Total N (%)	Org. C (%)	Carbonate C (%)	Moisture at MWHC (%)	Grid reference (all SU)
1	Black (A)	7.8	7.0	0.40	3.5	< 0.05	78.0	763762
2	Broad (A)	8.0	7.4	0.78	6.6	1.19	97.3	765765
3	Broadmoor (G)	8.3	7.5	0.53	5.7	4.03	110.8	572664
4	Bursledon (G)	6.0	5.1	0.29	3.0	< 0.05	71.6	530702
5	Charity (W)	6.1	5.3	0.37	3.9	< 0.05	76.6	278833
6	Colthrop (G)	8.1	7.3	0.65	6.6	2.90	93.4	552662
7	Denchworth (W)	5.7	5.1	0.40	4.1	< 0.05	78.1	264965
8	Evesham (W)	6.8	6.6	0.44	4.5	< 0.05	97.4	323982
9	Fyfield* (A)	7.6	6.9	0.24	2.2	< 0.05	51.1	297952
10	Gade (G)	8.2	7.6	0.49	4.8	1.01	64.4	544709
11	Grove (A)	7.4	6.6	0.39	3.5	< 0.05	70.4	388895
12	Hanney (A)	5.9	5.2	0.37	3.2	< 0.05	73.6	385905
13	Icknield (G)	8.0	7.4	1.20	10.2	0.70	89.0	398847
14	Southampton (W)	3.9	3.1	0.28	6.4	< 0.05	73.4	529693
15	Sulham (G)	6.3	5.5	0.59	5.7	0.11	105.1	641742

* synonymous with Faringdon series (Cawse, 1968).

The above series have been described by the following authors: Nos. 1 & 2 by Kay (1936), no. 7 by Avery (1964), no. 8 by Osmond et al. (1949), no. 9 by Jarvis (in the press), nos. 11 & 12 by Kay (1934), and the remainder by Jarvis (1968).

2-2 Irradiation techniques

The majority of irradiations were made on 10 - 50 g samples of moist soil in a "Mark IV Hotspot" apparatus which delivered 0.6 - 1 Mrad / hr. During irradiation the soil was kept at 22°C by circulating water from a refrigerated bath through a small tubular loop in the irradiation chamber, otherwise the temperature would have risen to 35 - 40°C. Irradiation and incubation of samples was usually carried out in glass, perspex or polypropylene containers having lids which were not completely air-tight, except where otherwise stated.

In experiments concerned with inoculation of nitrifying organisms into sterile soil it was necessary to treat 1 kg quantities with 8 Mrad. For this purpose the Irradiation Pond at Harwell was used; spent reactor fuel elements are utilised as radiation sources, and are capable of delivering high gamma dose rates from 3 - 6 Mrad / hr to aluminium irradiation canisters measuring 17 cm diameter X 53 cm deep, which are lowered into the water shield.

Radiation dosimetry at Wantage Research Laboratory is carried out by means of red polymethyl methacrylate sheet ("Red 400" - I.C.I. perspex). Irradiation causes darkening of the dye, which is 1-amino 4-hydroxy anthraquinone (Lacquer Red 2B), and the optical density of dosimeters measuring 38.1 X 11.1 X 3.2 mm thick is recorded at 635 mμ (Whittaker, 1964). A large number of dosimeters is prepared from one batch of red perspex, and specimens are calibrated for radiation darkening by ferrous sulphate dosimetry.

The soil thickness during irradiation in the "Hotspot" rarely exceeded 3 cm, and little attenuation of gamma-rays was expected; it was known that when the thickness was 6 cm the co-efficient of variation of mean dose received by the soil was within ± 10%. This

information was obtained by placing dosimeters sealed in polythene at the perimeter and centre of samples (at 50 - 80% MWHC) before irradiation. With the larger volumes of soil sent to the Irradiation Pond, it was not considered that accurate dosimetry was essential: the sole objective was adequate sterilisation, and the efficiency of penetration was sufficient to permit this.

2-3 Methods of chemical analysis

Practically all of the analytical results presented in the thesis are means of single determinations on duplicate samples, and in exceptional cases where the number of replicates was increased it is stated in the text.

2-3.1 Total nitrogen and mineral nitrogen

The total-N analysis reported in the soil properties Table 2-1 was performed by the semi-micro method of Bremner (1965a), which did not include nitrite or nitrate. In the ^{15}N tracer studies, mineral-N was extracted before determination of organic-N by the regular macro-method (Bremner, 1965a) with addition of 0.5 g glucose to the digestion mixture. According to Bremner and Fuhr (1966), glucose ensures the reduction of nitro- and nitroso-N, and was used in their Kjeldahl determinations to assess the fixation of nitrite by organic matter. Although Baker (1955) remarked that many nitro compounds give correct results without modification of the usual Kjeldahl digest, and mentioned that evidence in the literature indicated quantitative yields of ammonia from oximes and nitroso compounds without glucose, it was thought advisable to include it for acid digestion of organic matter in ^{15}N recovery studies.

When separate analysis of ammonium, nitrite and nitrate was

required on the same sample, 30 - 50 g of moist soil was shaken for 1 hr with 100 ml of N potassium chloride. Up to 1 ml of the filtered extract was taken for nitrite analysis by the colorimetric method of Crosby (1967), which utilises the reaction between 1-naphthylamine-7-sulphonic acid in the presence of sulphanilic acid. To the remainder of the filtered extract, usually 50 - 80 ml, 1 ml of 10% w/v sulphamic acid was added to remove nitrite, and was followed by distillation first with 4 g of magnesium oxide and then with 2.5 g of Devarda's alloy to recover ammonium- and nitrate-N respectively (Piper, 1950).

If nitrite was the only form of mineral-N to be determined, 10 g of fresh soil was shaken with 100 ml of water, followed by filtration and colorimetric assay. When nitrite and nitrate (but not ammonium) had to be analysed in a sample, water was again used as extractant; nitrite was found colorimetrically, and nitrate by double distillation after destruction of nitrite with sulphamic acid. The small amount of ammonia in the first distillation with magnesium oxide was thus rejected.

For determination of nitrate in soil solutions and perfusates where the volume of sample was very limited, it was found that the most suitable technique was measurement of ultraviolet absorption at 210 mμ (Cawse, 1967c).

If mineral-N extracts could not be analysed at once they were kept at 3°C for a maximum of 3 days, but nitrite in them was always analysed on the day of extraction.

2-3.2 ^{15}N Tracer determination in organic nitrogen and mineral nitrogen fractions

The techniques employed to separate the organic and mineral-N fractions for isotope ratio analysis showed very slight variation in the three tracer experiments, and are therefore reported in the respective Sections. To separate the mineral-N into ammonium-, nitrite- and nitrate-N, 0.7 - 2 mg N from the individual fractions was steam-distilled in an apparatus similar to that described by Bremner and Edwards (1965). An exception was the use of a silver condenser to reduce chemisorption of $^{15}NH_3$ by the cold borosilicate glass: Newman (1966) reported a ten-fold increase in retention with glass, and the small amounts of tracer which were retained by a silver condenser could be more easily removed by steaming for a few minutes between distillations.

If it was not desired to include nitrite, 1 ml of 2% w/v sulphamic acid was added to approximately 20 ml of extract before distillation. Ammonium-N was distilled by heating with 0.4 g of magnesium oxide, and nitrate-N was reduced to ammonia by a second distillation with 0.6 g of Devarda's alloy (<300 mesh). Approximately 30 ml of distillate was collected in 10 - 15 ml of 0.01 N sulphuric acid containing Tashiro's indicator, a 1 : 4 mixture of 0.1% w/v solutions of methylene blue and methyl red. The ammonium- and nitrate-N in the sample was then determined by back titration of the distillate with sodium hydroxide. The titrate was re-acidified with 0.5 ml of N sulphuric acid and evaporated down to approximately 2 ml at 28 - 30°C for isotope ratio analysis.

To carry out isotope ratio analysis of nitrite-N (Section 3-1), 60 ml of water extract was mixed with 2 g of alumina cream to remove organic substances which otherwise caused extensive foaming when

sulphamic acid was added to liberate nitrogen gas. The alumina cream and adsorbed organic matter was removed by centrifugation, and the supernatant solution was evaporated to approximately 2 ml at 28 - 30°C with a current of warm air passing over the surface: 0.1 ml of N potassium hydroxide was added to the solution to guard against instability of nitrite at low pH. With these conditions of evaporation it was possible to obtain 95% recovery of nitrite, but increasing the temperature to 50°C reduced this to 81%.

For conversion of the ammonium and nitrite in evaporated extracts to gaseous-N, special gas sample (reactant) tubes were used. The type usually employed is of inverted "Y" shape, originally described by Sprinson and Rittenberg (1949) and later used by Bremner (1965b). In the present work it was found that four freezing and thawing cycles were needed to thoroughly de-gas the extract, placed in one arm of the tube, and the reactant which was either hypobromite or sulphamic acid, in the opposite arm. In the course of the de-gassing procedure there was frequent breakage of the gas sample tubes, and even when the solutions were thawed extremely slowly the melting ice occasionally jumped into the opposite arm as entrapped air was suddenly released, causing premature evolution of nitrogen. In an attempt to avoid these problems, "Y" tubes were constructed with a larger bore, up to 3 cm internal diameter, but although an improvement they were not entirely satisfactory. Finally, a gas sample tube was designed to contain the solutions in a thin layer, permitting rapid freezing and thawing without breakage or severe jumping of melting ice, and the tube is shown in Figure 2-3.2.1. The reactants were separated by a cross wall during de-gassing, and were finally mixed by pivoting the B 19 cone of the tube in the



Figure 2-3.2.1 Gas sample tube

socket of the main gas handling apparatus.

Evaporated extracts containing ammonium were reacted in the gas sample tube with 2 ml of sodium hypobromite solution to yield nitrogen:

$$2NH_3 + NaOBr = N_2 + 3H_2O + 3NaBr$$

The hypobromite solution was prepared by adding bromine to concentrated sodium hydroxide solution at 5°C, as outlined by Bremner (1965b). For use, it was diluted with an equal part of 0.2% w/v potassium iodide to prevent evolution of traces of oxygen by decomposition of hypobromite when the ammonia is oxidised to nitrogen (Sims and Cocking, 1958). The diluted reagent was stored up to 6 months at 3°C in an air-tight glass bottle.

Evaporated extracts containing nitrite (Section 3-1) were reacted with 1 ml of 4% w/v sulphamic acid:

$$NH_2SO_3H + HNO_2 = N_2 + H_2SO_4 + H_2O$$

and as the nitrogen released would be derived in equimolar proportions from both reactants, the measurements of ^{15}N ratios were adjusted accordingly.

The complete apparatus used to prepare gaseous nitrogen samples is shown diagrammatically in Figure 2-3.2.2. De-gassing was achieved by freezing the contents of the gas sample tube with dry ice, pumping down the apparatus to 0.0005 mm mercury, closing the greaseless stopcock, and gradually thawing the solutions in the sample tube with a warm air blower. De-gassing twice reduced the air contamination of nitrogen samples to 1.5 - 2%, which was unsatisfactory owing to the contribution to the peaks measured at mass 28 $(N^{14}N^{14})^+$ and mass 29 $(N^{14}N^{15})^+$. De-gassing four times produced

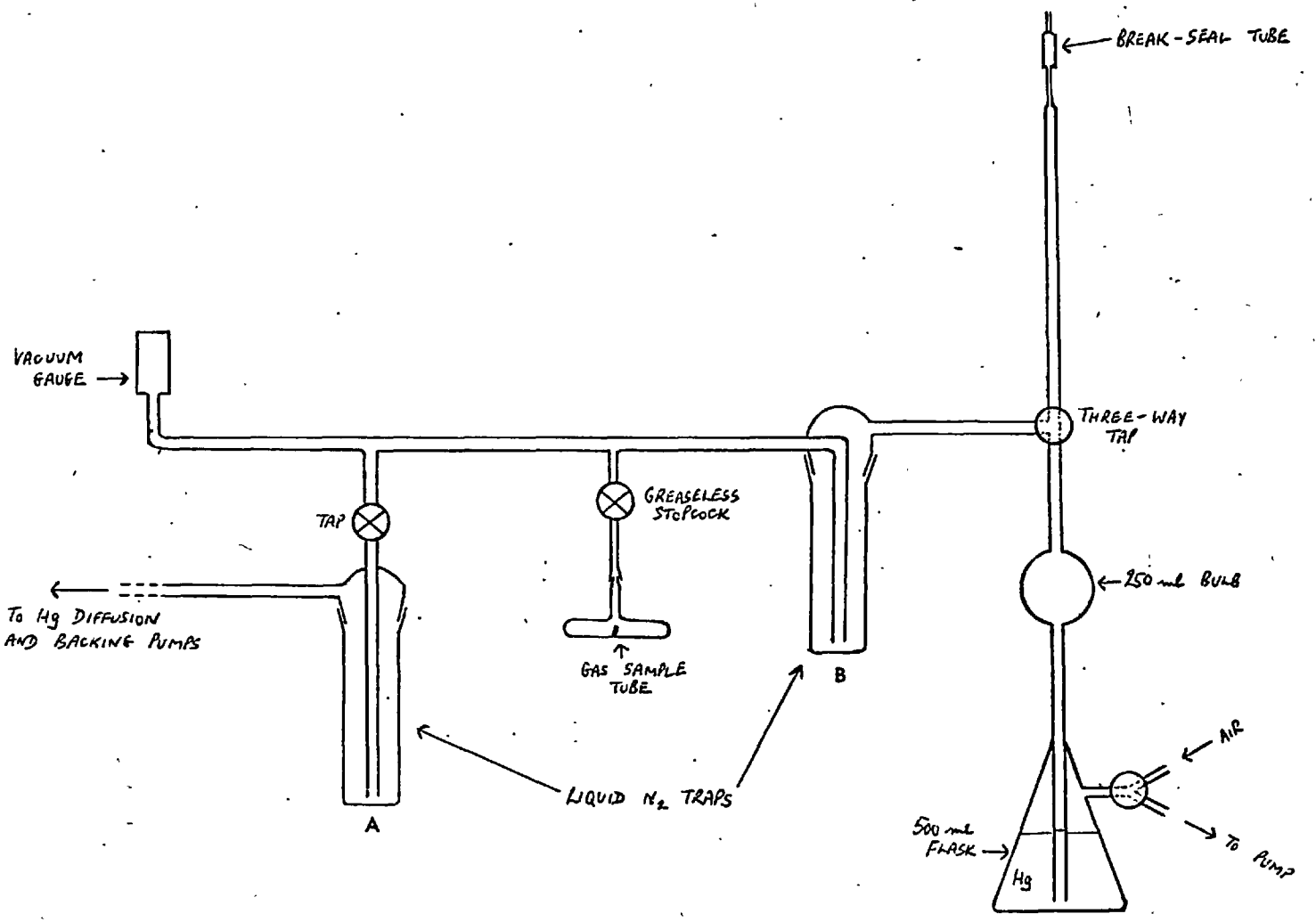


Figure 2-3.2.2 Scheme of apparatus for conversion of ammonium and nitrite to nitrogen gas.

nitrogen with <0.1% of air, and no corrections were deemed necessary for this low level of contamination (Bremner, 1965b). By means of a Toeppler pump, the nitrogen gas was transferred to a 10 ml break-seal tube to avoid leakage and air contamination before analysis on the mass spectrometer, an Atlas CH 4 model at Harwell. The liquid nitrogen trap at "A" prevented moisture from reaching the vacuum pumps, and trap "B" removed moisture, nitrous oxide, amines and carbon dioxide from the nitrogen sample.

Before the main experiments were started, the reliability of gas preparation and handling techniques was examined with solutions of ammonium chloride and sodium nitrite having 1 atom % excess ^{15}N. These were reacted with hypobromite and sulphamic acid respectively, and the mass spectrometer results for the gaseous nitrogen released agreed very closely with the expected percentage of ^{15}N, as calculated from the manufacturers (Prochem Ltd.) declared enrichment of the ^{15}N compounds.

2-3.3 Nitrous oxide

Nitrous oxide can be determined by infra-red spectroscopy, or by gas chromatography. An "Infracord" recording spectrophotometer was available, and enabled a closed system to be set up with periodical circulation of the atmosphere in and above the soil through an infra-red gas cell having a path length of 12 cm. In some subsidiary experiments, 50 ml break-seal ampoules were attached to the closed system so that the atmosphere could be analysed for oxygen and nitrogen by gas chromatography.

The two most sensitive infra-red absorption bands for nitrous oxide appear as doublets at wavelengths of 4.5 and 7.8 μ (2,224 and

1,285 cm^{-1} respectively). At 4.5 μ the minimum detectable concentration is 0.003 - 0.01% by volume at S.T.P., while at 7.8 μ sensitivity is reduced by a factor of ten: measurements were therefore made at the lower wavelength. The main absorption bands for nitric oxide and nitrogen dioxide are not sufficiently close to those of nitrous oxide to interfere (Cheng and Bremner, 1965), but with the apparatus subsequently described, nitric oxide would be slowly oxidised to nitrogen dioxide which would react with the soda lime trap used to remove carbon dioxide. Furthermore, there was the likelihood of extensive absorption of these two gases by the soil.

The outlet of an air circulating diaphragm pump (Dymax Mk. 1, Charles Austen Pumps Ltd.) was connected to the bottom inlet tube of the soil container shown in Figure 2-3.3.1: this was specially designed to permit periodical circulation of air through the soil, and to fit into the "Hotspot" irradiation source. Another object of the design was to make the vessel suitable for anaerobic studies as well, when the basal inlet tube could be sealed and air circulated through the top connections without disturbing the waterlogged soil. For work under aerobic conditions, one of the top connections was sealed off. Water vapour and carbon dioxide interfere with nitrous oxide absorption bands, and the sodium chloride windows of the infra-red cell would be damaged by moisture; to avoid these problems, the atmosphere in the soil container was passed through a U-tube containing magnesium perchlorate and soda lime (4 - 10 mesh self-indicating granules) before reaching the gas cell. Finally, the soil atmosphere flowed back to the diaphragm pump. The complete system was tested for leakage after assembly by introduction of nitrous oxide, and was calibrated by injecting 0.2 - 2 ml of nitrous oxide (99.9% purity).



Figure 2-3.3.1 Irradiation and incubation vessel for nitrous oxide experiments

During calibration, 30 g of dry sand was placed in the sample container to simulate the volume normally occupied by the soil. The atmosphere in the system was circulated for 5 minutes immediately before measurement of the infra-red spectrum, and when soil samples were under investigation the concentration of nitrous oxide was usually recorded daily.

2-3.4 Nitrogen dioxide and nitric oxide

At temperatures in the region of 20°C, mixtures of nitrogen dioxide and nitric oxide exist in equilibrium with dinitrogen trioxide (N_2O_3), but at 0.001 M concentration of N_2O_3 it is 99.95% dissociated: however, even if undissociated, this gas would be recovered by the absorbing reagent used. Pure nitrogen dioxide will be in equilibrium with dinitrogen tetroxide (N_2O_4), but since both are recovered as "nitrogen dioxide" by the absorbent they are referred to in the text as nitrogen dioxide.

Information on the rates of oxidation of nitric oxide by air at 25°C has been presented by Saltzmann (1965), and the conversion to nitrogen dioxide becomes extremely slow as the concentration of nitric oxide decreases. For example, 1.5 p.p.m. nitric oxide took 85 minutes for 2% conversion to nitrogen dioxide, and the concentration of nitric oxide in the soil air which passed through the flow system could not have exceeded 0.001 p.p.m., judging by the maximum amount of nitric oxide removed from the air stream in 24 hr at an approximate flow rate of 120 litres / day.

Nitrogen dioxide and nitric oxide were determined by the Saltzmann method (1965), designed for quantitative measurement of low atmospheric concentrations of these gases. A flow-through system

was used to obtain an idea of the rates of evolution from irradiated soils, usually over 14 days, but in some instances up to 21 days. The flow method had the advantage that it helped to prevent re-absorption of nitrogen dioxide by moist soil and microbial oxidation to nitrate, which is unavoidable with closed systems, and the soil was kept well aerated.

The flow apparatus consisted of a train of five gas-washing bottles connected by 5 mm internal diameter glass ball and socket joints. The first bottle contained 300 ml of water to humidify the incoming air which next passed into the basal inlet of a 250 ml capacity bottle containing the soil sample: this bottle was fitted with a sintered plate on which the soil rested, and air thus flowed upwards through the sample ensuring displacement of oxides of nitrogen. The gases were next drawn into a smaller wash bottle of 125 ml capacity, containing 100 ml of absorbing reagent to remove and react colorimetrically with nitrogen dioxide. Nitric oxide in the gas stream was oxidised by passage through a fourth bottle (125 ml) which contained 100 ml of slightly acidified potassium permanganate, and was then removed as nitrogen dioxide by 100 ml of absorbing reagent in the final 125 ml bottle. The last three bottles were fitted with fritted bubblers of 40 - 50 μ porosity. The final stage of the flow line was a vacuum pump which was adjusted by needle valves to maintain a slow flow of 4 - 5 litres / hr, and such conditions almost certainly permitted better absorption of gases than a rate of 24 litres / hr mentioned in the original procedure of Saltzmann (1965).

The reagents were made up as follows:

(i) Absorbing reagent for nitrogen dioxide

5 g of sulphanilic acid was dissolved in 900 ml of water

containing 140 ml of glacial acetic acid, 20 ml of 0.1% w/v N-(1-naphthyl)-ethylenediamine dihydrochloride was added, and the volume was adjusted to 1 litre with water. The reagent was stored up to 7 days in a tightly stoppered flask. The stable pink colour which develops on absorption of nitrogen dioxide was measured in 2 cm spectrophotometer cells at 550 mμ. For calibration, 2 - 10 μg nitrite-N was placed in 100 ml volumetric flasks, made to the mark with absorbing reagent, and measured after 15 minutes with a spectrophotometer. During experiments with soil, the absorbent was changed and measured every 1 - 3 days, depending on the colour intensity.

There is considerable variation in the literature on the yield of nitrite-N which is obtained on absorption of nitrogen dioxide-N, ranging from 72% conversion (Saltzmann, 1965) to 75 - 100% conversion reported by Thomas et al. (1956): the incomplete recovery arises from the partial formation of nitrate in the absorbent by decomposition of nitrous acid. The efficiency of reaction of nitrogen dioxide with the absorbing reagent was investigated under the conditions of the present flow system. 1 ml of nitrogen dioxide gas (99.5% purity) was injected with a gas chromatography syringe through the rubber seal cap of 4 fl. oz. medicine bottles (four replicates) containing 50 ml of N sodium hydroxide; after shaking for 30 minutes, the total nitrite + nitrate-N was determined by distillation with Devarda's alloy, and it appeared to be reasonable to assume an absorption efficiency of 100% (Burdick, 1922) with an equally good recovery by distillation. Another set of four bottles containing 100 ml of absorbing reagent received 12 μl of nitrogen dioxide, and were shaken for 30 minutes. The colour intensity was measured and the nitrogen was calculated from the nitrite-N calibration results.

the figure obtained being expressed as a percentage of the N recovered (per 12 µl of nitrogen dioxide) with the sodium hydroxide procedure. A recovery of 75% was obtained, and all calculations of gaseous N released from the soil as nitrogen dioxide and nitric oxide were corrected for the deficit.

(ii) Acidified permanganate

25 g of potassium permanganate was dissolved in 900 ml of water, acidified with 13 ml of concentrated sulphuric acid, and made to 1 litre with water. Fresh reagent was prepared every 2 weeks, and the permanganate in the flow system was changed after 7 days if much nitric oxide had been evolved, otherwise it remained in reasonable condition for 2 weeks with the slow flow rate used.

To examine the efficiency of the gas-washing bottles, nitrogen dioxide was passed through a series of three bottles each containing 100 ml of absorbent. Colorimetric measurements showed that 96.0% was removed by the first bottle. The efficiency of oxidation and absorption of nitric oxide was tested over 24 hr using irradiated Icknield soil with added nitrite: two extra gas-washing bottles were placed on the end of the flow line and contained permanganate and absorbing reagent respectively to oxidise and recover any nitric oxide which escaped, but colorimetric measurements indicated that there was 97.7% recovery by the main system. Minor additions were made to the soil results for nitric oxide to compensate for slightly incomplete absorption, and as far as the results for nitrogen dioxide were concerned, it was taken into account that the 4% which escaped the first absorber bottle would still be recovered with the nitric oxide. As the soil experiments proceeded, it became

clear that the best comparison was between nitrous oxide evolution and the sum of nitrogen dioxide and nitric oxide released; from this viewpoint it may seem that the absorption corrections were hardly warranted, but as the percentages of nitrogen dioxide and nitric oxide which contributed to their total sum were quoted, it was desirable to make them as accurate as possible.

A further important point to resolve was the efficiency with which the acidified permanganate solution oxidised nitric oxide. Very efficient oxidation would result in formation of nitrate instead of nitrogen dioxide, thus giving low readings for nitric oxide in the final bottle of absorbing reagent. Cheng and Bremner (1965) have stressed that widely different efficiencies have been reported with flow systems employing various types of gas-washing bottle, and can range from 3 - 100% conversion of nitric oxide to nitrogen dioxide.

The degree of oxidation of nitric oxide to nitrate by the acid permanganate was examined in two ways. Firstly, the permanganate was rendered alkaline with sodium hydroxide and distilled with Devarda's alloy to recover absorbed nitrogen at the end (14 days) of actual experiments with irradiated soil + nitrite, where an extensive release of nitric oxide took place. The mean of five determinations showed that the permanganate removed as much as 74% of the total nitric oxide released. This was confirmed by a second procedure, whereby 30 µl of nitric oxide (99.95% purity) was injected into the inlet of a permanganate gas-washing bottle connected to another vessel containing 100 ml of absorbing reagent to recover nitrogen dioxide passing through at a flow rate of 5 litres / hr: the flow was maintained for 1 hr after injection. The colour intensity which developed was expressed as a percentage of that given by injection of the same volume of gas into

a medicine bottle containing 100 ml of absorbing reagent, and subsequently shaken for 1 hr before measurement; a mean recovery of 28% was obtained from five determinations, thus indicating that 72% of the nitric oxide had been retained by the permanganate.

The results for nitric oxide-N were therefore corrected to 100% recovery on the basis of results from the soil procedure. Although there was such a low recovery of nitric oxide in the final bottle of absorbent, the flow system described still remained attractive because the intensity of colouration with the Saltzmann absorbing reagent provided a continuous visual check on the amounts of nitrogen dioxide and nitric oxide released; it enabled immediate comparisons to be made between treatments, which was an advantage in preliminary tests to decide what quantity of soil to irradiate.

2-3.5 Organic and inorganic carbon

Soil organic carbon was analysed by Bremner and Jenkinson's (1960) modification of the Tinsley wet oxidation procedure, using 0.1 - 0.5 g of air-dried soil which had been passed through a 0.6 mm sieve. The sample was boiled gently under reflux for 2 hr with acidified potassium dichromate, and the residual dichromate was finally titrated with ferrous ammonium sulphate using barium diphenylamine sulphonate as indicator.

Soluble organic carbon in irradiated soils was determined on 20 ml of water extract containing approximately 10 mg of carbon. The analytical procedure was identical to that employed for soil samples except that the sodium salt of N-phenylanthranilic acid was substituted as indicator; this compound was originally recommended by Syrokomsky and Stiepin (1936) and later used by Mebius (1960) for

organic carbon determination. The indicator was prepared by dissolving 0.2 g of N-phenylanthranilic acid (diphenylamine-2-carboxylic acid) and 0.2 g of sodium carbonate in 100 ml of water, 5 drops being sufficient for a titration. The end-point was marked by a sharp change from violet to bright green when the residual dichromate had been reduced.

Inorganic carbon was determined by the technique of Allison (1960). From 1 - 5 g of oven-dry soil, which would release approximately 40 mg of carbon dioxide, was acidified and heated for 7 minutes: the carbon dioxide evolved was swept into a previously weighed Nesbitt absorption bulb containing soda lime. The recovery of carbon dioxide by the apparatus was tested by analysis of 0.1 g "Specpure" oven-dried calcium carbonate, and the mean recovery from five determinations was 94.6%. This small deficit was taken into account in calculations of inorganic carbon present in soils.

2-3.6 Maximum water holding capacity (MWHC), moisture and pH

The MWHC or saturation value of soils was found by the method of Ekpete and Cornfield (1965), and the moisture content was determined by drying for 24 hr at 105°C. Soil acidity was measured with a calibrated glass electrode placed in both 1 : 2.5 soil : water and soil : 0.01 M calcium chloride mixtures that had been shaken for 30 minutes.

2-4 Pure culture of nitrifying bacteria

Nitrosomonas europaea (sewage strain) was obtained in pure culture from Dr. N. Walker, Rothamsted Experimental Station. The recommended liquid media had the following composition:

$(NH_4)_2SO_4$	0.5 - 1.0 g
KH_2PO_4	0.2 g
$CaCl_2 2H_2O$	0.05 g
$MgSO_4 7H_2O$	0.05 g
Fe-EDTA	0.5 mg
Phenol Red	4 mg
Distilled water to 1 litre	

15 ml of media in 4 fl. oz. flat medicine bottles was sterilised at 15 lbs / sq. in. for 15 minutes, and when cool, sterile 0.5 M sodium carbonate was added through a rubber seal cap by hypodermic syringe until the phenol red indicator was a rose-red colour; this corresponds to a pH of 7.5 - 8.0. After inoculation with Nitrosomonas, the pH was re-adjusted every 3 days during incubation at 25°C.

Nitrobacter winogradski was obtained in pure culture from Professor H. Engel, Hamburg Botanical Institute. He advised liquid media composed of:

$NaNO_2$	2.0 g	
NaCl	0.5 g	Adjust pH to 8.6 with
$MgSO_4 7H_2O$	0.05 g	N KOH before sterilisation;
K_2HPO_4	0.05 g	after, it should have
Fe-EDTA	0.5 mg	fallen to pH 7.6 - 7.8
$(NH_4)_6Mo_7O_{24}\ 4H_2O$	0.05 mg	
$CaCO_3$	8 mg	
Distilled water to 1 litre		

15 ml of media in 4 fl. oz. flat medicine bottles was

sterilised at 15 lbs / sq. in. for 15 minutes. After inoculation with Nitrobacter, incubation was carried out in darkness at 25°C. Light is unfavourable to Nitrobacter because of damage to cytochrome, which has maximum absorption at about 420 mμ.

Both bacteria showed better growth in "medical flat" bottles laid horizontally in the incubator, than in McCartney tubes. The former vessels provided a larger surface area, and Zobell (1943) reported many observations on the beneficial effect of solid surfaces. He believes that the surfaces concentrate nutrients by adsorption and retard the diffusion of exoenzymes away from the cell.

Sub-cultures of both bacteria were made every 3 weeks. After this time growth was visible by the turbidity of the medium, especially when the bottles were given a sharp shake to detach the organisms from the glass. To prevent contamination during sub-culturing, it was found best to use rubber capped bottles and carry out the transfer with a sterile disposable syringe.

The purity of the cultures was tested by inoculation of nutrient agar in petri dishes and bottles of nutrient broth, followed by incubation for 4 weeks and 2 weeks respectively at 25°C. With the sub-culturing technique described above, contaminants were not detected: Pseudomonas spp. often contaminate Nitrobacter cultures and should show in 24 hr incubation.

The agar and broth were made up as follows, both solutions being sterilised at 15 lbs / sq. in. for 15 minutes:

Nutrient Agar

"Lab-lemco" beef extract	1 g
Yeast extract	2 g
Peptone	5 g

NaCl	5 g
Agar	15 g

Distilled water to 1 litre (final pH should be approximately 7.4). After sterilisation the agar was poured into sterile petri dishes.

Nutrient Broth No. 2

"Lab-lemco" beef extract	10 g
Peptone	10 g
NaCl	5 g

Distilled water to 1 litre (final pH should be approximately 7.5). After sterilisation 10 ml aliquots were placed into 25 ml McCartney bottles. This broth complies with British Pharmacopoeia recommendations for a sterility test medium to verify the presence of aerobic organisms.

2-5 Perfusion technique

For initial experiments on the colonisation of nitrifying bacteria into radiation sterilised soil, the Sims and Collins (1958) modification of the Audus perfusion apparatus was used. Soil aggregates from 0.6 - 2 mm were contained in nylon sachets, as previously described (Cawse, 1968), because it was impracticable to irradiate the whole apparatus. In later experiments however, the amount of colonised soil obtained from each unit (20 g) was insufficient, and larger units were constructed to hold 100 g of dry aggregates in a nylon sachet measuring 5 X 15 cm (250 micron mesh). Both large and small units circulated 250 ml of perfusate, which was 0.01 M ammonium sulphate.

Perfusion units were sterilised by heating for 2 hr at 160°C,

and the perfusate was separately sterilised at 15 lbs / sq. in. for 15 minutes. Transfer of sterile soil and perfusate to and from the apparatus was accomplished in a sterile laminar air-flow cabinet. To commence perfusion, up to ten small or six large units were placed in an incubator at 25°C and connected between two manifolds; one led to an air suction pump and the other, on the incoming side, was connected to a filter system. The incoming air was first passed through three gas-washing bottles each containing 250 ml of sterile water to humidify it and remove dust-borne organisms. Secondly, the air passed through a cotton wool filter 15 X 1.5 cm before entering the manifold leading to separate units. Finally, a 6.0 X 1.5 cm cotton wool filter was placed in the air supply to each unit. The manifolds and filters were sterilised by irradiation with 2.5 Mrad.

During perfusion, the perfusates were prevented from falling below pH 7.5 by addition of sterile N potassium hydroxide, and slight moisture losses (detected by weighing the complete apparatus) were compensated by adding sterile water. For each set of experiments, two non-inoculated units were always included to confirm that the indigenous nitrifying bacteria had been completely eliminated, and that contamination of the air supply with unknown nitrifying species had not led to re-establishment of nitrification.

3 to 11 EXPERIMENTAL

3 Reduction of ^{15}N-labelled nitrate to nitrite by soils treated with 0.5 - 0.8 Mrad

3-1 Main ^{15}N tracer experiment

It was desirable to obtain more substantial evidence for the reduction of nitrate in irradiated Broad and Icknield soils, and thus confirm the results of leaching tests which strongly suggested that such a mechanism can occur (Cawse and Crawford, 1967). Two other series from Broadmoor and Colthrop were also included, because during an examination of production of nitrite by ten irradiated soils (apart from Broad and Icknield), they showed the greatest accumulation (Cawse and White, 1969b). By addition of potassium nitrate enriched in the stable ^{15}N isotope before irradiation, it was hoped to recover some tracer in the nitrite pool as a result of nitrate reduction over a short incubation period.

Materials and Methods:

Fresh samples of 50 g were taken from Broad, Broadmoor, Colthrop and Icknield series soils which had been separately analysed for nitrite and nitrate. It was calculated that addition of 200 µg ^{15}N tracer would give a concentration of at least 4 atom % ^{15}N in the nitrate pools of all soils, and a 2 ml aliquot of enriched potassium nitrate solution (96.71 atom % ^{15}N excess), which contained 214.2 µg ^{15}N, was added to each sample with sufficient water to raise the percentage moisture to the levels shown in Table 3-1.1. An equilibration period of 2 days was allowed before irradiation. The radiation dose and incubation time was chosen separately for each soil to

give maximum formation of nitrite as soon as possible (Table 3-1.1), and a control (without tracer or irradiation) was incubated and analysed for nitrite to ensure that the incubation conditions had not encouraged formation of nitrite.

Table 3-1.1 Experimental conditions for ^{15}N tracer investigation

Soil series	Moisture at irradiation (%)	Dose (Mrad)	Incubation time (hr)
Broad	70	0.8	64
Broadmoor	65	0.5	24
Colthrop	45	0.5	24
Icknield	70	0.6	64

After incubation, nitrite and nitrate were extracted by shaking the sample three times with 60, 20, and 20 ml of water respectively, centrifuging down the suspended particles for 10 minutes at 1000 G after each shaking period, and pouring off the supernatant. Clay particles remaining in the supernatant fraction were then removed by high speed centrifugation at 30,000 G for 40 minutes. This technique was preferred to a shaking and filtration procedure because it allowed slightly better recovery of nitrite and nitrate and thus reduced the amount of soil and tracer required. The nitrogen present in the nitrite and nitrate pools was determined by quantitative analysis of the extracts, followed by procedures to assess the relative enrichment of the pools with ^{15}N (Section 2-3.2).

Results:

The analytical results appear in Table 3-1.2. The atom % ^{15}N excess in the nitrate pool before irradiation (C_o) was calculated by the relationship described by Jansson (1958):

$$C_o = \frac{n_2 \times C_2}{n_1 + n_2}$$

where n_1 = µg N in the initial nitrate pool without ^{15}N excess.

n_2 = µg N added as $^{15}NO_3 + {}^{14}NO_3$.

and C_2 = atom % ^{15}N excess in n_2 (96.71%).

Following incubation of soil for time t after irradiation, the analyses showed the gross inorganic nitrate and nitrite pools represented as N_a and N_b µg nitrogen respectively, with a ^{15}N excess of C_a and C_b atom %. The recovery of ^{15}N in these pools was then calculated as:

$$\frac{(C_a \times N_a) + (C_b \times N_b)}{100}$$

and was expressed as a percentage of the tracer initially added (Table 3-1.2). The recoveries were satisfactory, and indicated that little nitrite was decomposed by chemical or microbial action during the incubation period.

To be strictly correct, the atom % figures should be transformed to weight percentage for the above calculations because:

$$\text{Weight \% } ^{15}N = \frac{1500 \times \text{atom\% } ^{15}N}{1400 + \text{atom\% } ^{15}N}$$

but as Jansson (1958) points out, samples containing 1 - 5 atom % ^{15}N only show a deviation between atom % and weight % of 6.8 and 7.1 relative % and can be considered constant; therefore no transformations were made. Furthermore, the normal atomic weight of nitrogen was used to calculate titration results, but at levels of 1.5 - 3 atom % ^{15}N the error in nitrogen weight would only be 0.1 - 0.2% low. In the calculation of

Table 3-1.2 The isotopic composition of nitrate and nitrite produced after irradiation of soils containing $^{15}NO_3$ tracer +

Soil series	Total µg mineral-N in sample						Total µg ^{15}N excess* in sample				
	nitrate pool			nitrite pool			nitrate pool			nitrite pool	
	(i)	(ii)	(iii)	(i)	(ii)	(iii)	(i) ^{15}N added	(ii)	lowest % expected after dilution	(ii)	% Recovery ^{15}N
Broad	4851	7572	4956	7.7	1429	68.9	214.2 (4.40)	186.6 (2.46)	(2.31)	23.0 (1.61)	97.9
Broadmoor	5340	6507	5424	11.0	1747	7.2	214.2 (3.99)	196.2 (3.02)	(2.52)	28.7 (1.64)	105.0
Colthrop	4783	5890	4677	35.6	1643	7.9	214.2 (4.46)	193.8 (3.29)	(2.75)	27.0 (1.64)	103.1
Icknield	2648	4492	2542	6.5	1381	22.8	214.2 (8.06)	169.5 (3.77)	(3.50)	75.4 (5.46)	114.3

where (i) = before irradiation, with contribution from tracer-NO_3 added.
(ii) = after irradiation and incubation with tracer.
(iii) = after incubation only, without tracer.

+ figures in parentheses denote atom per cent ^{15}N excess.

* natural abundance of ^{15}N = 0.366 atom per cent.
Co-efficient of variation of ^{15}N analysis = 0.77%.

^{15}N added and recovered, atom % was used, and there would be negligible difference from the use of weight percentage figures (Jansson, 1958).

Only Icknield series showed a higher percentage of ^{15}N in the nitrite as compared with the nitrate pool after incubation. The other soils had a lower ^{15}N content in the nitrite pool, and this might have been caused by microbial nitrate assimilation, oxidation of ammonium (unlabelled) to nitrite, and isotopic discrimination, these processes operating either separately or in combination. Overrein (1967) reported considerable immobilisation (assimilation) of nitrate in ^{15}N experiments, but such a mechanism is not favoured in the present study owing to the high recoveries of tracer in the inorganic pools.

Rapid nitrification of ammonium to nitrate was in evidence after irradiation of all soils, and would certainly lead to dilution of tracer in the nitrate pool. Assuming that all nitrite originated from nitrate reduction, the lowest expected dilution of ^{15}N tracer was calculated (Table 3-1.2); even so, the nitrite pools of Broad, Broadmoor and Colthrop soils still had 0.7 - 1.1 atom % ^{15}N excess under these values, which suggested a direct contribution of ammonium oxidation to nitrite pools.

Discrimination between ^{14}N and ^{15}N has been reported in pure culture experiments with denitrifying bacteria, and ^{14}N was utilised 2% faster (Wellman et al. 1968). Bowen (1960) remarked that the best conditions for fractionation require a small percentage uptake of the lighter isotope from the medium, and owing to the existence of a large soil nitrate pool after incubation (Table 3-1.2), it is possible that discrimination in favour of ^{14}N played a small

part in lowering the expected atom % ^{15}N in the nitrite pool. In the Icknield soil there was a greater percentage reduction of nitrate to nitrite, and discrimination would be expected to be less prominent; this series certainly gave a high ^{15}N percentage in the nitrite pool.

Finally, it was thought that faster reduction of the nitrate pool before it became diluted by nitrification could partly account for the higher percentage of ^{15}N in the nitrite pool of Icknield soil compared with the Broad series. To investigate this possibility, the two soils were irradiated and analysed for nitrite after incubation for 3, 6, 12 and 24 hr. The results (Table 3-1.3) showed little difference in rates of nitrite accumulation between soils:

Table 3-1.3 Rapidity of nitrite accumulation in irradiated Broad and Icknield soils

Time of incubation (hr)	mean p.p.m. nitrite-N	
	Broad 0.8 Mrad	Icknield 0.6 Mrad
3	18.3	14.3
6	18.3	16.4
12	22.2	19.0
24	26.7	23.6

The main conclusion to be drawn from the tracer experiment was that large amounts of nitrate were rapidly reduced to nitrite at medium soil moisture levels after treatment in the range 0.5 - 0.8 Mrad. The observation fully supported previous indications from leaching tests (Cawse and Crawford, 1967; Cawse and White, 1969b), where soil was leached immediately after irradiation with ammonium or nitrate ions: large increases in nitrite only appeared

during incubation of samples leached with nitrate. With regard to the actual mechanism of nitrite formation, it was thought that microbial action could be responsible for two reasons: firstly, because nitrite readily accumulated after leaching with nitrate (which would remove any nitrite formed during irradiation), and secondly, because increasing the radiation dose to 2.5 Mrad caused a gradual decline in nitrite formation (Cawse and Crawford, 1967; Cawse and White, 1969b). Nitrate reduction might be carried out by radioresistant enzyme systems of denitrifying bacteria that cannot proliferate after radiation treatment, in a similar manner to the rapid nitrification which occurs after many soils have received 0.2 Mrad, apparently by continued oxidation processes within non-proliferating cells of nitrifying bacteria (Cawse, 1968).

If oxidation of ammonium did contribute directly to the nitrite pool in some irradiated soil, it should at least be possible to demonstrate an increase in nitrite following ammonium addition together with radiation treatment, provided that the oxidation was not already proceeding at its maximum rate. Use of ^{15}N-labelled ammonium was not practicable because some would also be completely nitrified to $^{15}NO_3$, and therefore $^{15}NO_2$ could arise from nitrate reduction as well as ammonium oxidation.

3-2 Effect of ammonium addition on accumulation of nitrite

As a supplementary experiment to the main ^{15}N tracer study, it was decided to compare the extent of nitrite accumulation after treatment of soils with (i) ammonium alone, in case toxicity towards Nitrobacter blocked nitrite oxidation (Stojanovic and Alexander, 1958), (ii) irradiation, and (iii) ammonium + irradiation.

Materials and Methods:

Broad and Icknield soils were taken from the same batch used to supply samples for the ^{15}N tests which had been made 6 months previously. For Broadmoor and Colthrop types, further samples had to be obtained from the field. The mineral-N and moisture was determined, and sufficient water and ammonium sulphate solution (1000 p.p.m. ammonium-N) was added to samples of 30 g to obtain 100 p.p.m. ammonium-N on a soil dry weight basis, and final moisture levels equivalent to those in the ^{15}N experiment. To avoid nitrification during an equilibration period, the ammonium and water had to be added immediately before irradiation. The radiation dose and incubation time for each soil was identical to that employed in the tracer test (Table 3-1.1). After incubation the soils were analysed for ammonium and nitrate as well as nitrite, in order to follow changes in mineral-N composition between treatments.

Results:

The results in Table 3-2 clearly show that in all irradiated soils the presence of extra ammonium increased the accumulation of nitrite, and it seemed very probable that some nitrite had been produced by oxidation of ammonium in the tracer tests. It was impossible to obtain an exact quantitative indication of the contribution to the nitrite pool from this pathway relative to the nitrate reduction mechanism, because there were two main features which complicated the situation.

The first problem was that nitrite accumulated in unirradiated soil from Broadmoor and Colthrop series following ammonium addition only, and such behaviour almost certainly represents damage to Nitrobacter. However, it was not to be expected that so much nitrite

Table 3-2 Effect of ammonium addition on nitrite accumulation

Soil series	Form of mineral-N	p.p.m. mineral-N before treatment	after incubation: NH_4 added	after incubation: irradiated	after incubation: NH_4 + irradiation
Broad	NH_4	2.6	3.2	6.3	46.1
	NO_2	0.4	1.4	48.6	69.5
	NO_3	155.3	258.8	183.5	160.8
Broadmoor	NH_4	3.0	32.8	15.2	108.8
	NO_2	0.3	22.6	29.3	34.8
	NO_3	41.0	99.6	65.4	61.0
Colthrop	NH_4	5.4	40.5	7.0	90.0
	NO_2	0.5	24.2	26.4	40.4
	NO_3	43.5	76.9	73.2	62.4
Icknield	NH_4	4.2	1.2	1.3	74.9
	NO_2	0.6	2.8	41.2	52.1
	NO_3	169.5	267.3	233.8	229.6

L.S.D.	NH_4	NO_2	NO_3
p 0.05	4.2	3.0	4.0
p 0.01	9.6	6.8	9.2

would originate from ammonium after irradiation alone, because the soil ammonium concentration was lower. In addition, the sum of nitrite present in the separate "ammonium added" + "irradiated" treatments exceeded the nitrite found after "ammonium + irradiation", and this observation could be explained by a limited inhibitory effect of 0.5 Mrad on ammonium oxidation to nitrite; the results do show that large amounts of ammonium remained unoxidised after incubation.

The second complication refers to the behaviour of Broad and Icknield series. Although they did not accumulate nitrite following addition of ammonium, unlike the other two soils, it was still impossible to accurately assess the amount of nitrite which had been produced by ammonium oxidation as opposed to radiation. For example, if the nitrite concentration after irradiation was deducted from that found after "ammonium + irradiation", the minimum increase from ammonium oxidation in Broad soil would be 69.5 - 48.6 = 20.9 p.p.m. nitrite-N, or 30% of the nitrite pool: the assumption is made that all nitrite in the "irradiated" treatment originates from nitrate reduction. This percentage would be too high if addition of ammonium to the pool of radiation-released ammonium had accentuated accumulation of nitrite by inhibition of Nitrobacter. The smaller amount of ammonium produced by radiation alone would be less likely to cause an equivalent check to nitrite oxidation.

It was thought that nitrification inhibitors might be used to eliminate the contribution of ammonium oxidation to the nitrite pool, but the idea was not pursued because addition of 4 - 25 p.p.m. (soil dry weight basis) of "N-Serve" (2-chloro-6-(trichloromethyl)

pyridine) only gave 75% reduction in nitrification (Cawse, 1967b), and there is no detailed knowledge on the response of denitrifying organisms to this compound.

Alternatively, since reduction of nitrate had been confirmed as the major pathway leading to accumulation of nitrite in irradiated soils, further effort was directed towards a study of the way in which various soil factors influenced the extent of nitrite formation, perhaps by affecting the proliferation and metabolism of nitrate reducing bacteria.

4 Factors affecting the formation of nitrite in soils after irradiation in the region of 0.75 Mrad

4-1 Moisture

Experiments on irradiation of dry, medium and wet Broad soil up to 88% MWHC have shown that increasing moisture favours production of nitrite (Cawse and Crawford, 1967), but the effect of a complete range of moisture from air-dry to waterlogging has not been previously examined. More detailed information was required, especially under very wet conditions that would encourage denitrification, and the behaviour of Broad and Icknield soils was examined.

Materials and Methods:

(i) Preliminary tests were carried out to ensure that an incubation period of 64 hr at 25°C following irradiation would give maximum nitrite accumulation in Icknield soil which, unlike Broad series, had not been analysed at different times after irradiation in the course of earlier studies.

Samples of 10 g were raised to 60% moisture, and treated with 0.6 Mrad. Nitrite was determined after periods of incubation from 16 - 168 hr, and the results showed that 64 hr would also be a suitable length of time for the occurrence of maximum nitrite concentration in irradiated Icknield series:

incubation time (hr) after irradiation	mean p.p.m. nitrite-N
16	21.3
32	28.1
64	33.3
128	29.4
168	28.8

(ii) For the main experiment, samples of 30 g were taken. Soils were air-dried at 20°C for the treatments at 10, 20 and 30% moisture, and water was added to obtain moistures from 40% - 120%, in increments of 10%. It was decided to include heavily waterlogged soils because studies by Cady and Bartholomew (1960), Greenwood (1962), and Mahendrappa and Smith (1967) have found an increase in denitrification rate from addition of water above the saturation point.

Water was added only 1 hr before irradiation to reduce the danger of denitrification in some treatments, as this would complicate the experiment if it occurred prematurely; the main disadvantage would be removal of nitrate which is, of course, the substrate for formation of nitrite. Unirradiated control samples were also prepared at all moisture levels.

Radiation doses of 0.6 and 0.8 Mrad were applied to Icknield and Broad series respectively, because these amounts gave maximum accumulation of nitrite in relation to dose at medium moisture (Cawse and Crawford, 1967). Samples were incubated at 25°C in 40 ml polypropylene tubes, and after 64 hr the ammonium, nitrite and nitrate was extracted.

Results:

The analytical results are presented in Figures 4-1.1 (Broad) and 4-1.2 (Icknield). Both soils gave a similar response to radiation over the entire range of moisture. Irradiation of samples from air-dry to near 75% MWHC gave increasing stimulation of nitrite and nitrate production. Above 75% MWHC there was a severe fall, first in nitrate and then nitrite present after incubation, with a rise in ammonium encouraged by waterlogging. Control treatments did not show such severe losses of nitrate, and ammonium failed to increase under

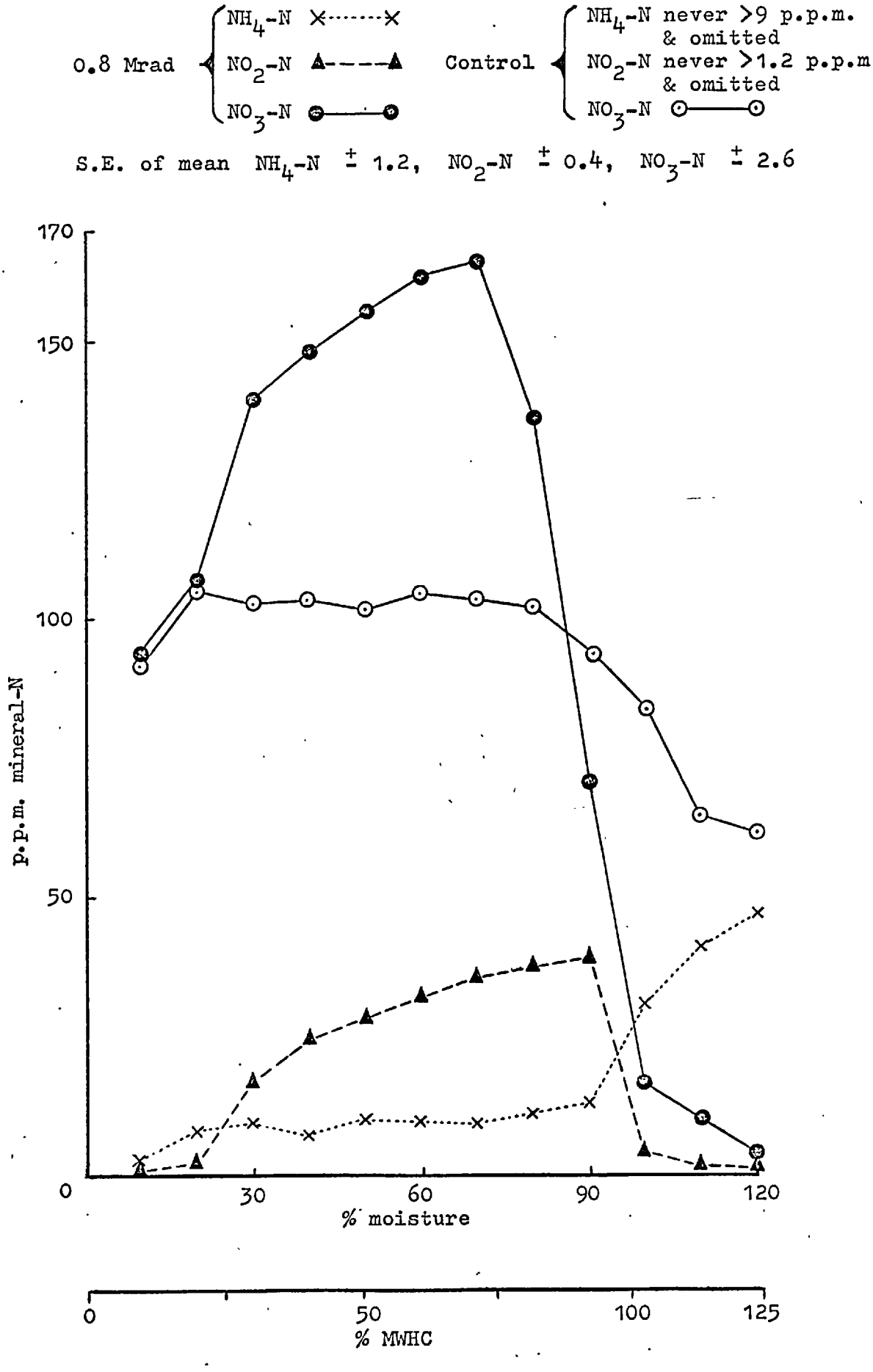


Figure 4-1.1 Effect of moisture on mineral-N accumulation in irradiated Broad soil after 64 hr incubation

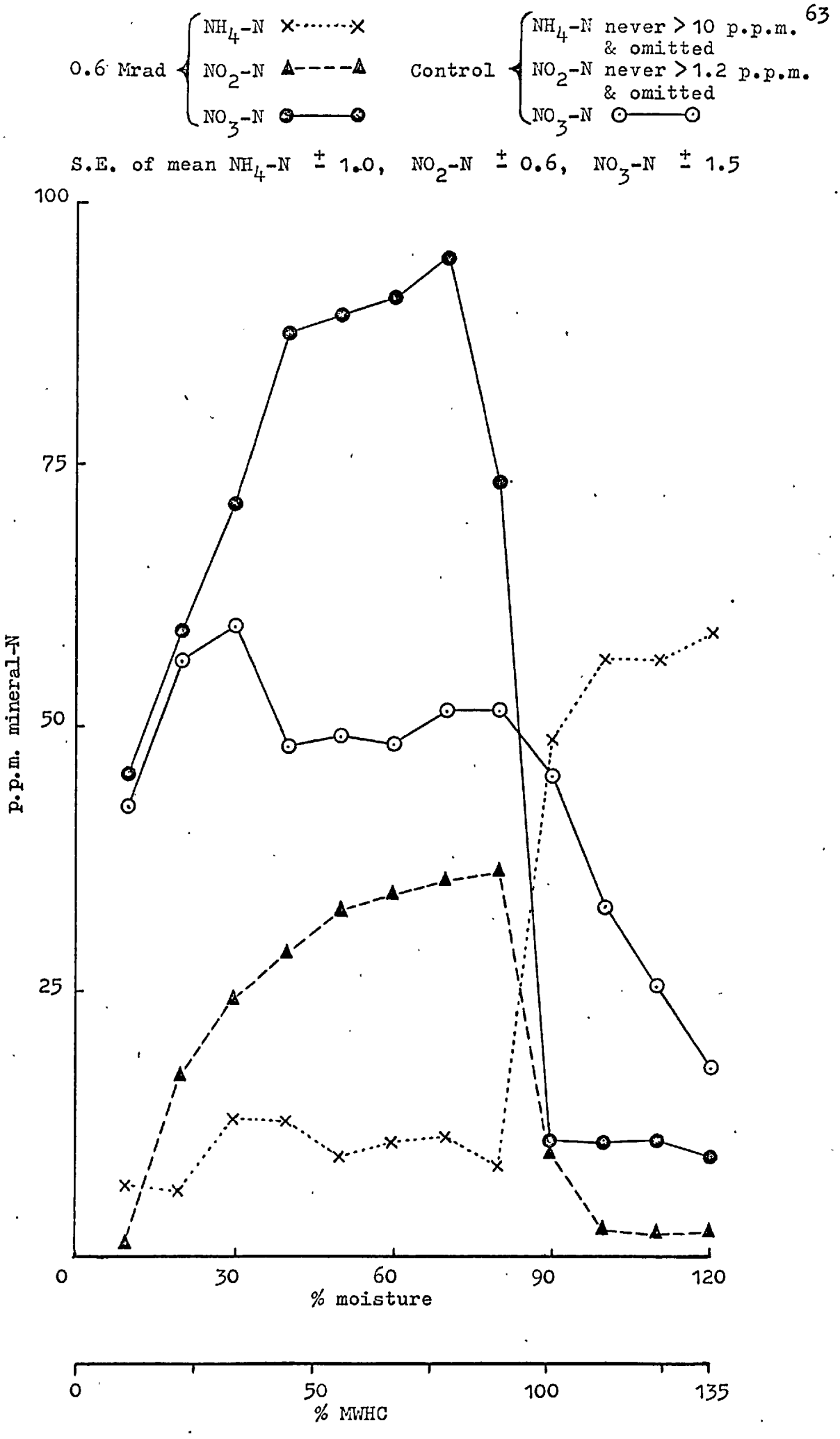


Figure 4-1.2 Effect of moisture on mineral-N accumulation in irradiated Icknield soil after 64 hr incubation

waterlogging; nitrite-N did not appear above 1.2 p.p.m. at any moisture level.

It can therefore be said that as well as stimulating nitrification under aerobic conditions, radiation increases the rate of nitrate reduction under anaerobiosis in relation to unirradiated control soil. The latter effect might result from radiation-released carbon compounds acting as highly available substrate for denitrifying organisms.

There have been reports of increases in ammonium in soil during denitrification (Bremner and Shaw, 1958a; Ekpete and Cornfield, 1966), but the origin has not been clearly established. It appears that the majority of investigators have been more concerned with measurement of oxides of nitrogen, and ammonium receives no mention (Allison et al. 1960; McGarity, 1961; Cooper and Smith, 1963; Mahendrappa and Smith, 1967; Jordan et al. 1967).

Firstly, the ammonium could arise from a block to nitrification under waterlogging (Waring and Bremner, 1964; Blasco and Cornfield, 1967), and this suggestion allows a convenient explanation of ammonium occurrence only in irradiated soil, because lysis and mineralisation of damaged organisms would be expected to be greater than the control. In both soils the increase in ammonium is less than the decrease in nitrite and nitrate, and no doubt nitrite is further reduced to nitrous oxide and nitrogen.

Secondly, a mechanism of dissimilatory nitrate reduction could be responsible for ammonium increases. Verhoeven (1956) reported production of ammonia by Bacillus spp. when grown under anaerobic conditions with nitrate as the sole nitrogen source, and the higher concentration of nitrate available as an electron acceptor in irradiated soil would intensify this mechanism.

Thirdly, it might be proposed that nitrate is assimilated by microorganisms and ammonia is subsequently released by death and mineralisation of organic-N; Overrein (1967) added $^{15}NO_3$ tracer to forest humus (50% MWHC) and reported such a pathway after 10 days' incubation at 20°C, but it is considered unlikely to occur during only 64 hr incubation of irradiated soil, and the control sample should have behaved in a similar manner.

Fourthly, ammonium can be produced from reduction of nitrate and nitrite by Azotobacter spp. during nitrogen fixation, as shown in Azotobacter vinelandii by Azim and Saraf (1956). Ammonia was formed in the culture medium at almost the same rate that nitrite was taken up by these bacteria; in addition, nitrite and then ammonia appeared as nitrate was removed. The presence of ammonia in waterlogged irradiated soil would, according to this behaviour, result from stimulation of Azotobacter by radiation-released carbon.

The present experiment makes it clear that extensive reduction of nitrate under anaerobic waterlogged conditions does not encourage accumulation of nitrite in irradiated soil, rather the reverse. The possibility that radiation blocks nitrite reduction and thus causes nitrite to accumulate under aerobic conditions is hardly likely, because there is definitely a very rapid loss of nitrite in irradiated soil at saturation. The speed of nitrate reduction observed is supported by the work of Turner and Patrick (1968) with waterlogged soils, where little nitrate remained after 60 hr incubation.

4-2 Temperature

4-2.1 Effect of temperatures up to 60°C

The reduction of nitrate to nitrite in irradiated soils was examined up to 60°C to see if inhibition would occur at temperatures known to exert a harmful effect on denitrifying bacteria and soil enzymes.

Materials and Methods:

Samples of Broad and Icknield soils were raised to 55% and 70% moisture respectively. After equilibration for 2 days, samples of 10 g were treated with 0.8 Mrad for Broad series and 0.6 Mrad for Icknield series. The soils were then incubated for 64 hr at 0, 10, 20, 30, 40, 50 and 60°C. Nitrite was analysed before irradiation, immediately after irradiation (within 5 minutes), and after the period of incubation.

Preliminary tests with irradiated samples from both soils gave very erratic nitrite analyses at temperatures of 30 and 40°C, and in some cases the readings differed by approximately 30 p.p.m. nitrite-N. This was a similar observation to that reported in subsequent work on freezing and thawing (Section 4-4), where the problem was eliminated by using containers which allowed air diffusion, and a build-up of carbon dioxide was suspected. It was found that use of these containers for the temperature work also improved the replication. The occurrence of nitrite in irradiated soil under a high concentration of carbon dioxide is reported later (Section 4-6).

Results:

The concentration of nitrite found in the irradiated soils is shown in Table 4-2.1. As the temperature was increased to 30 - 40°C

Table 4-2.1 Effect of temperature on nitrite accumulation in irradiated Broad and Icknield soils

Incubation temperature (°C)	p.p.m. nitrite-N after 64 hr incubation	
	Broad (0.8 Mrad)	Icknield (0.6 Mrad)
Immediately before irradiation	0.2	0.4
Immediately after irradiation	8.7	3.0
0	9.1	5.2
10	14.9	18.7
20	22.4	32.3
30	42.0	48.2
40	49.5	23.5
50	0.4	0.7
60	0.3	0.6

L.S.D. p 0.05 = 1.0, p 0.01 = 2.2

there was a definite rise in the amount of nitrite formed, but a further increase of 10°C resulted in a very sharp decrease in nitrite, almost to the trace levels present in normal soil.

The denitrification studies of Bremner and Shaw (1958b) have indicated the stimulatory effect of increasing temperature up to 25°C, but there was an abrupt cessation of nitrate loss at 70°C. Nommik (1956) found that the optimum temperature for denitrification was in the region of 65°C. Alexander (1961) states that mesophyllic bacteria, which are predominant in soil, have optima at 25 - 35°C and cannot grow above 45°C: therefore it appears that thermophyllic denitrifying organisms are responsible for the reports of nitrate loss at high temperature. In fact, enrichment cultures for thermophyllic bacteria incubated at 55 - 65°C frequently yield Bacillus spp. which are well recognised as important denitrifiers (Alexander, 1961).

The above information from the literature broadly lends itself to interpretation of the present results with irradiated soils if nitrate is being reduced to nitrite biologically. Thermophyllic bacteria are presumably absent, and mesophyllic organisms are being checked by heat, i.e. their proliferation is inhibited as well as their reducing enzyme component. With regard to soil enzymes in general, Porter (1965) reported that many are denatured at temperatures in excess of 60°C.

4-2.2 Effect of autoclaving

Materials and Methods:

Soils from Broad, Broadmoor and Icknield series, which had been found to form most nitrite after treatment of fifteen series with 0.75 Mrad, were selected to examine the effect of

autoclaving. Samples at 60% MWHC were autoclaved for 1 hr at 15 lbs / sq. in., and then exposed to 0.75 Mrad. Nitrite was analysed immediately after irradiation and after 7 days' incubation at 25°C; it was also determined immediately after autoclaving the soils, without further treatment with radiation.

Results:

The results in Table 4-2.2 show that small amounts of nitrite were produced by irradiating autoclaved soils (before incubation began), but autoclaving alone failed to produce such a rise. Analysis after 7 days proved that the increases which occur in irradiated soils, reaching a maximum nitrite concentration after 3 days of incubation (Table 4-2.2), had not taken place; it was therefore concluded that biological pathways of nitrate reduction had been eliminated. The apparent formation of nitrite, by 0.75 Mrad even after autoclaving, suggested that non-biological pathways may be reducing nitrate during irradiation, and this feature was borne in mind for detailed investigation at a later stage (Section 10).

Table 4-2.2 Effect of autoclaving on nitrite increases in soils treated with 0.75 Mrad

Soil series	p.p.m. nitrite-N				
	Control	3 days after 0.75 Mrad	immediately after autoclaving	immediately after autoclaving + 0.75 Mrad	7 days after autoclaving + 0.75 Mrad.
Broad	0.6	41.6	0.4	9.6	9.2
Broadmoor	0.5	53.2	0.7	10.0	8.7
Icknield	0.8	44.6	0.4	7.1	6.0
L.S.D. p 0.05 = 0.6, p 0.01 = 1.5					

4-3 Aggregate size

The size of soil aggregates has often been studied in relation to nitrification and nitrate reduction processes, and some positive effects have been reported. In view of these findings, it was thought that the accumulation of nitrite in irradiated aggregates could well be influenced by their diameter, and experiments were designed to investigate this point.

Materials and Methods:

Broad and Icknield soils were taken at 35% and 51% moisture respectively, and then sieved to obtain aggregates of <0.5, 0.5 - 1, 1 - 2, 2 - 3 and 3 - 4 mm. 10 g samples of each size were adjusted to 55% and 70% moisture for each soil series, and allowed to equilibrate for 2 days before irradiation. Broad series was treated with 0.8 Mrad and the Icknield type with 0.6 Mrad, and after 64 hr of incubation at 25°C the accumulated nitrite was analysed.

Results:

Table 4-3 shows the results of all nitrite analyses. At 55% moisture, there were slightly significant variations from the nitrite formed in aggregates <0.5 mm diameter as aggregate size increased. However, a more definite pattern was evident at 70% moisture, where there was a significant decrease in the amount of nitrite detected in both soils as the aggregate diameter increased to 3 mm. This was followed by an increase in nitrite again in the 3 - 4 mm range, almost certainly by reduction of nitrate.

These findings are in close agreement with the work of Nommik (1956), who measured the evolution of nitrous oxide and nitrogen in water-saturated aggregates which varied in size from <0.3 - >4 mm. He found that the denitrification rate fell as

Table 4-3 Effect of aggregate size on nitrite accumulation in irradiated Broad and Icknield soils

Diameter of aggregates (mm)	p.p.m. nitrite-N after 64 hr incubation			
	Broad (0.8 Mrad)		Icknield (0.6 Mrad)	
	55% H_2O	70% H_2O	55% H_2O	70% H_2O
<0.5	26.0	40.9	25.2	42.9
0.5 - 1	28.8	41.3	23.6	42.9
1 - 2	29.3	34.0	22.5	37.5
2 - 3	29.5	35.3	21.6	34.8
3 - 4	32.5	43.6	26.9	41.5
L.S.D. p 0.05 = 2.9, p 0.01 = 6.8				

aggregate size increased to the 2 - 4 mm group, but then it showed a sharp rise in the >4 mm group. To explain this behaviour, Nommik (1956) proposed that air pores are so small in the poorly aggregated fraction that they become filled with water and interrupt oxygen diffusion, whereas in a well aggregated soil at the same moisture there are larger pores and thinner water films which do not impede oxygen diffusion so greatly. The sudden increase in denitrification in very large aggregates was thought to result from the distance that oxygen must travel to the centre.

Currie (1961) has presented a detailed paper on the kinetics of gaseous diffusion in aggregated soils, and it was suggested that an aggregate has a critical radius at which it is just wholly aerobic, and any further increase in size would lead to the existence of anaerobic zones.

Greenwood (1962) has shown that very low oxygen concentrations, in the region of 5×10^{-6} M, are needed to encourage rapid nitrate dissimilation. However, even an oxygen concentration of $2 - 5 \times 10^{-6}$ M could still support nitrification at 50% of its normal level, probably because the cytochromes present in Nitrosomonas and Nitrobacter have such a great affinity for oxygen. Greenwood and Goodman (1964) later reported that the respiration rate of a soil suspension determined in the presence of excess oxygen was halved when oxygen concentration was reduced to $2 - 6 \times 10^{-6}$ M in the aqueous phase.

In a discussion of the oxygen supply to soil microorganisms, Greenwood (1968) gave a diffusion equation to define the volume of the spherical aggregate where anaerobic processes occur. Oxygen must reach the organism by diffusion through gas and liquid phases, and evidence to support the equation was obtained by measurement of

oxygen concentration at different depths within spherical aggregates (Greenwood, 1967). For a Wellesbourne soil which is water-saturated, the equation indicates that any zone greater than 2 mm from a gas / water interface would be anaerobic (Greenwood, 1968).

The effect of a wide range of soil moisture on nitrate concentration has been studied by Ekpete and Cornfield (1966), using aggregates <2 mm diameter. When moisture exceeded 50% of MWHC the nitrate concentration decreased rapidly, denitrification being mainly responsible. Reference to Table 4-3 of the present work does show that there was stimulation of nitrite formation in both soils when their moisture was increased from 55% to 70%, whatever the aggregate size. This is in good agreement with the above experiments on nitrate loss in unirradiated soils.

4-4 Age of sample, and storage by freezing and drying

4-4.1 Age of sample

Broad soil taken from Reading University Farm at Sonning in January 1967 was placed in a small 1 m^2 X 15 cm deep plot at Wantage, and kept permanently under fallow conditions. It was noticed that the rise in nitrite after treatment with 0.8 Mrad was considerably less after 7½ months of fallowing, but showed some recovery after a further 3½ months had elapsed. The nitrate present before irradiation was determined in case it had limited nitrite formation:

Irradiation date	Fallowing period	NO_3-N pre γ	NO_2-N 64 hr after 0.8 Mrad
29/3/67	2 months	41.2	29.9
12/9/67	7½ months	166.5	4.5
2/1/68	11 months	39.0	16.8

When a fresh sample was obtained from the field in the same month (Sept. 1967), the accumulation of nitrite on irradiation was high, and approached the level observed in the first sample (March, 1967). The nitrogen mineralisation rates for "high" and "low" nitrite soils did not differ greatly, and showed 14.7 and 11.5 p.p.m. mineral-N increase respectively in 7 days.

Several possibilities might account for the detection of only small amounts of nitrite. For example, there could have been differences in nitrite accumulation in relation to dose or incubation time, causing a shift in the "peak" previously found at 64 hr after 0.8 Mrad; other factors could be an increase in numbers of

nitrifying bacteria leading to the presence of more survivors after irradiation (capable of oxidising nitrite), or a decrease in the initial population of nitrate-reducing organisms. Some experiments were undertaken to clarify these aspects.

4-4.1.1 Nitrite accumulation in relation to dose and incubation time

Materials and Methods:

Samples of 10 g Broad soil which had been fallowed for 11 months were treated with six doses in the range 0.25 - 1.5 Mrad after adjustment of the moisture level to 55%. The irradiated soil was incubated for various times ranging from 0.5 - 168 hr at 25°C, and the patterns of nitrite accumulation were compared with earlier data obtained from soil fallowed for not more than 2 months (Cawse, 1967b).

Results:

The differences in nitrite accumulation between samples are compared in Figures 4-4.1.1 (dose relationship) and 4-4.1.2 (time relationship); nitrite concentrations at doses and times of incubation other than those shown in the Figures were lower, and this data is not presented. The observation that both short-fallowed and long-fallowed soils gave good agreement in their peak of maximum nitrite production in relation to dose (0.8 Mrad) and time of incubation (64 hr), if not in the maximum concentration reached, proved that there was no alteration in the influence of these factors which could be held responsible for the decreased formation of nitrite in one of them.

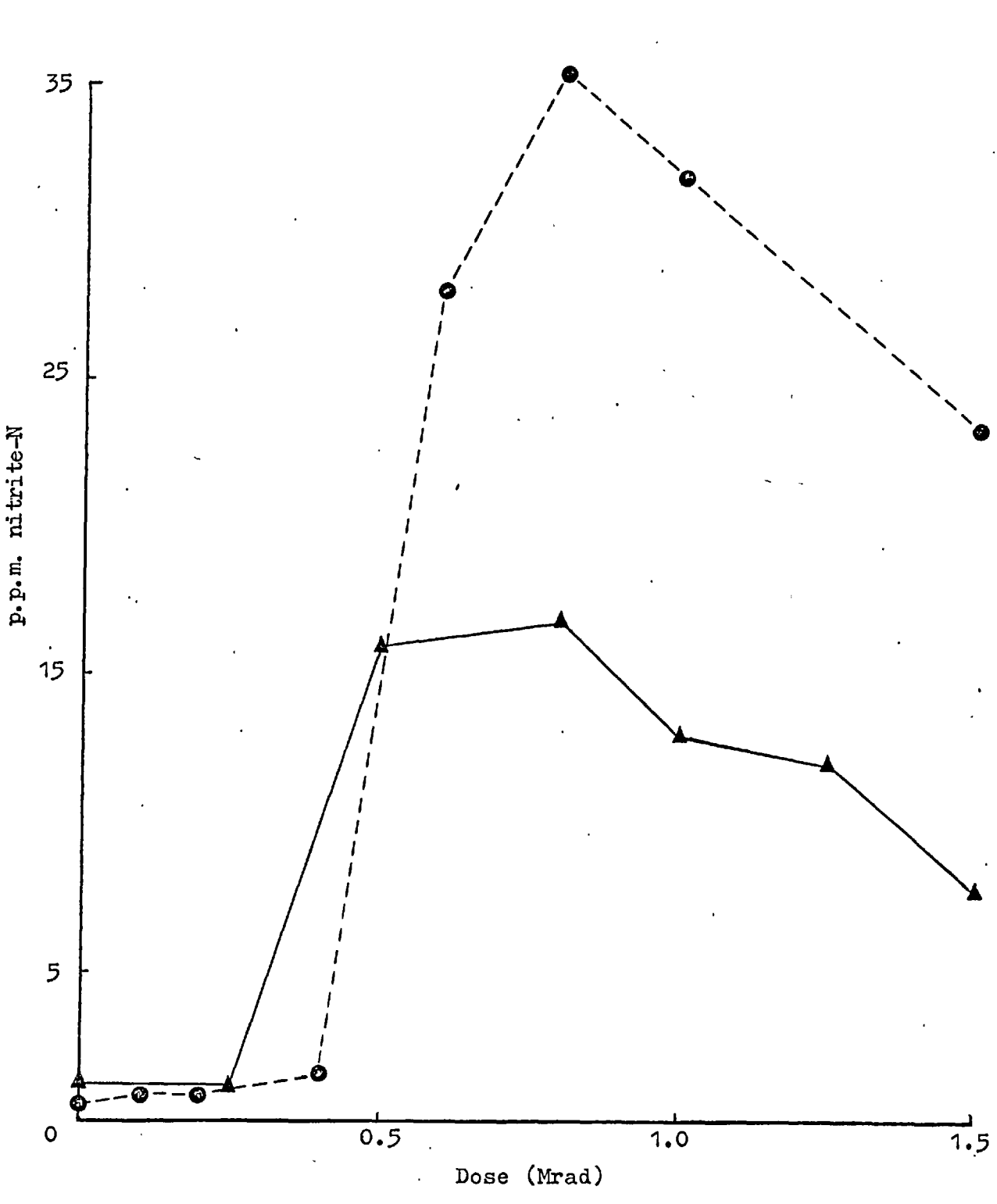


Figure 4-4.1.1 Nitrite accumulation at 64 hours after irradiation of different samples of Broad soil

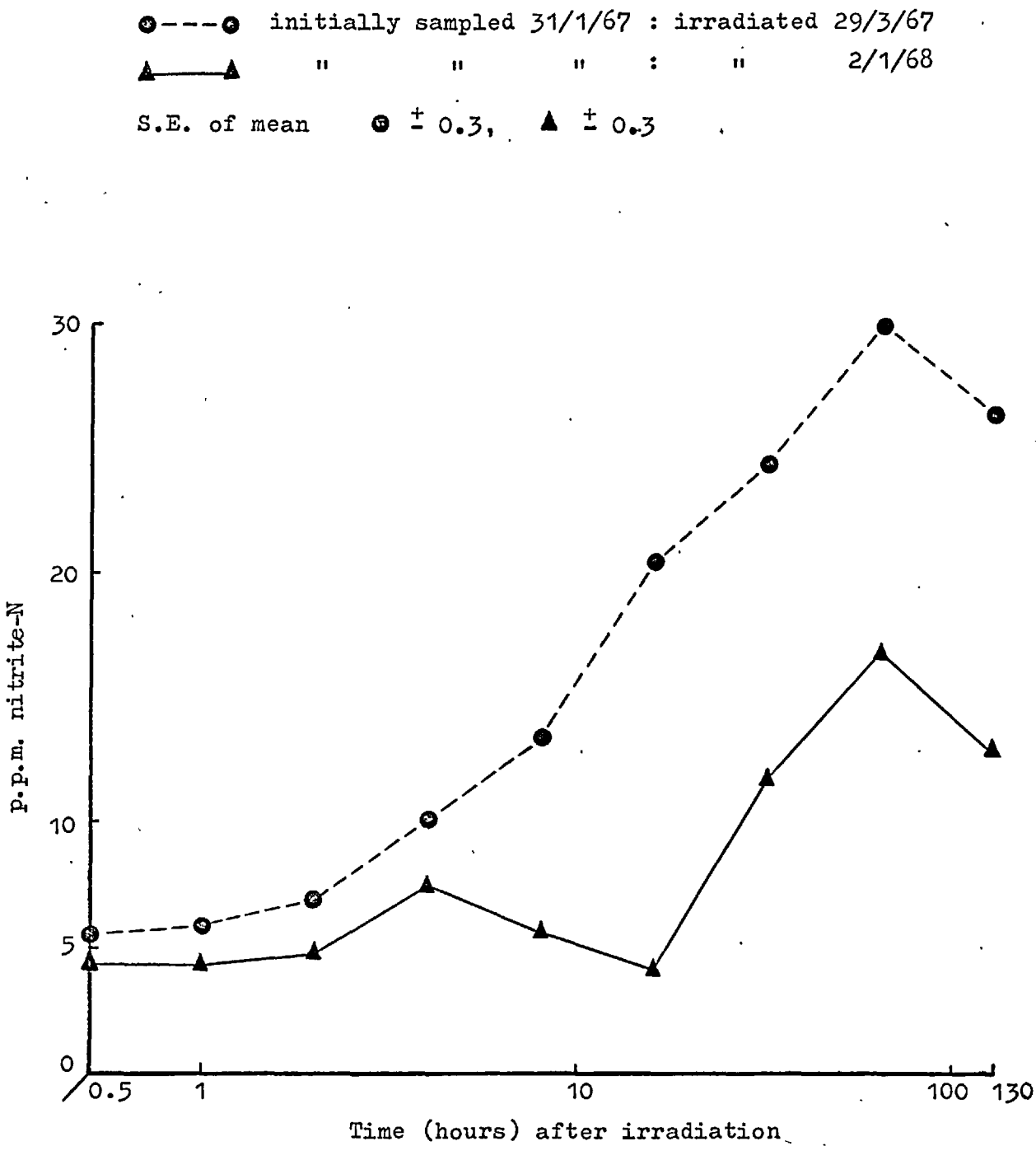


Figure 4-4.1.2 Nitrite accumulation in different samples of Broad soil according to incubation time after treatment with 0.8 Mrad

4-4.1.2 Nitrite accumulation in relation to fallowing and cropping

The plot of Broad soil originally sampled from Sonning on 31/1/67 was divided and subjected to either continued fallowing or cropping for 2 years. The object was to compare nitrite increases when soil was irradiated after cropping with that in the fallowed sample, because the presence of plant roots may encourage a microflora capable of reducing nitrate to nitrite. Such behaviour could explain the variation in nitrite level found in Broad soil irradiated after different periods of fallowing.

The positive influence of the rhizosphere on denitrification has been established (Woldendorp, 1962), and oxygen tension near to and on the root surface was much reduced by root uptake and rhizosphere organisms. When Bacillus licheniformis was added to sterile cultures of pea plants, large amounts of nitrite appeared (Woldendorp, 1963). In addition, Rovira and McDougall (1967) have discussed several reports concerning high populations of denitrifying organisms in the rhizosphere.

Materials and Methods:

For the cropped treatment, barley variety "Maris Badger" was sown in late March and cut in late August for 2 years, 1968 and 1969, leaving the roots in the soil. Broad soil had supported continuous barley since 1960, and it was inadvisable to alter the crop for the present type of study in view of Woldendorp's (1963) finding that greater loss of nitrogen occurred under peas than ryegrass.

Samples were taken from cropped and fallowed soils at 1 month after harvesting, and water was added to obtain 55% moisture. Irradiations (0.8 Mrad) were made in triplicate, followed by

incubation for 64 hr to investigate the nitrite formed, and for 7 days to assess the extent of nitrification. Nitrite and nitrate were also analysed before irradiation, and after incubation of unirradiated samples. Other samples were taken immediately before sowing and examined for nitrite formation after irradiation, under the same conditions as those sampled in the Autumn.

It was desirable to know the effect of cropping on nitrate increases after irradiation as well as nitrite behaviour, because nitrification can sometimes be suppressed by plants (Theron, 1951).

Results:

The results of nitrite and nitrate determinations are given in Table 4-4.1.2. Both fallowing and cropping for 6 months or more caused extra nitrite to be formed after irradiation compared with the figure of 11.3 p.p.m. nitrite-N obtained on 20/3/68, but cropped soil still gave more nitrite than the fallowed. To summarise the response given by the fallowed soil over 2½ years:

Irradiation date	p.p.m. nitrite-N 64 hr after 0.8 Mrad
29/3/67	29.9
12/9/67	4.5
2/1/68	16.8
20/3/68 pre-sowing	11.3
1/10/68	19.5
26/3/69 pre-sowing	17.1
1/10/69	25.9
31/3/70	26.5

The above differences almost certainly find explanation in variation of microbial numbers. The most interesting observation

Table 4-4.1.2 Effect of fallowing and cropping over 2 years on nitrite formation in irradiated Broad soil

Treatment	p.p.m. nitrate-N before irradn.	p.p.m. nitrate-N 7 days after irradn. control	p.p.m. nitrate-N 7 days after irradn. 0.8 Mrad	p.p.m. nitrite-N 64 hr after irradn. control	p.p.m. nitrite-N 64 hr after irradn. 0.8 Mrad
1968					
fallowed	37.7	47.1 (+9.4)	96.2 (+58.5)	0.4	19.5
cropped	28.0	32.3 (+4.3)	85.7 (+57.7)	0.4	26.1
1969					
fallowed	33.6	38.5 (+4.9)	83.5 (+49.9)	0.4	25.9
cropped	20.6	26.8 (+6.2)	75.0 (+54.4)	0.4	30.8

L.S.D. nitrate-N p 0.05 = 3.1, p 0.01 = 7.2

L.S.D. nitrite-N p 0.001 = 3.9

was that the nitrite which accumulated in the cropped soil in 1968 (Table 4-4.1.2) fell little short of the original response of 29.9 p.p.m. on 29/3/67 and was virtually identical to it in 1969, implying restoration of the microflora to the original numbers, or balance.

The increase in nitrate over the amount present immediately before irradiation (shown in parentheses) was similar in fallowed and cropped samples, although the latter possessed a lower nitrate content, no doubt owing to plant uptake. In an attempt to relate differences in nitrite accumulation after irradiation to microbial populations in soil, it was decided to examine the effects of prolonged freezing and storage of soil in an air-dry condition.

4-4.2 Storage of frozen soil

Storage of soils by freezing has been investigated by several workers in relation to nitrogen mineralisation rates on thawing, and in most experiments, significant changes in mineralisation have been reported, although Gasser (1958) stored fresh soil at -10°C for 32 days without effect on the ammonium- and nitrate-N produced on subsequent incubation. Allen and Grimshaw (1962) found that fresh soils kept at -15°C showed increases in ammonium immediately on thawing, but nitrate remained fairly constant. Munro and MacKay (1964) kept a fine sandy loam in moist condition at -20°C and -5°C up to 15 weeks; subsequent incubation revealed constant nitrate production with increasing storage time, but -20°C storage caused 2 - 3 p.p.m. more nitrate-N production (approximately 10%). Microbiological studies by Grossbard and Hall (1964) on the effect of storing soils up to 60 weeks at -15°C demonstrated considerable differences in microbial numbers upon thawing; while the numbers of actinomycetes and bacteria tended to fall with increased storage time, a similar response was not detected for fungi.

McGarity (1962) studied the effects of freezing on denitrification down to -78°C; he concluded that the increase in nitrate reduction rate on subsequent thawing and incubation indicates that the organisms are more able to use oxidisable substrate. This ability could arise from the removal of competition from other organisms, or an increase in availability of organic matter owing to lysis of the damaged biomass.

Materials and Methods:

An experiment was undertaken to examine the effects of freezing Broad soil, at a moisture content of 39.2%, on the nitrite

rise in 64 hr of incubation at 25°C after 0.8 Mrad. On 26/10/67, samples of 10 g were placed in 25 ml McCartney bottles for freezing at -15°C, and every month for a year, two bottles were removed for thawing (1 hr at 20°C), irradiation and incubation for 64 hr. At the beginning of the experiment the soil contained 92.9 p.p.m. nitrate-N.

Results:

The amounts of nitrite that formed are shown in Table 4-4.2. After 6 months of freezing, a sudden fall in nitrite accumulation was noticed, and appeared again on the subsequent month. It was thought that carbon dioxide may have increased to such a high concentration on thawing, as observed by Soulides and Allison (1961), that it induced the reduction of nitrite by denitrifying bacteria.

When four 1 mm diameter holes were drilled in the caps of the McCartney bottles to allow gaseous diffusion after thawing, and incubation was carried out under these conditions, it was found that nitrite formed in the usual high concentration of 28.2 p.p.m. compared with only 3.4 p.p.m. nitrite-N in the sealed tubes.

It was concluded that provided frozen samples were exposed to the atmosphere after thawing, then freezing for 12 months would not inhibit the formation of nitrite after irradiation, and it appeared that reducing enzymes remained undamaged. In fact, a slightly significant increase in nitrite was indicated during the intermediate months.

4-4.3 Storage of air-dry soil

Storage of air-dried Broad soil over 12 months at room temperature (starting date 29/5/68) was compared with a second sample

Table 4-4.2 Effect of freezing on nitrite formation in thawed and irradiated (0.8 Mrad) Broad soil

Storage time (months) at -15°C	p.p.m. nitrite-N after 64 hr incubation	
	tubes air-tight	tubes not air-tight
0	21.0	
1	26.7*	
2	29.4*	
3	28.6*	
4	26.7*	
5	16.0*	
6	8.9**	
7	3.4**	28.2*
8		25.8*
9		29.3*
10		25.8*
11		23.9
12		21.2

L.S.D. p 0.05* = 4.3, p 0.01** = 9.9, excluding results for 6 and 7 months storage in air-tight tubes.

kept at 30% moisture, nitrite being measured after the same dose and incubation time as in the freezing experiment. It was reasonable to expect that there would be some differences in accumulation of nitrite from the work of several authors. For example, Soulides and Allison (1961) found that drying was more destructive to bacteria than freezing, and resulted in increased nitrification on re-moistening. When the air-dried soil in the present experiment was re-wetted before irradiation, a release of carbon dioxide would probably occur, and air-tight containers were avoided: according to Birch (1958), the burst of respiration is caused by surviving cells which enter a log phase with a high metabolic rate.

Materials and Methods:

Samples of 10 g air-dried soil (6.5% moisture) and soil at 30% moisture were raised to 55% moisture 4 hr before irradiation; they were treated with 0.8 Mrad, in tubes with perforated caps, and nitrite was determined after 64 hr of incubation at 25°C.

Results:

Table 4-4.3 shows that both storage conditions resulted in a significant change in accumulation of nitrite, which is more marked than that obtained by freezing. Air-drying for 12 months produced a gradual decline in nitrite formed, an opposite response to the soil stored in moist condition. These effects are probably best explained by an inhibitory, slightly destructive and stimulatory action of soil moisture content during storage on microorganisms which contain enzyme systems capable of reducing nitrate. It is not possible to make a direct comparison of the absolute nitrite concentrations in freezing and air-drying experiments because they were started at different times, requiring separate samples from the field.

Table 4-4.3 Effect of storage at different moisture on nitrite accumulation in irradiated (0.8 Mrad) Broad soil

Storage time at room temperature (months)	p.p.m. nitrite-N after 64 hr incubation	
	stored air-dry†	stored at 30% moisture†
0	34.3	34.2
1	30.5*	32.3
2	31.0*	36.4
3	31.7*	34.5
4	30.6*	41.2*
5	28.6*	39.9*
6	24.3**	41.8*
7	24.4**	40.2*
8	23.6**	43.8**
9	24.4**	45.8**
10	20.3**	44.9**
11	19.1**	44.4**
12	16.5**	45.1**
L.S.D. p 0.05* =	3.3	3.7
p 0.01** =	7.6	8.6

† all samples adjusted to 55% moisture before irradiation

There are some interesting reports in the literature dealing with lethal effects of air-drying on the biomass. Stevenson (1956) found that air-drying four soils for only 48 hr at room temperature reduced bacterial numbers to 26 - 73% of the initial population. Keeney and Bremner (1968) used air-dried soils stored up to 48 weeks for assessment of various soil nitrogen availability tests; there were marked effects of storage on the total mineral nitrogen produced over an incubation period of 14 days under aerobic conditions at 30°C, and average mineralisable nitrogen values could be up to $3\frac{1}{2}$ times greater than moist soil. The major contributory factor is almost certainly rapid mineralisation of the damaged microflora, and this contention is supported by the work of Jenkinson (1966) on partial sterilisation of soil in which ^{14}C-labelled ryegrass had been allowed to decompose. Air-drying and incubation gave an increase in the percentage of $^{14}CO_2$, and it was concluded that the heavily labelled soil biomass was decomposed to a greater extent than the non-biomass fraction of the organic matter. In addition, Harada and Hayashi (1968) reported that nitrogen recently immobilised by microorganisms was particularly affected by air-drying (mainly amino acid and amino sugar nitrogen), and was more decomposable than other soil organic nitrogen fractions on re-wetting.

4-5 Presence of soluble carbon

In the first instance, the effect of soluble carbon on formation of nitrite in Broad soil was studied under conditions of medium moisture, because radiation-released carbon compounds may encourage the accumulation of nitrite by their availability as an energy source for microorganisms capable of reducing nitrate. Secondly, it had been established that nitrite did not accumulate (over 64 hr) if Broad soil was irradiated above 110% MWHC (Section 4-1), and it was thought that the presence of carbon compounds had encouraged very rapid reduction of both nitrate and nitrite by the surviving organisms under anaerobic conditions. Initially, it was decided to investigate the effect of adding carbon to soil under aerobic conditions, before introducing radiation to the system, and the behaviour under waterlogging was examined later.

4-5.1 Addition of glucose-carbon under aerobic conditions

Materials and Methods:

Broad soil was taken at 38.3% moisture, and found to contain 104.1 p.p.m. nitrate-N; samples of 20 g fresh weight were placed in 40 ml polypropylene tubes together with sufficient carbon and nitrate-N to obtain a final C : N ratio of 3 : 1, which Bremner and Shaw (1958a) found to be optimal for nitrogen loss in some waterlogged soils. Carbon was added from a glucose solution containing 15 mg C / ml (3.75 g glucose / 100 ml), and nitrate from a solution containing 3 mg nitrate-N / ml (2.1666 g potassium nitrate / 100 ml). Finally, water was added to adjust the moisture content to 70%. Two levels of nitrate were investigated by addition of 200 and 400 p.p.m. of nitrate-N to the amount already present, so that there was an ample

concentration available for reduction. Samples were incubated in the stoppered tubes (not air-tight) at 25°C, and analysis for nitrite and nitrate was made after 1, 2 and 3 days of incubation.

Results:

The results presented in Table 4-5.1 show that nitrite failed to accumulate above 0.7 p.p.m. nitrite-N, and although some nitrate disappeared during the first 24 hr of incubation, the loss was not sustained; however, this was to be expected under the aerobic conditions of the experiment. Quite clearly, the presence of highly available carbon does not cause nitrite to appear in Broad soil under aerobic conditions, without radiation treatment.

4-5.2 Addition of glucose-carbon to irradiated soil under aerobic and anaerobic conditions

Secondly, the effect of carbon addition on accumulation of nitrite in irradiated soil was investigated. It was necessary to add the glucose after irradiation in view of reports on the toxicity of irradiated glucose. In particular, Molin and Ehrenberg (1964) stated that irradiated 1% glucose solution has a strong antibacterial action on Pseudomonas, but at present there is no general agreement on the radiolysis product/s responsible for this effect (see Section 1-3.2). Similar observations were made some time ago on the inhibitory effects of heat-sterilised glucose solutions applied to cultures of Cytophaga spp., which are responsible for anaerobic decomposition of cellulose (Stanier, 1942), and also to mixed cultures of nitrifying bacteria (Meiklejohn, 1951).

Materials and Methods:

Samples were taken according to the previous experiment, and

Table 4-5.1 Effect of glucose carbon (C : NO_3-N ratio of 3 : 1) on nitrite accumulation in Broad soil under aerobic conditions

p.p.m. nitrate-N initially present	p.p.m. nitrate-N present after:			p.p.m. nitrite-N present after:		
	1 day	2 days	3 days	1 day	2 days	3 days
311.3	280.0	291.5	295.5	0.6	0.3	0.2
518.4	451.9	452.2	442.9	0.7	0.3	0.3
	L.S.D. p 0.05 = 25.9			L.S.D. p 0.05 = 0.3		

Table 4-5.2 Effect of glucose carbon (C : NO_3-N ratio of 3 : 1) on nitrite accumulation in irradiated Broad soil under aerobic and anaerobic conditions

Treatment	mineral-N present after 64 hr incubation*			
	70% moisture		120% moisture	
	nitrate	nitrite	nitrate	nitrite
0.8 Mrad	171.2	31.2	12.3	2.9
0.8 Mrad + carbon	138.8	11.5	8.8	2.6
L.S.D. nitrate-N p 0.05 = 10.1				
L.S.D. nitrite-N p 0.05 = 2.6				

* initially present: 120.9 p.p.m. nitrate-N, 0.5 p.p.m. nitrite-N

after treatment with 0.8 Mrad at 60% moisture, sufficient glucose was added to achieve a C : NO_3-N ratio of 3 : 1 with the nitrate naturally present, and moisture levels were raised to 70% and 120% (73% and 124% MWHC). Incubation was carried out for 64 hr at 25°C, a period known to give maximum accumulation of nitrite in irradiated soil without carbon addition (Cawse and Crawford, 1967), then nitrite and nitrate were determined.

Results:

Table 4-5.2 shows that under aerobic conditions, irradiation gave the expected increase in nitrate over the amount initially present (in agreement with the moisture experiments in Section 4-1); addition of available carbon checked this increase, most probably by stimulation of organisms that carry out nitrate reduction. Complete denitrification of nitrate is suggested rather than just reduction to nitrite, because the nitrite level in irradiated soil was lowered by 65% in the presence of added carbon. This implies that soils possessing a large amount of soluble carbon following irradiation under aerobic conditions are likely to show more rapid reduction of nitrite that is formed, and possibly no nitrite would remain after incubation for 64 hr.

Under anaerobic conditions, denitrification was virtually complete in both treatments, and the suggestion that a radiation block to the mechanism of nitrite removal is responsible for its accumulation under aerobic soil conditions seems very unlikely, in agreement with the conclusion from the moisture studies (Section 4-1). Further experiments were undertaken under anaerobic conditions to examine the extent of nitrite formation and denitrification in irradiated and non-irradiated soil which had levels of carbon known

to be optimal and sub-optimal for denitrification. For a start, three C : NO_3-N ratios were tested on waterlogged soil (without irradiation) to prove if the 3 : 1 optimal ratio for denitrification found by Bremner and Shaw (1958a) also applied to Broad soil.

4-5.3 Effect of different glucose-C : nitrate-N ratios on nitrate reduction under anaerobic conditions

Materials and Methods:

400 p.p.m. of nitrate-N was added to 20 g samples of moist soil, together with sufficient glucose to obtain C : NO_3-N ratios of 3, 6 and 10 : 1, and the moisture content was finally adjusted to 120%. Analysis of nitrite and nitrate was made after 1, 2 and 3 days of incubation.

Results:

The results presented in Table 4-5.3 indicate that a 3 : 1 ratio was not optimal for nitrate reduction, but all levels of carbon caused nitrite to accumulate. It is interesting to note that Bremner and Shaw (1958a) also found about 150 p.p.m. nitrite-N 2 days after addition of glucose to waterlogged soils, whereas experiments under waterlogging but without added carbon have not induced nitrite formation (Ekpete and Cornfield, 1965; Mahendrappa and Smith, 1967). No doubt this is because nitrite removal is the rate-limiting step in denitrification in alkaline soils, as suggested by Cooper and Smith (1963), and the rate of nitrate reduction to nitrite is so much faster when soluble carbon and nitrate are abundant that it exceeds the rate of nitrite removal. In agreement with this scheme, radiation alone (without glucose or nitrate being added) fails to produce extensive nitrite formation in waterlogged soil (see moisture experiment in

Section 4-1, and experiment 4-5.2) because removal of nitrite is not rendered rate-limiting.

Nitrite accumulated to almost 100 p.p.m. after incubation for 2 days at a C : N ratio of 3 : 1, therefore there seems little chance that chemical decomposition limits the concentration; Jones (1951) did not find any reaction between ammonium and nitrite in waterlogged soil at pH 4 - 6, and although Wahzab and Uddin (1954) reported significant losses from interaction at alkaline pH, this only occurred in desiccated soils.

It was thought that if radiation was applied to a waterlogged soil system, where nitrite was already being formed after addition of carbon and nitrate, then the presence of extra radiation-released carbon may perhaps stimulate nitrate reduction to nitrite even more. Experiments were made at a C : NO_3-N ratio of 3 : 1 because while it encourages the formation of nitrite, it does not provide optimal carbon for denitrification, and any stimulatory effect from the additional radiation-released carbon compounds is more likely to be observed than at higher C : N ratios.

Table 4-5.3 Effect of C : N ratio on denitrification in waterlogged Broad soil

glucose-C : nitrate-N ratio	p.p.m. nitrate-N present* after:			p.p.m. nitrite-N present* after:		
	1 day	2 days	3 days	1 day	2 days	3 days
3 : 1	363.4	140.3	50.6	49.4	98.0	15.1
6 : 1	358.4	63.1	7.1	49.6	65.0	2.0
10 : 1	353.0	45.4	11.9	52.0	65.8	2.3
	L.S.D. p 0.05 = 16.6			L.S.D. p 0.05 = 10.1		

* initially present: 518.5 p.p.m. nitrate-N, 0.9 p.p.m. nitrite-N

4-5.4 Effects of glucose-carbon and radiation on nitrite accumulation at a C : NO_3-N ratio of 3 : 1, under anaerobic conditions

Materials and Methods:

400 p.p.m. of nitrate-N was added to 20 g samples of Broad soil which were then waterlogged, and the following treatments applied:

(i) untreated control

(ii) 0.8 Mrad

(iii) glucose added to obtain a C : NO_3-N ratio of 3 : 1

(iv) 0.8 Mrad + treatment (iii) after irradiation

All treatments were incubated at 120% moisture, and analysis of nitrite and nitrate was made after 1, 2, 3 and 4 days.

Results:

The results are presented in Figures 4-5.4.1 (nitrate) and 4-5.4.2 (nitrite). Comparison of treatments (i) and (ii) shows that radiation alone could encourage nitrate reduction and increase nitrite without addition of carbon; stimulation from radiation-released carbon compounds was strongly suggested, and comparison of treatments (ii) and (iv) shows that the effect could be intensified by addition of carbon in the form of glucose to irradiated soil.

Comparing treatments (iii) and (iv), where carbon was added to both, radiation slowed down the loss of nitrate and nitrite in relation to incubation time and indicates that carbon (radiation-released as well as added) was optimal for nitrate reduction. This situation can be compared with the response of nitrification to gamma treatment (Cawse, 1968): when carbon (or ammonium) is abundant, the depressing effect of radiation on microbial processes is observed, but when carbon (or ammonium) is in short supply the stimulatory effect of radiation-released compounds becomes evident. The rapidity

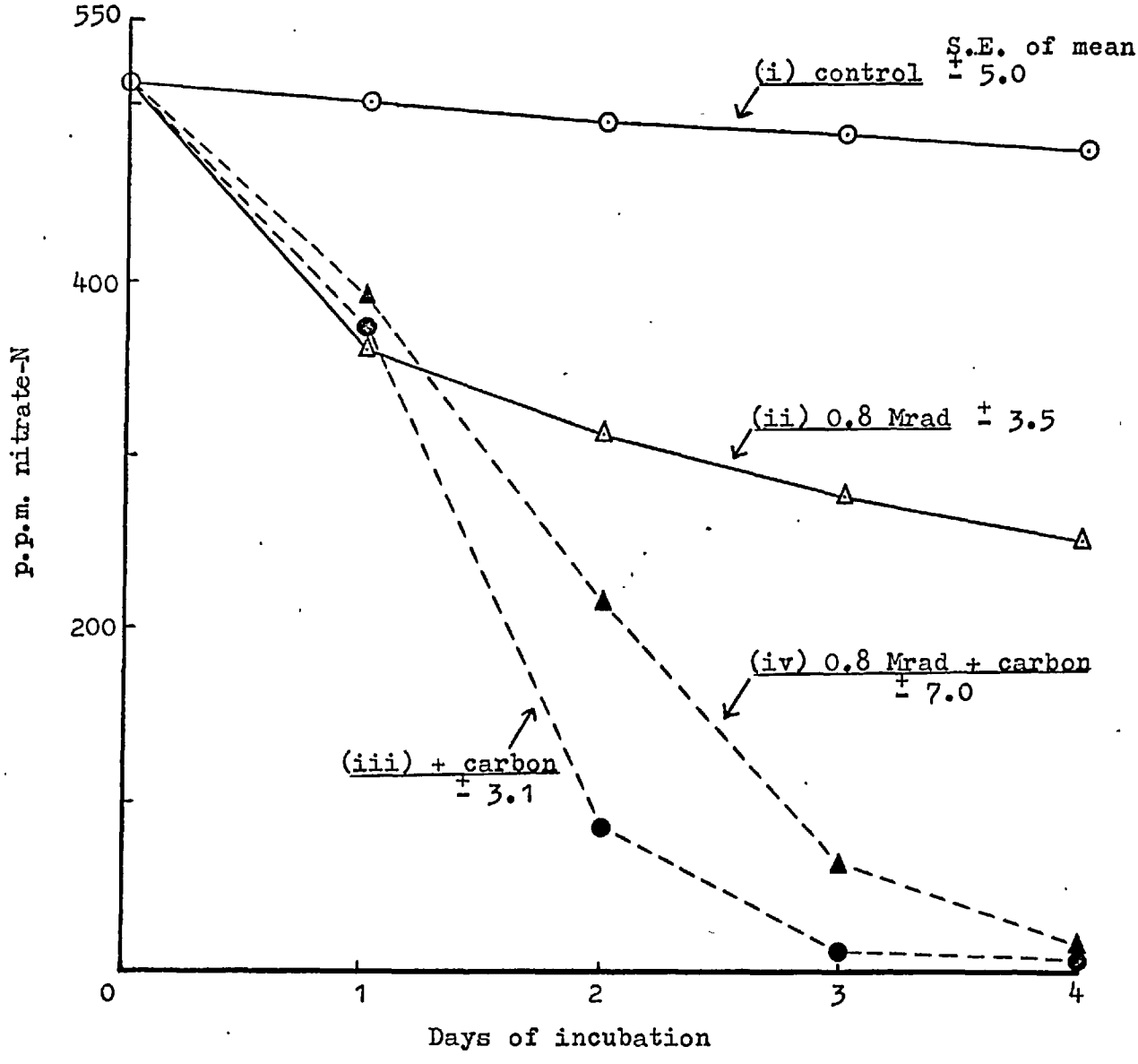


Figure 4-5.4.1 Effects of glucose-carbon and radiation on nitrate reduction at 3:1 C:NO_3-N ratio under anaerobic conditions

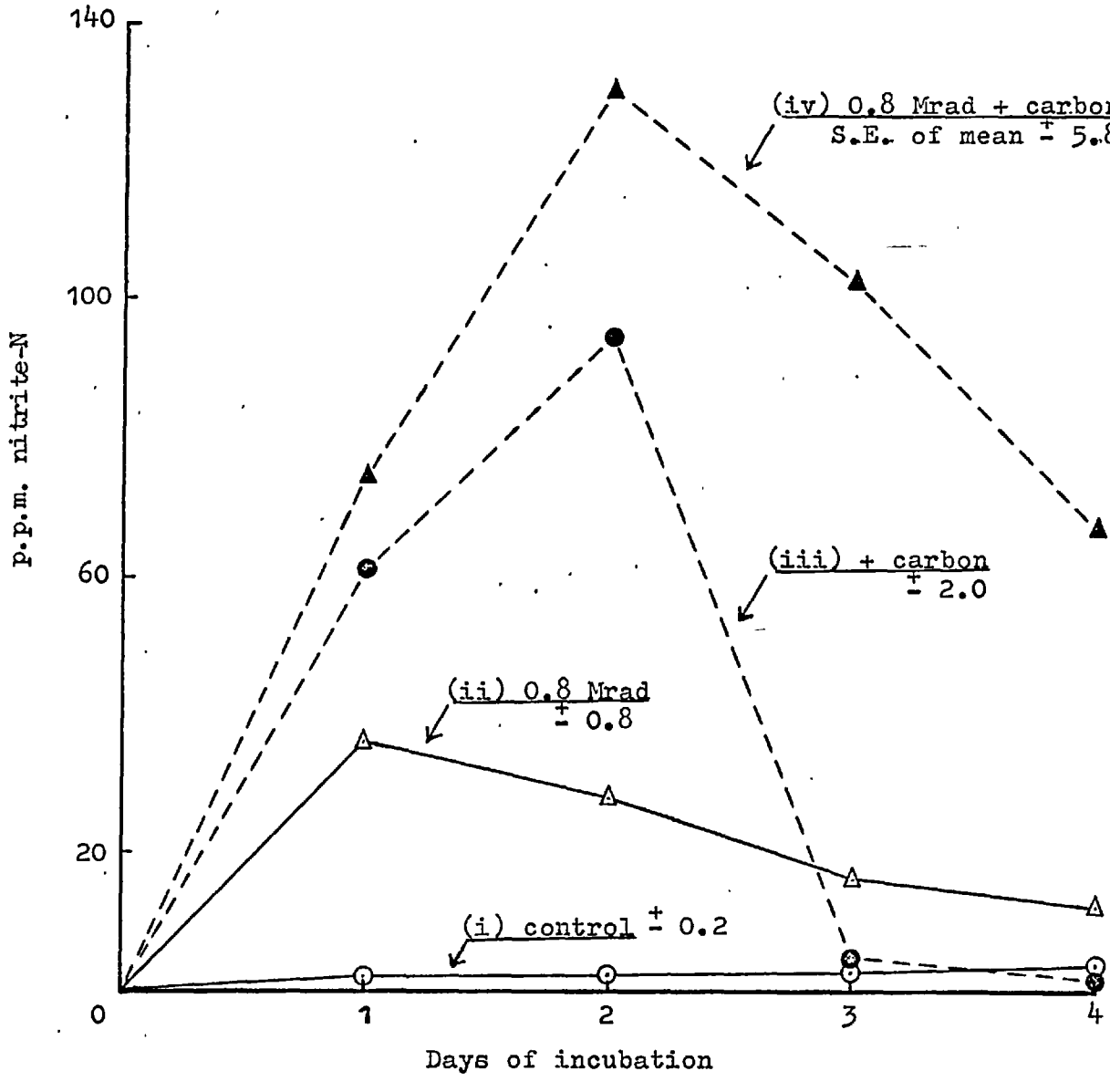


Figure 4-5.4.2 Effects of glucose-carbon and radiation on nitrite accumulation at 3:1 C:NO_3-N ratio under anaerobic conditions

of denitrification agrees with the results of Cooper and Smith (1963), where it was found that several soils could completely reduce 300 p.p.m. nitrate-N to nitrogen over 28 - 96 hr at 30°C.

It was decided to investigate the extent to which radiation delayed denitrification at a C : NO_3-N ratio of 10 : 1, where it was certain that carbon would not be limiting to reduction, and stimulation from radiation-released carbon would be most unlikely to occur.

4-5.5 Effect of radiation on nitrite accumulation at a C : NO_3-N ratio of 10 : 1 under anaerobic conditions

Materials and Methods:

The experiment was set up and analysed under identical conditions to the previous tests, and the treatments were:

(i) glucose added to obtain a C : NO_3-N ratio of 10 : 1

(ii) 0.8 Mrad + treatment (i) after irradiation

Results:

Table 4-5.5 shows that radiation checked the removal of nitrite and nitrate, but there was not a complete block, and both were almost completely removed by the fourth day of incubation. At such a high C : NO_3-N ratio, a temporary radiation check to organisms responsible for nitrate and nitrite reduction seems the most probable explanation. Although such inhibition could be expected to occur under aerobic conditions as well, the stimulatory effect of radiation-released carbon compounds (in the absence of added carbon) on surviving organisms would probably be predominant.

Table 4-5.5 Effect of radiation on nitrite accumulation at a C : NO_3-N ratio of 10 : 1 under anaerobic conditions

Treatment	p.p.m. nitrate-N present* after:				p.p.m. nitrite-N present* after:			
	1 day	2 days	3 days	4 days	1 day	2 days	3 days	4 days
+ carbon	371.4	30.5	9.5	9.9	57.9	63.0	0.3	0.4
0.8 Mrad + carbon	392.2	232.3	62.0	6.7	69.4	86.5	135.6	1.6
	L.S.D. p 0.05 = 20.5				L.S.D. p 0.05 = 12.8			

* initially present: 527.1 p.p.m. nitrate-N, 0.3 p.p.m. nitrite-N

4-5.6 Formation of nitrous oxide after irradiation under anaerobic conditions

According to the results on nitrite levels in waterlogged soil after 0.8 Mrad (treatment (ii) in Figure 4-5.4.2), there was a gradual decline in nitrite commencing 24 hr after irradiation. Reduction of nitrite to nitrous oxide and nitrogen was suspected, but to obtain more definite evidence for this pathway of denitrification, the evolution of nitrous oxide from irradiated and unirradiated soil was compared.

Materials and Methods:

Samples of 30 g dry weight were treated with 0.8 Mrad at 60% moisture. Immediately afterwards, the irradiated soil and two unirradiated samples were placed in specially designed vessels (Figure 2-3.3.1) and were waterlogged to 120% moisture; sufficient nitrate was

included to adjust the soil concentration to 500 p.p.m. nitrate-N. The vessels were connected to a closed system incorporating an infra-red gas cell (Section 2-3.3), and the release of nitrous oxide was recorded at 20°C, usually every day. Such large quantities of the gas were produced from the irradiated samples over 4 days that the incubation period was extended to determine the maximum concentration which formed.

Results:

Table 4-5.6 shows that the release of nitrous oxide from irradiated soil greatly exceeded the evolution from the control, which is in accordance with the relative rates of nitrate reduction and occurrence of nitrite previously observed (Figures 4-5.4.1 and 4-5.4.2). The irradiated soil rapidly produced nitrous oxide, and 67% of the maximum concentration observed was detected after the first 4 days of incubation. The loss of nitrous oxide-N at 4 days exceeded the nitrite-N deficit by 78.5 - 23.8 = 44.7 p.p.m. N, and suggests that nitrite undergoes such rapid reduction that its accumulation is limited. The experiment indicated that the decline in nitrite observed in irradiated soil under anaerobic conditions involves further reduction, probably of a microbial nature, which is stimulated by radiation-released carbon compounds.

Experimental summary to carbon studies

The main findings from all the carbon studies at 0.8 Mrad were:

(i) A severe block to nitrite removal did not occur following irradiation under aerobic or anaerobic conditions, although in the latter there was a slight delay in denitrification.

(ii) Addition of carbon to irradiated aerobic soil lowered the nitrite

concentration, therefore a large amount of radiation-released carbon is more likely to reduce the persistence of nitrite rather than increase it.

(iii) If soluble carbon was abundant in waterlogged soil, the introduction of radiation depressed the denitrification rate; in soil to which carbon was not added, radiation increased reduction of nitrate to nitrite and nitrous oxide, most probably by stimulation of surviving microorganisms with released carbon compounds.

Table 4-5.6 Formation of nitrous oxide by waterlogged soil after irradiation

Days of incubation	µg N_2O-N released from 30 g dry soil after 0.8 Mrad
1	273
2	831
3	1185
4	1587
6	1987
7	2096
8	2207
9	2281
10	2356
11	2252
14	2220

Control soil produced a maximum of 157 µg N_2O-N at day 8
S.E. of mean ± 19.4

4.6 Effect of saturation with carbon dioxide

Some investigators have reported that irradiation of soil can increase the evolution of carbon dioxide. For example, Coleman and Macfadyen (1966) found a very high evolution rate (approximately four times greater than control) over 24 hr after treatment with 2.5 Mrad, although this fell to slightly less than the control after 2 days. In addition, Cawse (1967a) reported that five soil series (including the Broad type) produced more carbon dioxide than unirradiated controls over one week after treatment with a sub-sterilising dose of 0.2 Mrad.

It was thought desirable to examine the effect of replacing the soil atmosphere with carbon dioxide on the formation of nitrite (after 0.8 Mrad at medium soil moisture), to confirm suggestions made following experiments on temperature and freezing effects that a high concentration of carbon dioxide in air-tight vessels caused less nitrite to accumulate.

Materials and Methods:

Broad and Icknield soils were raised to a moisture content corresponding to 60% MWHC. After 2 days of equilibration, 10 g samples were weighed into 25 ml McCartney bottles having screw caps with rubber inserts. Through each cap was inserted two hypodermic needles to act as gas inlet and outlet. The inlet needle was 12.8 mm long, and reached to the bottom of the bottle. The outlet needle was 2.5 mm long, and just projected into the air space above the soil sample. Carbon dioxide was passed through the soil for 24 hr at a flow rate of 0.3 litres / hr, the needles were removed and the cap was sealed with hot paraffin wax. Immediately after sealing, the samples were irradiated with 0.8 Mrad for Broad series and a slightly

lower dose of 0.6 Mrad for the Icknield soil: a set of gassed samples of each soil was left unirradiated.

After an incubation period of 64 hr, the soils were analysed for nitrite and nitrate and the amounts detected were compared with the levels present in aerobic samples which had been irradiated and incubated. The amount of nitrate in aerobic control soils was also determined before incubation to assess the extent of nitrification over 64 hr.

Results:

The results appear in Table 4-6. The atmosphere of carbon dioxide reduced the extent of nitrification during incubation of control samples, and in the irradiated treatments it considerably lowered the amounts of nitrite and nitrate present. The latter behaviour strongly suggested that denitrification was increased by irradiating soil saturated with carbon dioxide, and this agrees with the response obtained from argon-saturation tests reported later in Section 7-1.

It was concluded that a large increase in the concentration of carbon dioxide in irradiated soil is unlikely to encourage the formation of nitrite, rather the reverse. It seemed almost certain that accumulation of carbon dioxide was responsible for the bad replication and sudden fall in nitrite observed during the temperature response experiments (Section 4-2.1) and freezing tests (Section 4-4.2).

Table 4-6 Effect of carbon dioxide saturation on soil nitrite increase after irradiation

Soil series	Treatment	p.p.m. mineral-N after 64 hr incubation			
		Aerobic		CO_2 saturated	
		nitrite	nitrate*	nitrite	nitrate
Broad	Control	0.3	211.0	1.4	158.5
	0.8 Mrad	37.0	243.2	7.6	89.1
Icknield	Control	0.3	163.0	1.4	96.9
	0.6 Mrad	33.9	219.0	11.1	36.3

L.S.D. nitrite-N p 0.05 = 5.7, p 0.01 = 13.1

L.S.D. nitrate-N " = 24.8, " = 57.3

* increase in nitrate-N during incubation of control (Broad = 4.9 p.p.m. Icknield = 3.9 p.p.m.

4-7 Effect of calcium carbonate

Owing to the instability of nitrous acid at pH values below 5.5 - 6.0 (Allison, 1963), there was little point in examining the effect of acidifying Broad and Icknield soils on the accumulation of nitrite after irradiation in the region of 0.8 Mrad. Of greater interest was the effect of raising the pH on nitrite accumulation, particularly in the case of some neutral and acid soils which are known to give very little nitrite after irradiation compared with more alkaline series (Cawse and White, 1969b).

Raising the pH of an acid or neutral soil may result in the accumulation of nitrite in two ways:

(a) by improving the stability of any nitrite that does form after irradiation,

or (b) by an inhibitory effect on Nitrobacter. Aleem and Alexander (1960) report that inhibition results from the action of free ammonia, and the phenomenon is known to occur in alkaline soils after addition of ammonium fertilisers (Chapman and Liebig, 1952; Justice and Smith, 1962; Meek and MacKenzie, 1965). In addition, Lees (1963) found that nitrite can inhibit its own oxidation if the concentration exceeds 140 p.p.m. nitrite-N in a soil perfusate. Experiments on perfusion of soil from Grove series (Cawse, 1967b) showed that 140 p.p.m. nitrite-N caused only a slight delay in oxidation to nitrate, but 280 p.p.m. nitrite-N produced severe inhibition. Since the latter concentration in the perfusate represents as much as 3,500 p.p.m. nitrite-N on a soil dry weight basis ($\frac{280\ \mu g \times 250\ ml\ per}{20\ g\ soil}$ ... nitrite accumulation is unlikely to affect Nitrobacter in the present experiment, where the concentration was not expected to exceed 50 p.p.m. nitrite-N in any soil series after irradiation.

If there was a contribution to the formation of nitrite by mechanism (b), it could be detected by incubation of unirradiated soils to which calcium carbonate had been added. If the contribution was insignificant, then 'irradiation' and 'calcium carbonate + irradiation' treatments could be compared.

A single radiation dose of 0.75 Mrad was chosen because a study by Cawse and White (1969b) over the range 0.5 - 1.5 Mrad had shown that it was optimal for maximum nitrite accumulation in five alkaline soils; the investigation also indicated that nitrite formed rapidly, and there would be little or no further increase in concentration with incubation for longer than 3 days.

Materials and Methods:

Fifteen soil series were taken for this experiment, and are listed in Table 4-7. For each series, two fresh samples were prepared: (i) 140 g, to which 1.4 g of calcium carbonate was added, and (ii) 70 g, at normal pH.

All series were raised to 60% of MWHC, and allowed to equilibrate for 2 weeks at 5°C. After this time, samples of 20 g were taken from treatments (i) and (ii) for determination of pH in water extracts. Sub-samples of 10 g were taken from treatment (i) + calcium carbonate, for incubation without irradiation, and further sub-samples were treated with 0.75 Mrad. From treatment (ii) at normal pH, sub-samples of 10 g were taken for irradiation. All treatments were allowed 3 days of incubation before analysis for nitrite.

Results:

Table 4-7 shows that addition of calcium carbonate alone failed to induce a large accumulation of nitrite in any soil; this was most satisfactory. The fact that the six series having a normal pH of

<6.4 produced almost the same small amount of nitrite whether irradiated at their normal pH, or at alkaline pH in the region 7.8 - 8.2, rules out any suggestion that soils of lower pH fail to accumulate nitrite after irradiation because of instability. It seems that such soils do not contain the enzyme system/s which carry out the reduction of nitrate.

In the five alkaline soils which normally gave 31 - 53 p.p.m. nitrite-N after 0.75 Mrad, there was no consistent effect produced by addition of calcium carbonate: Icknield series showed little difference in nitrite formation, but Broad and Broadmoor gave about 12% less with calcium carbonate present, whereas Colthrop and Gade showed slight gains. It is difficult to account for these observations, but an explanation to account for the detection of more nitrite after irradiation in the presence of calcium carbonate could be that nitrate-reducing bacteria proliferated during the equilibration period. Less nitrite could result from increased numbers of bacteria responsible for nitrite reduction to oxides of nitrogen and gaseous nitrogen, relative to those organisms responsible for nitrate reduction to nitrite.

It should perhaps be mentioned that considerable amounts of ammonium will be produced in the 'calcium carbonate + irradiation' treatment by microbial damage (Cawse and White, 1969a). Although these conditions are more favourable to a build-up of nitrite by ammonium toxicity to Nitrobacter than the '+ calcium carbonate' alone treatment, the results provide no evidence that soils which accumulated little nitrite after 0.75 Mrad were encouraged to produce greater concentrations by damage to Nitrobacter.

Table 4-7 Effect of calcium carbonate on nitrite accumulation after irradiation

Soil series	pH in water		p.p.m. nitrite-N after 64 hr incubn.*+		
	normal	with $CaCO_3$	with $CaCO_3$	with $CaCO_3$ + 0.75 Mrad	0.75 Mrad alone
Black	7.8	8.2	0.9	3.7	4.9
Broad	8.0	8.0	0.4	36.4	41.6
Broadmoor	8.3	8.3	1.4	47.2	53.2
Bursledon	6.0	8.2	0.7	1.3	1.2
Charity	6.1	8.1	0.7	1.9	2.5
Colthrop	8.1	8.1	1.8	38.0	34.6
Denchworth	5.7	8.0	1.8	3.8	1.6
Evesham	6.8	8.1	1.0	3.7	4.5
Fyfield	7.6	8.1	0.8	2.3	2.6
Gade	8.2	8.2	0.8	33.2	31.4
Grove	7.4	8.2	0.8	2.2	2.4
Hanney	5.9	7.9	0.5	2.2	1.2
Icknield	8.0	8.0	0.6	44.9	44.6
Southampton	3.9	7.8	0.7	2.4	1.6
Sulham	6.3	7.8	0.4	2.5	2.9

L.S.D. nitrite-N p 0.05 = 1.6, p 0.01 = 3.8

* untreated soils all contained 0.7 p.p.m. nitrite-N

+ soils all at 60% MWHC

5 Relationship between nitrite formation by soils after 0.75 Mrad and their capacity to reduce nitrate when waterlogged

If the reduction of nitrate to nitrite in certain irradiated soils under aerobic conditions was being accomplished by microbial enzyme systems, as suggested by experiments in Section 4, it was thought that the extent of nitrite accumulation should show agreement with the potential of unirradiated soils to carry out denitrification in the presence of ample nitrate and carbon (termed "denitrifying potential"), so that the numbers of denitrifying organisms would be the critical factor controlling the rate of nitrate reduction. This rate would be most interesting over the first 24 and perhaps 48 hr of incubation, which should give little opportunity for the organisms to proliferate too extensively. If they did, it could give a false idea of the initial capacity of a soil to reduce nitrate, but relative differences in rates of reduction between series would still be expected to be apparent.

5-1 Denitrification rates

5-1.1 Preliminary tests on rates of nitrate loss

A preliminary experiment was carried out to compare the loss of nitrate in Broad, Fyfield, Grove and Icknield soils following addition of either nitrate, or nitrate and available carbon. It was hoped to prove the necessity of adding carbon to achieve a fast denitrification rate, and provide information on the incubation time which would be most suitable for soils enriched in carbon and nitrate.

Materials and Methods:

To 20 g samples of the four soil series described above was

added (i) glucose and nitrate, or (ii) nitrate alone. The glucose solution contained 15 mg C / ml, and the nitrate solution (from potassium nitrate) had 3 mg nitrate-N / ml: 2 ml of each solution was added, thus obtaining a C : N ratio of 5 : 1 ignoring the nitrate naturally present, and the samples were waterlogged to a depth of 1 cm. Nitrate was determined at the beginning of the experiment to enable the nitrate content at the start of waterlogging to be calculated, allowing for the addition of 6 mg nitrate-N.

Nitrate was analysed in samples receiving glucose after 3 days of incubation, but those without glucose were analysed after 3, 7 and 14 days. As there had been virtually complete loss of all nitrate present in the samples with glucose by 3 days, there was no point in continuing the incubation and analysis beyond this time.

Results:

The percentages of initial nitrate lost on incubation are shown in Table 5-1.1, and make it clear that firstly, the presence of glucose is essential for rapid denitrification. Secondly, for the main experiment to follow, there would be no object in continuing the incubation for more than 3 days after adding glucose and nitrate.

Table 5-1.1 Rates of nitrate loss in waterlogged soils with and without addition of glucose

Soil series	% initial p.p.m. nitrate-N lost after incubation for:			
	with glucose	without glucose		
	3 days	3 days	7 days	14 days
Broad	96.7	14.4	15.9	22.6
Fyfield	96.5	6.6	10.3	12.9
Grove	86.3	9.4	14.0	18.3
Icknield	92.5	0	8.4	11.1

5-1.2 Denitrification rates in fifteen waterlogged soil series

Materials and Methods:

Fifteen soil series were selected for denitrification studies. All of them had been previously examined under aerobic conditions with respect to the response of nitrification and nitrite accumulation (Cawse, 1967b; Cawse and Crawford, 1967; Cawse and White, 1969a and 1969b).

Samples of 20 g were waterlogged after the addition of 30 mg glucose-C and 6 mg nitrate-N. Nitrate was determined in the fresh samples to allow calculation of the total amount present immediately before waterlogging. After 1, 2 and 3 days of incubation, nitrite and nitrate were determined. Although it was only possible to rely on the nitrate analyses for calculation of denitrification losses, nitrite was estimated because of interest in the stability and concentration that could be reached in the various soil series. In addition, the appearance of considerable amounts of nitrite simultaneously with severe nitrate loss would help to confirm that true denitrification was occurring; all soils showed a "frothiness" in the surface layer of water, caused by evolution of gas during incubation.

Results:

The figures in Table 5-1.2 show that nitrate was lost very rapidly in the alkaline series, and the fastest was Broadmoor, where 220 p.p.m. nitrate-N (a total of 2.65 mg) disappeared in the first 24 hr. Such rapid losses in the presence of glucose have already been reported by Bremner and Shaw (1958a), McGarity (1961) and Greenland (1962).

The results obtained for the alkaline soils agree with reports on the abundance of denitrifying organisms in soils of high pH, and

their sensitivity to low pH. Valera (1959) detected very few denitrifying bacteria in soils of pH 4.0, and later, Valera and Alexander (1961) found that in alkaline soils fewer than 5% of the total flora could liberate nitrogen from nitrate: exceptionally, the number could reach 30%. The acidic Southampton series certainly gave least nitrate loss, in agreement with these authors, and the second lowest was Denchworth series with a pH of 5.7.

The losses of nitrate over the first 24 hr were compared with the amounts of nitrite that accumulated after irradiation, the latter figure appearing in Table 5-1.2 for convenience (taken from experiment 4-7). It was clear that five out of six soils producing the greatest loss of nitrate also formed most nitrite after 0.75 Mrad, the exception being Sulham series, where more rapid reduction of nitrite may have prevented accumulation. The nitrite analyses on waterlogged soils showed that concentrations were highest in those series which gave the most rapid loss of nitrate; this conforms with the finding by Cooper and Smith (1963) that nitrite removal can become the rate-limiting step in denitrification.

There was good agreement between the denitrifying potential of waterlogged soils and the amount of nitrite formed after 0.75 Mrad at medium moisture, but three other variables could also be well related to nitrate loss; they are the soil pH and inorganic carbon level, which would favour denitrifying organisms, and the carbon released by lysis of irradiated microbes.

Released carbon may directly stimulate formation of nitrite at medium moisture, and could therefore be a better indicator of nitrate reduction than nitrite itself if the latter was removed rapidly in certain soils. Although experiments with one irradiated

Table 5-1.2 Production of nitrite in irradiated soils at medium moisture, and nitrate losses in unirradiated soils under waterlogging

Soil series	pH in water	% inorg. C	after 0.75 Mrad at 60% MWHC: p.p.m. NO_2-N 64 hr post γ	after 0.75 Mrad at 60% MWHC: p.p.m. soluble C immed. post γ	p.p.m. NO_3-N loss when waterlogged*: day 1	day 2	day 3
Black	7.8	<0.05	4.9	276	95.3	226.2	392.4
Broad	8.0	1.19	41.6	317	141.0	307.9	518.6
Broadmoor	8.3	4.03	53.2	549	220.4	420.7	511.0
Bursledon	6.0	<0.05	1.2	540	43.4	106.0	318.1
Charity	6.1	<0.05	2.5	650	72.8	143.6	268.9
Colthrop	8.1	2.90	34.6	604	214.2	352.5	453.0
Denchworth	5.7	<0.05	1.6	547	17.3	69.3	243.0
Evesham	6.8	<0.05	4.5	680	77.5	331.3	381.5
Fyfield	7.6	<0.05	2.6	408	57.7	193.6	358.5
Gade	8.2	1.01	31.4	465	156.2	313.7	433.8
Grove	7.4	<0.05	2.4	341	82.6	195.1	372.6
Hanney	5.9	<0.05	1.2	329	38.0	72.3	132.5
Icknield	8.0	0.70	44.6	427	183.7	325.0	606.6
Southampton	3.9	<0.05	1.6	1074	9.7	19.0	20.7
Sulham	6.3	0.11	2.9	445	140.6	349.7	524.0

L.S.D. nitrite-N p 0.05 = 1.6, p 0.01 = 3.8

L.S.D. soluble C " = 84.3, " = 194.5

* 30 mg glucose-C and 6 mg nitrate-N added

soil (Section 4-5) led to the conclusion that a large amount of carbon was more likely to reduce persistence of nitrite under aerobic conditions, it was decided to establish the exact concentrations of carbon in all series immediately after irradiation; 30 g samples were exposed to 0.75 Mrad at 60% MWHC, and were shaken with 40 ml of water. The suspensions were centrifuged for 40 minutes at 30,000 G, and 20 ml of the supernatant fraction was analysed for carbon. The results are included in Table 5-1.2. It was found that the amount of soluble carbon present was considerable: for instance, 400 p.p.m. C represents approximately 8 mg C in the 30 g of moist sample, which would hardly be very limiting to microbial nitrate reduction, and no doubt explains the poor correlation with nitrite formation.

It is assumed that a large proportion of radiation-released carbon compounds are available to microorganisms. Skyring and Thompson (1966) have shown that Pseudomonas denitrificans is capable of faster nitrate reduction in a soil treated with 0.5 - 5 Mrad, and conclude that an increase in availability of organic matter may be responsible. The work of Boyer et al. (1966) and Bowen and Cawse (1964b) has indicated that such heavy doses as 4 - 5 Mrad give a greater increase in the fulvic fraction of soil organic matter than in humic substances, and this effect is also possible to a limited extent at lower levels of radiation. Considerable amounts of carbon are normally found in both fulvic and humic fractions; Kononova (1961) quoted the elementary composition of fulvic acids from a humus-illuvial horizon, and 45% carbon was present with only 1.1% nitrogen. These figures compare with 57% carbon and 3.9% nitrogen for humic acids from different soils.

The contribution of all four variables to nitrate loss (y) after 1, 2 and 3 days was analysed by multivariate regression, and 0.05% inorganic C was accepted as the true amount for soils where determinations had set it as the upper limit. It was first established that with all the data, replacement of nitrite by log nitrite (to base e) gave a better fit; for example, a 't' test on the significance of the regression co-efficients showed t = 6.20 for nitrite, and t = 7.70 for log nitrite using day 1 nitrate loss figures (15 observations). Secondly, the significance of individual regression co-efficients suggested that nitrate loss was independent of inorganic carbon or soluble carbon levels, and the best relationship appeared to be that of nitrate loss on log nitrite formed (a) and pH (b):

Over 1 day	y =	35.3a	+	10.8b	- 34.5
" 2 days	y =	42.2a	+	40.3b	- 126.2
" 3 days	y =	27.2a	+	78.0b	- 220.9

The best fitting line was given by day 1 results with 84% of the total variation explained, compared with 72% and 71% for days 2 and 3. The significance of the regression co-efficients of log nitrite and pH was examined by a 't' test, and the values were:

Independent variable	Day 1	Day 2	Day 3	Significance
log p.p.m. nitrite	4.00**	2.03	1.03	** 5% level
pH	1.06	1.67	2.56*	* 2% level

The greater significance of the contribution of log nitrite to the regression obtained over the first day agrees with comments in the introduction, which stressed that extensive proliferation of

denitrifying bacteria during incubation could obscure the original capacity of a soil to reduce nitrate; it seemed that in particular, Bursledon and Denchworth series were behaving in this way, with increasing nitrate loss in relation to incubation time. Results over the first day are also more reliable because many series had reduced so much nitrate after 2 - 3 days that it would limit further microbial activity.

6 Influence of soil type on radiosensitivity of Nitrosomonas and Nitrobacter up to 1 Mrad

One explanation to account for the accumulation of nitrite in some soils (Broad, Icknield) but not others (Black, Grove) after irradiation in the 0.75 Mrad region might be that nitrification is inhibited to a greater extent than nitrate reduction, and thus the latter process becomes noticeable by formation of nitrite. In a soil with more radioresistant Nitrobacter, any nitrite would be rapidly oxidised. Differences in radiosensitivity of nitrifying bacteria could arise from:

(a) environmental effects, e.g. soil chemical and physical status, or

(b) the presence of different nitrifying species.

The effect of soil environment can only be determined by studying the same species of nitrifying bacteria in both "nitrite-forming" and "non nitrite-forming" (0.75 Mrad) soils. If pure cultures of Nitrosomonas and Nitrobacter were introduced into sterile soils and colonised successfully, they could then be irradiated over a range of doses up to 1 Mrad to obtain nitrification response curves: this approach was undertaken.

A study of (b) would entail isolation and identification of the indigenous nitrifying species, followed by measurement of their radiosensitivity in pure culture, but it was not possible to proceed with such an experiment owing to the attention given to other aspects of nitrite behaviour.

6-1 Introduction of nitrifying bacteria into sterile soil columns

Materials and Methods:

It was thought that the colonisation of nitrifiers in sterile

soil would best be achieved by the use of soil columns with free drainage: 400 g of fresh Grove soil (340 g dry weight) was placed in a glass tube measuring 45 cm long X 4 cm I.D., as shown in Figure 6-1. Four replicate treatments were applied according to the details in Table 6-1. For inoculation, Nitrosomonas europaea and Nitrobacter winogradskyi were cultured for 2 weeks in advance, then 10 ml of each culture was injected through the sand on to the surface of the soil, and was washed further down with 50 ml of sterile water. The water added raised the soil moisture to 54% MWHC in treatments (i), (ii) and (iv). All columns were incubated for 25 days at 25°C, and air was passed through them every week to avoid anaerobic conditions.

The leachate from treatment (iii) was examined for toxicity of radiation-released organic substances towards the nitrifiers, because positive evidence would have been useful to substantiate any later results suggesting enhanced nitrification in leached columns; 15 ml of leachate was added to separate 15 ml aliquots of Nitrosomonas and Nitrobacter culture media which were immediately inoculated with the respective bacteria. After incubation for 7 days at 25°C, the Nitrosomonas media was analysed for ammonium and the Nitrobacter media for nitrite, but extensive oxidation of the substrates had occurred, without inhibition:

Media analysed:	Nitrosomonas media	Nitrobacter media
Before inoculation	105.6 p.p.m. NH_4-N	200.5 p.p.m. NO_2-N
After incubation	7.4 " "	0.03 " "

After incubation, each column was leached with 800 ml of sterile water at pH 7, and the treatment was repeated 7 days later;

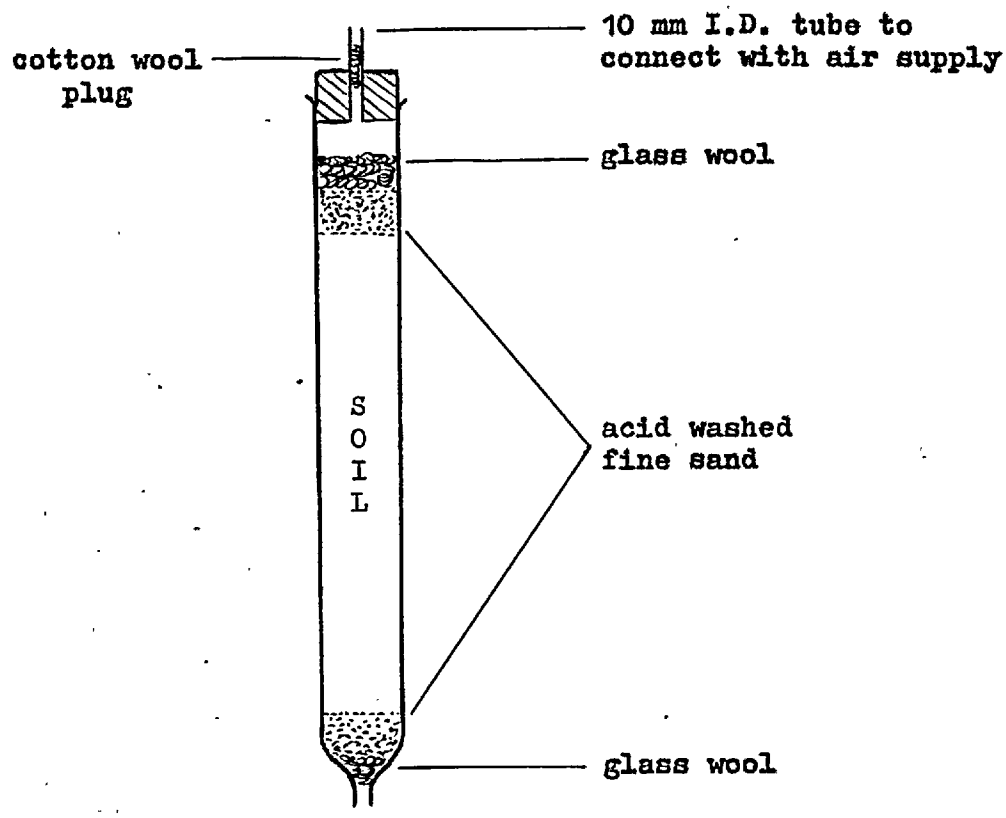


Figure 6-1 Soil column for colonisation of nitrifying bacteria

Table 6-1 Nitrifying capacity of inoculated soil columns

Treatment	Total µg mineral-N in leachate expressed as mean p.p.m. soil in column			
	Nitrate-N			Nitrite-N
	present at irradn.	up to 25 days post-irradn.	25-32 days post-irradn.	25 days post-irradn.
(i) 4 Mrad, then inoculated	13.2	13.5	2.0	0.3
(ii) 4 Mrad, air passed through column, then inoculated		15.8	2.7	0.1
(iii) 4 Mrad, leached with 800 ml water, air passed, then inoculated		15.7	1.6	0.1
(iv) Not irradiated or inoculated, but 70 ml water added		43.4	9.7	5.4
L.S.D. nitrate-N p 0.05 = 7.1, p 0.01 = 16.5				

nitrite and nitrate were determined in the leachates and compared with the amount present in soil leached immediately before irradiation.

Results:

The extent of nitrification is shown in Table 6-1. Inoculated soils gave little increase in nitrate, but in unirradiated columns nitrification proceeded readily under the physical conditions of the experiment. Far more ammonium was present in irradiated soils, and could not have limited nitrification. The poor colonisation obtained led to the consideration that conditions under perfusion would be intermediate between pure culture and soil environments, and may permit more gradual adaptation to soil conditions with less check to the organisms.

6-2 Introduction of nitrifying bacteria into sterile perfusion systems

Materials and Methods:

Nitrosomonas and Nitrobacter were cultured for 2 weeks to obtain dense populations, then 5 ml of each culture was placed on the surface of 20 g sterile Broad and Grove soils contained in nylon sachets in the small perfusion units (Section 2-5). At the same time, 100 µg nitrite-N was added (from 100 p.p.m. NO_2-N potassium nitrite solution) to the perfusate of ammonium sulphate, to provide substrate for Nitrobacter until Nitrosomonas functioned well. The units were incubated for 10 days, nitrite and nitrate being determined at frequent intervals.

Results:

Nitrification proceeded rapidly in inoculated soils (Table 6-2), and the perfusate was replaced with fresh solution after 10 days.

Table 6-2 Nitrification in sterile and inoculated soils during perfusion

Soil and treatment	DAYS OF PERFUSION							
	p.p.m. nitrate-N				p.p.m. nitrite-N		p.p.m. nitrate-N	
	1	4	7	10	4	10	1	5
Sterile:								
Broad	18.0	19.2	19.4	20.1	0.6	0.7		
Grove	9.8	10.4	10.8	11.3	0.4	0.5		
							perfusion continued with fresh perfusate	
Inoculated:								
Broad	26.5	38.6	164.7	256.8	16.1	0.1	46.8	318.8
Grove	17.9	24.9	34.3	41.5	0.2	0.1	8.6	19.9
L.S.D. nitrate-N	p 0.05 = 5.4, p 0.01 = 12.5							
L.S.D. nitrite-N	" = 0.9, " = 2.0							

Further analysis established that nitrate still accumulated rapidly, therefore activity of nitrifying bacteria in the perfusate rather than the soil could be discounted. The perfusion technique permitted extensive colonisation of radiation-sterilised soil, and was far more suitable than soil column inoculation; however, only 20 g of soil could be placed in each unit, and at least 1 kg of colonised soil was needed for a dose / nitrification response study. It was thought that it might be possible to use perfused soil for inoculation of larger quantities which could be incubated in glass jars, and larger volumes of perfused soil at "Bacterial Saturation" (Lees and Quastel, 1946) for inoculum could be obtained by construction of larger units.

6-3 Irradiation of inoculated soil

Experiments were designed to compare the effect of 0.05 - 1 Mrad on nitrifying bacteria established in Broad and Grove series soils, known to be "positive" and "negative" nitrite-formers respectively after 0.75 Mrad. Later, it was necessary to substitute Black series for Grove to obtain more reliable results.

Perfusion units were constructed to hold 100 g of soil, and once the cultures of nitrifying bacteria could be established it was intended to mix the whole sachet of perfused soil with 1 kg of radiation-sterilised soil for colonisation to proceed, and then to irradiate the soil supporting the bacteria.

6-3.1 Inoculation and irradiation of Grove series

Materials and Methods:

Preliminary tests with Grove soil showed that although the nitrifiers could be successfully colonised in perfused soil, when the latter was mixed with 1 kg of sterile soil, the colonisation and formation of nitrate was poor. In an attempt to overcome this problem, double the quantity of perfused soil (200 g) was used to inoculate the main bulk of 1 kg.

Sachets containing 100 g of air-dry soil were treated with 4 Mrad, and placed into sterile perfusion units each containing 250 ml of ammonium sulphate as perfusate. One pair of units was inoculated with 25 ml of separate *Nitrosomonas* and *Nitrobacter* cultures, and 100 µg nitrite-N was added. The other pair of units were kept as sterile controls. After 2 weeks of perfusion, analysis showed that 70 p.p.m. nitrate-N had been formed in the perfusate from inoculated soil; the organisms appeared to be well established, therefore 200 g

of perfused soil was immediately mixed with 1 kg of Grove soil which had been treated with 4 Mrad in a 2 litre glass jar fitted with an air inlet plugged with cotton wool. The soil moisture was raised to 45% with sterile water, and the jar was incubated at 25°C for further colonisation to proceed. Samples were removed for analysis of nitrate and nitrite after 1 and 6 weeks of incubation, and at the end of the 8th week, duplicate samples of 50 g were taken for irradiation over the range 0.05 - 1 Mrad. Soil was also analysed for nitrate immediately before irradiation.

Results:

The extent of nitrification in the 1 kg bulk of inoculated soil is shown in Table 6-3.1, but as the earlier analysis had proved it to be slow, samples were incubated for 6 weeks in the hope that nitrate levels in irradiated treatments would show a greater difference from control. The final nitrate concentrations found (Table 6-3) demonstrate the harmful effect of radiation on nitrification even at 0.05 Mrad, in agreement with perfusion experiments where ammonium was also unlimiting to nitrification (Cawse, 1968). At the same time, the absence of nitrite indicated that there was little possibility of selective radiation damage to Nitrobacter.

There was a tendency (p 0.05) towards nitrate loss below the level present before irradiation, being most noticeable at 1 Mrad. Contamination of the sterile jars with nitrifying or denitrifying organisms was proved absent by analysis of nitrate in soil 24 weeks after the start of incubation: Table 6-3.1 shows that sterility was very satisfactory, and the inoculated sample had nitrified slowly to give an extra 65.3 p.p.m. nitrate-N.

In view of the above observations, and subsequent findings on

Table 6-3 The effect of radiation on Grove, Black and Broad soils inoculated with nitrifying bacteria

Dose (Mrad)	mean p.p.m. nitrate-N after incubation for: 6 weeks Grove*	3 weeks Black*	2 weeks Broad*
0	95.4	199.3	275.7
0.05	79.3	174.1	197.5
0.1	76.3	175.2	197.3
0.2	75.7	168.0	190.8
0.4	75.1	167.9	189.9
0.6	74.7	171.3	188.2
0.8	75.4	167.7	187.2
1.0	72.8	165.2	183.6
pre-irradn.	81.5	153.1	171.6
L.S.D. p 0.05=	8.4	9.6	19.1
L.S.D. p 0.01=	19.3	22.1	44.1

* nitrite-N never >0.3 p.p.m. at any dose

Table 6-3.1 Nitrate accumulation in Grove soil inoculated with nitrifying bacteria

Treatment	% moisture	mean p.p.m. nitrate-N* 1	6	8	24 weeks after inoculation
sterile	45	32.1	32.9	35.0	32.8
inoculated	45	48.0	76.1	81.5	146.8
L.S.D. p 0.05	sterile = 4.4,	inoculated = 13.5			

* nitrite-N never >0.1 p.p.m. at any time

radiolytic reduction of nitrate in Section 10, it is extremely likely that the latter mechanism was responsible for these small losses of nitrate. Radiolysis produced 13.8 p.p.m. nitrite-N immediately after 1 Mrad, and by 7 days this had fallen to 0.8 p.p.m. According to the data in Table 6-3, there was a deficit of 81.5 - 72.8 = 8.5 p.p.m. nitrate-N after 1 Mrad, similar to the amount reduced to nitrite by radiolysis. In a soil which supported more vigorous nitrification after inoculation, it is possible that such a nitrate loss would be non-evident, because a dose of 1 Mrad would fail to check nitrification so effectively as in Grove soil.

6-3.2 Inoculation and irradiation of Black series

The poor ability of Grove soil to support growth of nitrifying organisms led to the choice of Black series, also a soil that failed to form nitrite over the dose range 0.05 - 1.0 Mrad (Cawse, 1968).

Materials and Methods:

The inoculation and subsequent irradiation techniques were identical to those previously described for Grove soil, except that a sterilising dose of 8 Mrad was required before inoculation, and less perfused soil was used to inoculate the 1 kg bulk. Perfusion units were analysed for nitrite and nitrate at 7 days after inoculation, and were then allowed to perfuse for a further 5 days to achieve more dense colonisation: analysis showed that nitrification was proceeding well -

Treatment	mean p.p.m. nitrate-N in perfusate after:	
	7 days	12 days
Sterile	32.8	32.0
Inoculated	109.8	286.2

therefore the perfusion sachets containing 100 g (dry weight) of soil were placed into jars containing 1 kg of sterilised (8 Mrad) Black soil, with the moisture content finally at 45%. Samples were removed from the jars after 7 and 20 days of incubation to assess the extent of nitrification:

Treatment	mean p.p.m. nitrate-N in soil after:		p.p.m. nitrite-N
	7 days	20 days	
Sterile	49.1	51.1	never >1.5
Inoculated	92.4	153.1	never >0.2

Nitrification had been well established with the pure cultures, and duplicate samples of 50 g were weighed out immediately for treatment with 0.05 - 1 Mrad. Irradiated samples were analysed for nitrate and nitrite after 3 weeks of incubation.

Results:

The dose response obtained is included in Table 6-3. The complete absence of nitrite formation throughout the dose range indicated that there was no selective radiation damage to Nitrobacter as compared with Nitrosomonas, but inhibition of nitrification did definitely occur.

6-3.3 Inoculation and irradiation of Broad series

Materials and Methods:

Broad soil treated with 8 Mrad was inoculated with nitrifying organisms in perfusion units in the same manner as Black soil. After 7 days the colonisation was satisfactory:

Treatment	mean p.p.m. nitrate-N in perfusate after 7 days
Sterile	32.1
Inoculated	238.0

Each 100 g perfused sachet was transferred to 1 kg of sterile (8 Mrad) Broad soil, and the moisture was adjusted to 60%. Samples were analysed after 10 and 20 days of incubation, which proved that nitrification was established:

Treatment	mean p.p.m. nitrate-N in soil after:		p.p.m. nitrite-N
	10 days	20 days	
Sterile	38.2	40.5	never >0.2
Inoculated	123.0	171.6	never >0.2

Duplicate samples of 50 g were treated with 0.05 - 1 Mrad, and analysed for nitrate and nitrite after 2 weeks of incubation.

Results:

The response obtained was similar to that of Black series, and is included in Table 6-3. Firstly, there was no evidence to suggest that the cultures of nitrifying organisms were more radiosensitive to doses between 0.4 - 1.0 Mrad in Broad soil compared with Black series, a factor which would have allowed any nitrite that was formed by microbial reduction of nitrate to accumulate. In both soils, nitrification was so severely checked by 0.05 Mrad that there was little extra inhibition as the dose increased to 1 Mrad. Thus the soil environment has no influence on the radiosensitivity of these nitrifying species over the radiation dose range which is particularly noted for nitrite formation by microbial action.

Secondly, because nitrite failed to accumulate in the sterilised, inoculated and irradiated Broad soil, it seems unlikely that greater radiosensitivity of Nitrobacter could lead to the nitrite accumulation observed after 0.8 Mrad (Cawse and Crawford, 1967), although separate species of bacteria having different radiosensitivities may be involved. In addition, the absence of nitrite formation means that microbial enzyme systems must have been inactivated by the sterilisation treatment with 8 Mrad prior to inoculation; this feature was hoped for, and was essential to the experiment.

Thirdly, the low concentrations of nitrite found after incubation of all radiation-sterilised but non-inoculated soils suggested that radiolytic reduction of nitrate was probably limited by the small amount of nitrate present: in any event, it was later shown (Section 10) that nitrite so formed gradually declined during incubation.

Finally, the technique developed to establish nitrifying bacteria in irradiated soil may be of value in other research programmes. All three soil series in the present study were successfully inoculated, although nitrification was slow in Grove soil.

6-3.4 Clay mineral analysis of Broad and Grove series

The influence of clay minerals on microbial development has been reviewed by Stotzky (1967), and some recent papers have drawn further attention to the way in which montmorillonite can fix enzyme protein (Sorensen, 1969), affect antibiotic tolerance of the soil microflora (Soulides, 1969), and become adsorbed on to cells of Rhizobium spp. (Marshall, 1968).

Differences in the ratio of montmorillonite to kaolinite clay were therefore of particular interest in the present inoculation work

owing to a possible influence on recolonisation by nitrifying bacteria. Air-dried and ground samples of Broad and Grove soils were subjected to mechanical and x-ray diffraction analysis by Mr. E.C. Ormerod at Rothamsted Experimental Station. The results in Table 6-3.4 show that the percentage clay is very similar in both soils, but there are differences in the composition of the clay fraction.

Expanding minerals are dominant in both clays, and consist mainly of minerals that expand like montmorillonite, but which can contain some randomly interstratified non-expanding layers. However, the Grove soil contains up to 25% of vermiculite in the expanding mineral fraction which irreversibly fixes added potassium, whereas Broad series only contains a trace of this particular mineral. There was less kaolinite present in the clay fraction from Grove soil.

Table 6-3.4 Mechanical and x-ray diffraction analysis of Broad and Grove soils

Fraction		% of whole soil	
		Broad	Grove
Coarse sand	>200 μ	3.6	9.8
Fine sand	200 - 50 μ	12.7	21.6
Silt	50 - 2 μ	19.1	20.7
Clay	<2 μ	39.8	38.5
		% of clay fraction	
Mica		13	18
Kaolinite		13	5
Expanding minerals		72	75
Quartz, Feldspar, Iron oxides		2	2

It is difficult to explain how the differences in clay minerals which have been found could account for such poor nitrification in Grove soil. The most closely related study appears to be that of Stotzky (1967), who reported increased oxidation of ammonium to nitrate by autotrophic bacteria when montmorillonite was added to soils not containing this mineral; nevertheless, it is impossible to correlate his finding with the present work from the results of the clay analysis.

Merzari and Broeshart (1968) also discussed the failure of nitrifying organisms to colonise a soil sterilised with 2.5 Mrad (a Philippine rice soil with pH 6), but details of the clay mineral composition and other basic properties were not given. As far as other organisms are concerned, Peterson (1962b) reported rapid establishment of a Pseudomonas sp. and an Arthrobacter sp. in irradiated soil, and Salonius et al. (1967) found greater cell yields of Pseudomonas fluorescens and Arthrobacter globiformis in soils previously treated with 3 Mrad than in autoclaved material. However, Peterson (1962b) did establish that Myxococcus fulvus died when introduced into either radiation-sterilised or autoclaved soil.

7 Release of nitrous oxide by soils after 0.75 Mrad

If nitrite in soils treated at the 0.75 Mrad level was existing as an intermediate in the nitrate reduction pathway to gaseous nitrogen, it should be possible to detect decomposition to nitrous oxide as well as nitric oxide, nitrogen dioxide and nitrogen in the soil atmosphere. Earlier work (Cawse and White, 1969b) established that nitrite formed on irradiation of two alkaline soils (Broadmoor and Colthrop series) with 0.75 Mrad did decline in concentration after 3 days' incubation: this could occur either by reduction, or by oxidation back to nitrate. Losses of nitrite have also been observed following treatment of Broad and Icknield soils with 0.75 Mrad (Section 10), but a longer time elapsed before the decline began: after 14 days for Broad series and 7 days for Icknield.

Gilmour et al. (1958) suggested that a refined Warburg technique might be useful to study the evolution of gases during anaerobic denitrification. Such a method was developed by McGarity (1961), using potassium hydroxide in the centre well of the Warburg flask to absorb carbon dioxide and hydrogen sulphide, and displacing the soil atmosphere with argon. It was assumed that nitrogen and nitrous oxide would be the gases most likely to give large positive pressure readings, and only trace amounts of ammonia, hydrogen and methane were expected to be present: this was inferred by experiments which showed a close relationship between nitrate removal and gas production. The method would be useful to study the decomposition of nitrite in irradiated soil if the argon atmosphere did not greatly alter the concentrations of nitrite which initially appeared, and this aspect was examined.

7-1 Effect of argon saturation on nitrite increase after irradiation

Materials and Methods:

The moisture content of Broad and Icknield soils was adjusted to 60% MWHC, and 10 g samples were weighed into 25 ml McCartney bottles, as used for the carbon dioxide experiment described in Section 4-6. Argon of 99.995% purity was passed through the soil for 24 hr at an approximate flow rate of 0.3 litres / hr. After this period the bottle caps were sealed with hot paraffin wax, and irradiated with 0.8 Mrad for Broad series and 0.6 Mrad for Icknield. Another set of argon-gassed samples was left unirradiated. An incubation period of 64 hr was allowed before the samples were extracted with 100 ml of water for nitrite and nitrate analysis. The concentrations of nitrite and nitrate in the argon-saturated soils were then compared with the amounts present in samples which had been irradiated and incubated under aerobic conditions.

Results:

The results in Table 7-1 prove that displacement of the soil atmosphere with argon caused much less nitrate to accumulate after irradiation, and nitrate concentrations were also greatly different from the aerobic irradiated samples.

The concurrent fall in nitrate and nitrite levels strongly suggested that reduction mechanisms were operating under the argon atmosphere in both irradiated soil types, and the Warburg technique was therefore unsuitable to study decomposition of nitrite. A more direct approach was required with individual measurement of the gaseous products likely to be encountered, such as nitrous oxide, nitric oxide and nitrogen dioxide.

Table 7-1 Effect of argon saturation on nitrite increase after irradiation

Soil series	Treatment	p.p.m. mineral-N after 64 hr incubation			
		Aerobic		Argon saturated	
		nitrite	nitrate*	nitrite	nitrate
Broad	Control	0.3	211.0	1.9	157.6
	0.8 Mrad	37.0	243.2	2.1	21.2
Icknield	Control	0.3	163.0	0.8	111.8
	0.6 Mrad	33.9	219.0	0.6	16.4
L.S.D. nitrite-N	p 0.05 = 2.1,		p 0.01 = 5.0		
L.S.D. nitrate-N	" = 22.2,		" = 51.2		

* increase in nitrate-N during incubation of control (Broad = 4.9 p.p.m. Icknield = 3.9 p.p.m.

An additional finding from the results in Table 7-1 is that denitrification at 60% MWHC under argon becomes greatly stimulated by irradiation with 0.6 - 0.8 Mrad, in agreement with data presented in Section 4-5.6, where Broad soil showed an increase in denitrification under waterlogged conditions if treated with 0.8 Mrad.

7-2 Production of nitrous oxide

7-2.1 Nitrous oxide formed after irradiation of three soils

The evolution of nitrous oxide from soil under conditions of medium moisture has been studied by Arnold (1954), Burford and Millington (1968), and Overrein (1969), but since the amounts released are small and often undetectable, most investigators have concentrated on the intensive release of the gas by the activity of denitrifying bacteria in waterlogged anaerobic soil. There is no information on the release of nitrous oxide by irradiated soil under any conditions of moisture.

Materials and Methods:

Broad, Broadmoor and Icknield soils were chosen for measurement of nitrous oxide release because they produced most nitrite after 0.75 Mrad, and would be very likely to yield nitrous oxide if any further reduction took place. Their nitrate content was determined at the start of the experiments (included in Table 7-2.2) to ensure that sufficient was available for reduction to nitrite, but large increases in nitrate could be expected during the incubation period following irradiation. During the course of these tests (lasting approximately 14 weeks) the nitrate levels increased owing to nitrification, but as the soils were stored below 12°C, such increases did not exceed 10 p.p.m. nitrate-N.

The soils were raised to 60% MWHC and equilibrated for 2 days. Sufficient moist soil calculated to contain 30 g dry weight was placed in the irradiation vessel (Figure 2-3.3.1) for treatment with 0.75 Mrad. Immediately afterwards, the vessel was connected to a circulatory system for measurement of nitrous oxide (Section 2-3.3), and the rate

of evolution was studied over 14 days. Owing to the size of the complete apparatus it was not possible to incubate at 25°C, and laboratory temperature of approximately 20°C had to be used. On average, there was 7% decrease in the percentage soil moisture during incubation.

Results:

All irradiated soils produced considerable amounts of nitrous oxide with a maximum concentration after 8 - 9 days of incubation, followed by a slight decline which was probably due to further reduction (Figure 7-2.1). Unirradiated control soils produced < 20 µg nitrous oxide-N / 30 g dry weight over the whole period of incubation.

On a quantitative basis, the release of nitrous oxide from irradiated samples was very large for soil kept under aerobic conditions. For example, the maximum concentration produced by Broadmoor soil represents 1.72 mg nitrous oxide-N / 100 g dry soil: this is equivalent to 63 kg nitrous oxide-N / hectare (assuming that 1 acre to 5" depth = 2×10^6 lbs), and can be compared with losses of only 0.12 - 1.2 kg nitrous oxide-N / hectare over 13 days from a fine sandy loam kept under conditions of medium moisture where the oxygen level never fell below 17% (Burford and Millington, 1968). With very favourable conditions for nitrate reduction, when soil is waterlogged and nitrate is added as substrate for denitrifying organisms, Cooper and Smith (1963) report that 8 - 11.5 mg nitrous oxide-N / 100 g dry soil can be released (under pure helium atmosphere, pH of soils from 6.8 - 7.8).

To obtain proof that the release of nitrous oxide was directly related to microbial formation of nitrite in irradiated soil, all three series were autoclaved for 1 hr at 15 lbs / sq. in. before treatment with 0.75 Mrad: on subsequent incubation for 14 days, nitrous oxide

could not be detected. It was expected that small amounts of nitrite would be produced by radiolytic reduction of nitrate, and analysis of autoclaved soils immediately after 0.75 Mrad showed this to be within 7.1 - 10.0 p.p.m. nitrite-N: 14 days later the concentration was from 5.7 - 7.7 p.p.m. nitrite-N for the three soil series. It could therefore be concluded that in the absence of microbial formation of nitrite in response to a dose of 0.75 Mrad, there is insignificant production of nitrous oxide.

7-2.2 Nitrous oxide formed after addition of nitrite to irradiated soils

The addition of nitrite to that already produced by the irradiated soils should demonstrate that the presence of nitrite was directly related to nitrous oxide release, and at the same time it was decided to record the oxygen concentration to examine whether it had fallen sufficiently to greatly encourage microbial reduction of nitrite. Furthermore, addition of nitrite to autoclaved and irradiated soil should indicate if microbial reduction was more important than chemical decomposition in the formation of nitrous oxide.

Materials and Methods:

The conditions of experimentation were similar to the preceding work, except that less water was added for equilibration to achieve 60% MWHC when nitrite was added. 3 mg of nitrite-N was applied to 30 g dry weight of soil (from a sterile potassium nitrite solution containing 1 mg nitrite-N / ml) immediately after (i) autoclaving + 0.75 Mrad, and (ii) 0.75 Mrad alone. In the case of the second treatment, break-seal ampoules were attached to the circulation system.

Broad and Icknield soils were incubated for 14 days, and the

ampoules were removed after 7 and 14 days for analysis of oxygen and nitrogen. With Broadmoor series, the last ampoules were not removed until 21 days of incubation, by which time the concentration of nitrous oxide had fallen considerably; in this way it was hoped to find whether lack of oxygen was leading to microbial reduction of nitrous oxide.

The effect of soil moisture on the release of nitrous oxide was examined by addition of 3 mg nitrite-N to 30 g dry weight of irradiated (0.75 Mrad) Broadmoor soil, followed by incubation for 7 days at 20, 30 and 40% MWHC. At the end of incubation, moisture and residual nitrite were determined in all experiments described.

Results:

Addition of nitrite to all irradiated soils increased nitrous oxide evolution (Figure 7-2.2) above the rate observed after irradiation alone (Figure 7-2.1), and the rise in nitrous oxide-N was significantly less than the nitrite-N deficits which appear in Table 7-2.2, together with oxygen and nitrogen concentrations in the soil atmosphere. The nitrite deficits were obtained by deducting the residual nitrite from the 100 p.p.m. added nitrite-N + the amount of nitrite present at 3 days after 0.75 Mrad (Table 4-7).

The reduction of nitrate to nitrite, and the formation and removal of nitrous oxide were proceeding in the presence of quite high concentrations of oxygen, although the respiratory activity of the soil removed more oxygen towards the end of incubation. The detection of small changes in nitrogen levels was rendered difficult by the high base value, but a normal atmosphere was used to ensure that the pattern of nitrite formation and decline after irradiation remained unaltered.

Apart from microbial reduction, two alternative pathways which

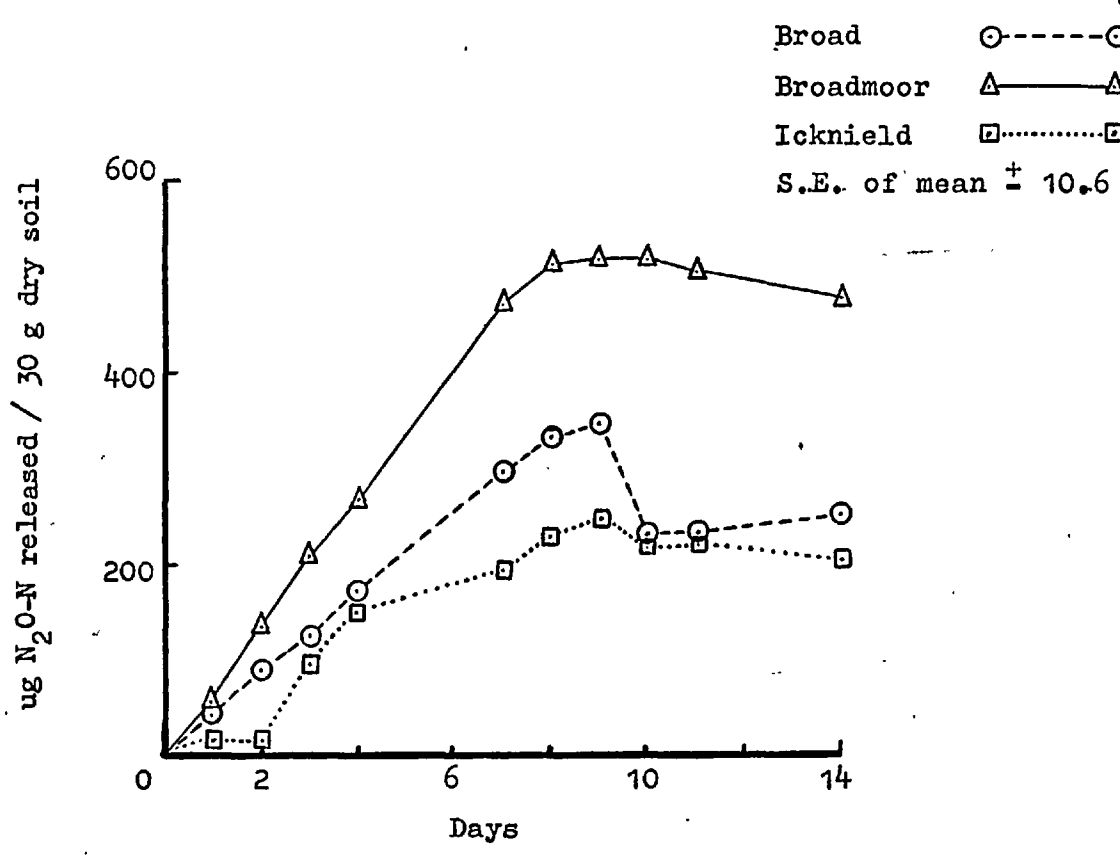


Figure 7-2.1 Nitrous oxide formed after treatment of three soils with 0.75 Mrad

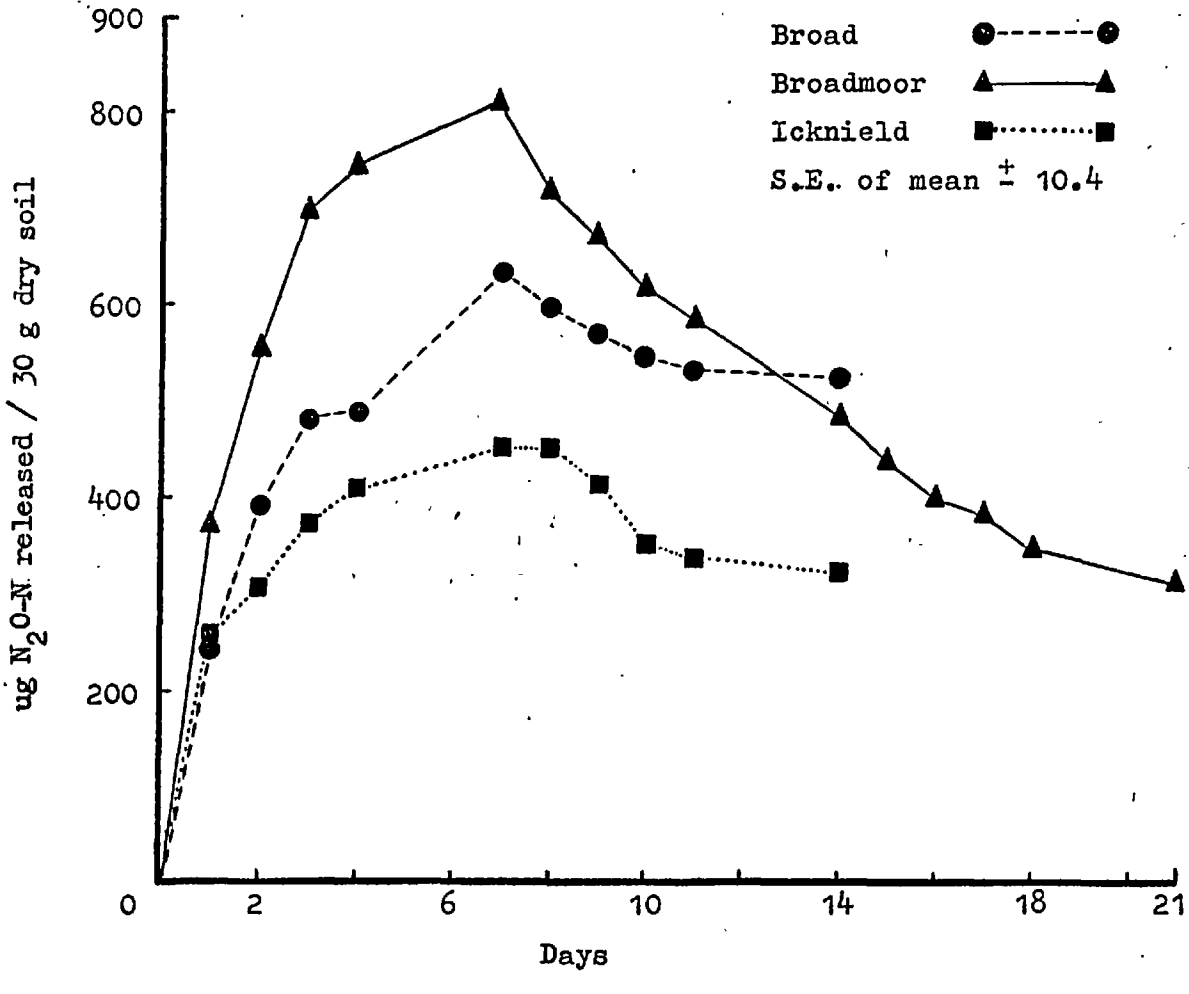


Figure 7-2.2 Nitrous oxide formed after treatment of three soils with 0.75 Mrad and addition of 100 p.p.m. nitrite-N

Table 7-2.2 Residual nitrite concentrations and soil atmosphere composition after incubation of soils treated with 0.75 Mrad and 100 p.p.m. nitrite-N

Soil series	p.p.m. nitrate-N before irradn.	p.p.m. nitrite-N: expected 3 days after 0.75 Mrad	p.p.m. nitrite-N: 14* or 21+ days after 0.75 Mrad & NO_2 addition	Deficit	Maximum p.p.m. N lost as N_2O
Broad	98.5	41.6	68.9*	72.7	21.2
Broadmoor	58.0	53.2	61.0+	92.2	27.1
Icknield	71.5	44.6	12.7*	131.9	15.1

Soil series	Incubation time (days)	% by volume at 20°C & 760 mm Hg†: Nitrogen	Oxygen
Broad	7	81.19	16.14
	14	79.09	14.06
Broadmoor	7	81.95	15.69
	21	81.79	13.57
Icknield	7	79.35	12.51
	14	81.55	10.34
Normal atmospheric concentrations		78.11	20.96
L.S.D. p 0.05 =		5.55	3.02
L.S.D. p 0.01 =		12.80	6.97

†in atmosphere within closed system for N_2O determination by infra-red absorption method

may remove nitrite are oxidation back to nitrate by Nitrobacter, which is known to be active after such a dose (Cawse and White, 1969a), or the organic matter fixation and chemical decomposition mechanism proposed by Nelson and Bremner (1969).

The decline in nitrous oxide in all soils after 7 - 9 days was similar to the behaviour without added nitrite (Figure 7-2.1), and the decrease after 21 days' incubation of Broadmoor series was just over 50% of the maximum concentration observed. As far as anaerobic conditions are concerned, it is known that nitrous oxide can be rapidly reduced to nitrogen (Cady and Bartholomew, 1960), and Cooper and Smith (1963) suggest that organisms utilise nitrous oxide in preference to nitrite; microbial action is almost certainly responsible for such reduction, and it has been demonstrated that resting cells of Pseudomonas denitrificans can reduce nitrous oxide to nitrogen in pure culture (Sacks and Barker, 1952). In the present work, considerable amounts of nitrite still remained throughout the period of nitrous oxide decline, and nitrite concentration could not have been limiting gas production.

Meek and MacKenzie (1965) reported that addition of 100 and 200 p.p.m. nitrite-N to a calcareous soil (pH 7.8) at field capacity resulted in extensive formation of nitrous oxide over 7 days of incubation, followed by a decline after 21 days. This observation compares closely with the behaviour of nitrous oxide in irradiated soils, and it is also interesting to compare some quantitative data. For example, application of 200 p.p.m. nitrite-N to their soil type gave a nitrous oxide loss amounting to 0.6 mg nitrous oxide-N / 100 g dry weight, or 3% of the nitrite which was added, whereas Broadmoor series had approximately 150 p.p.m. nitrite-N and released a maximum of 2.7 mg nitrous

oxide-N / 100 g dry soil, or 18% of the nitrite present after irradiation. Meek and MacKenzie (1965) also failed to detect significant alterations in the concentration of gaseous nitrogen when nitrite was applied.

The similarity between release of nitrous oxide in relation to time either with or without added nitrite did not suggest that chemical decomposition of nitrite was very important, otherwise more rapid evolution of the gas should have followed the application of 100 p.p.m. nitrite-N after irradiation; the gradual increase in nitrous oxide after irradiation was more typical of microbial action.

The unimportance of chemical decomposition was confirmed by the behaviour of autoclaved and irradiated soils to which 100 p.p.m. nitrite-N had been added; they did not release detectable (<20 µg) amounts of nitrous oxide-N, and after 14 days of incubation the remaining nitrite-N was within the range 101 - 113 p.p.m.

With regard to the decrease in evolution of nitrous oxide at low soil moisture (Table 7-2.3), it was again noted that sufficient nitrite was present to avoid any possibility that it was limiting reduction to nitrous oxide. Consequently, the absence of water may have restricted substrate (nitrite) movement to sites of reduction, or may have permitted gaseous diffusion of oxygen to anaerobic zones and lessened the need for microbial reduction of nitrite.

In conclusion, although nitrous oxide was readily released from irradiated soils kept under aerobic conditions, almost certainly by microbial reduction of nitrite, the amount of nitrite-N lost in this gaseous form only accounted for part of the total calculated deficit (especially in Icknield series), and the emission of other nitrogen oxides was studied next.

Table 7-2.3 Nitrous oxide released from irradiated Broadmoor soil at different moisture*

Days after irradiation (0.75 Mrad)	µg N_2O-N / 30 g dry soil		
	20	30	40% MWHC
1	<20	285	387
2	<20	434	563
3	112	534	675
4	-	563	769
6	149	511	737
7	147	491	717
	p.p.m. nitrite-N		
7	105.0	109.0	77.1

L.S.D. N_2O-N p 0.05 = 65, p 0.01 = 150

L.S.D. NO_2-N " = 5.9, " = 13.5

* 100 p.p.m. nitrite-N added

8 Release of nitrogen dioxide and nitric oxide by soils after 0.75 Mrad

8-1 Nitrogen dioxide and nitric oxide formed after irradiation of three soils

Nitrogen dioxide and nitric oxide are known to be released from soil during denitrification (Cady and Bartholomew, 1960; Cooper and Smith, 1963), but the amount which appears is usually small relative to nitrous oxide and nitrogen. This is not surprising in view of the solubility of nitrogen dioxide in water, but it was thought that the aerobic nature of the soils at 60% MWHC and the presence of nitrite after irradiation might result in greater evolution of gases, and the possibility was examined.

Materials and Methods:

Broad, Broadmoor and Icknield soils having the same initial nitrate content as indicated in Table 7-2.2 were raised to 60% MWHC, and samples containing 30 g dry weight were treated with 0.75 Mrad. Immediately after irradiation, they were connected to a flow-through system for measurement of nitrogen dioxide and nitric oxide (Section 2-3.4) released over a period of 14 days at laboratory temperature of approximately 20°C: during this time, the percentage soil moisture fell by 8%.

Another set of samples was autoclaved before irradiation to prove that nitrite accumulation and emission of nitrogen dioxide and nitric oxide were related, in a similar manner to the nitrous oxide studies.

Results:

The cumulative evolution of nitrogen dioxide and nitric oxide

with time is shown in Figure 8-1 together with the relative percentage of nitric oxide, the main component. The slow oxidation of low concentrations of nitric oxide has already been discussed in Section 2-3.4. The ratio of total N released as nitrogen dioxide and nitric oxide over 14 days relative to the maximum nitrous oxide-N released was low in Broad and Broadmoor soils (0.9 and 1.3 : 1 respectively), but as high as 3.2 : 1 in Icknield series, and may have resulted from less intensive reduction to nitrous oxide or more rapid release of gas from the latter soil type.

Unirradiated treatments never released >20 µg N as nitrogen dioxide and nitric oxide over the whole 14 days. When soils were autoclaved before irradiation the release never exceeded 33 µg N, proving that prevention of nitrite accumulation by microbial action also inhibited the formation of nitrogen dioxide and nitric oxide, as well as the production of nitrous oxide.

8-2 Nitrogen dioxide and nitric oxide formed after addition of nitrite to irradiated soils

Materials and Methods:

Broad, Broadmoor and Icknield soils were treated with (i) autoclaving + 0.75 Mrad, and (ii) 0.75 Mrad alone, and 3 mg of nitrite-N was immediately added to each sample of 30 g dry weight, giving a final moisture of 60% MWHC. The release of gases was measured over 14 days in the case of Broad and Icknield soils, but was extended to 21 days for Broadmoor series to correspond with nitrous oxide studies. Finally, the residual nitrite was determined for calculation of the deficits.

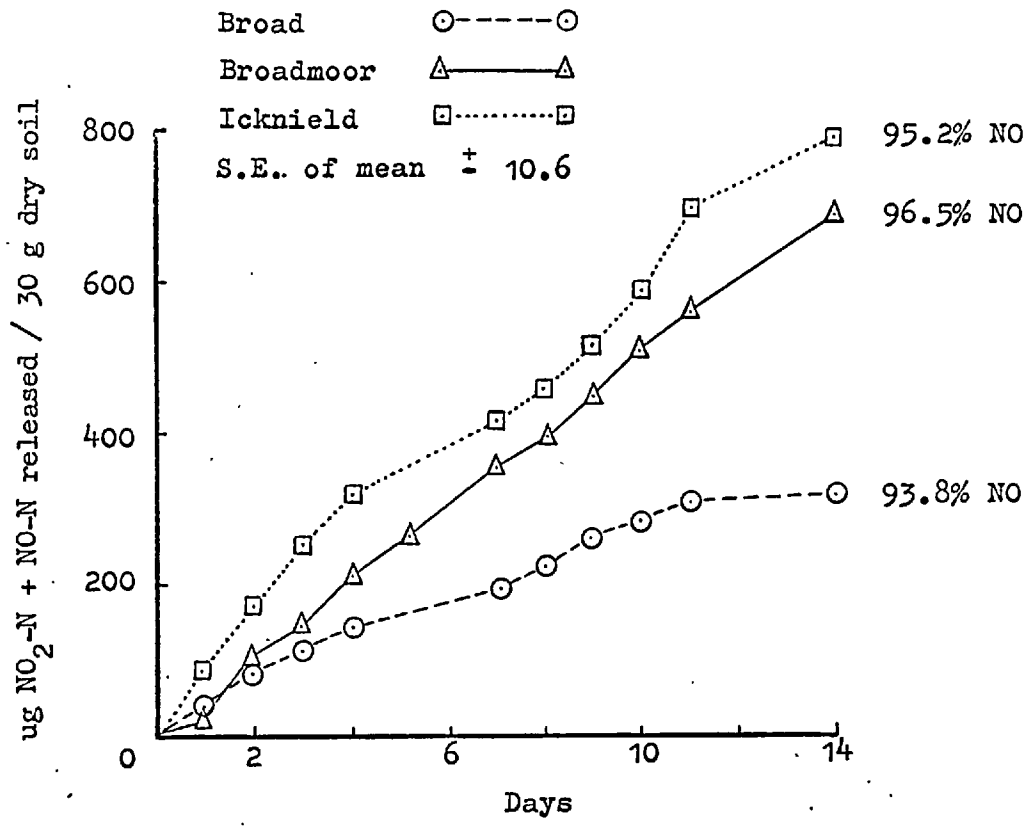


Figure 8-1 Nitrogen dioxide and nitric oxide formed after treatment of three soils with 0.75 Mrad

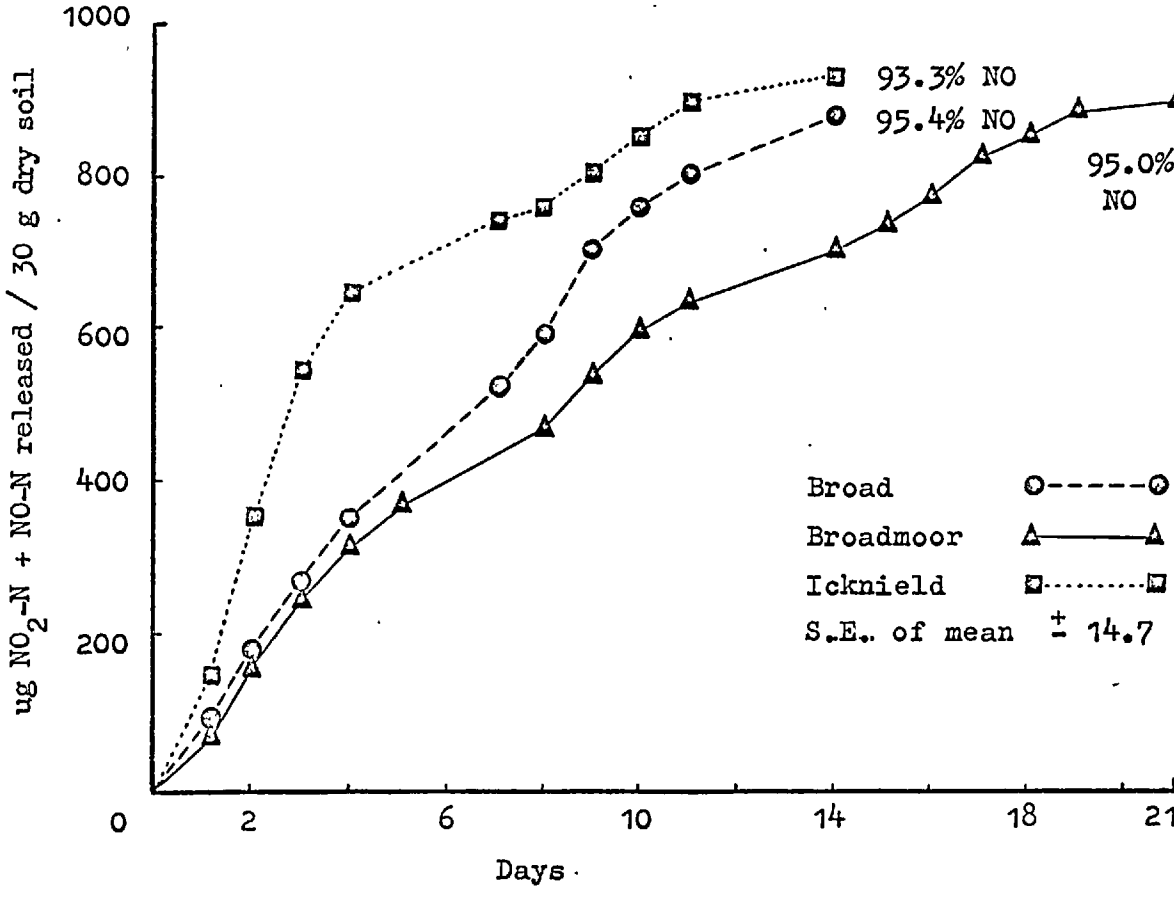


Figure 8-2 Nitrogen dioxide and nitric oxide formed after treatment of three soils with 0.75 Mrad and addition of 100 p.p.m. nitrite-N

Results:

Addition of nitrite to two of the irradiated soils increased the release of nitrogen dioxide and nitric oxide above the rate observed after irradiation alone (Figure 8-2), but little extra gas appeared in Broadmoor series and may perhaps be explained by rapid reduction, suggested by the high yield of nitrous oxide in this soil (Figures 7-2.1 and 7-2.2). In all series, the nitrogen released as nitrogen dioxide and nitric oxide was considerably less than the calculated deficits of nitrite-N, and when the nitrogen detected as nitrous oxide was added it was evident that the calculated deficits were still larger, especially in Icknield soil (Table 8-2). Release of gaseous nitrogen and oxidation of nitrite to nitrate were suspected as principal factors contributing to removal of nitrite, and were studied by means of ^{15}N tracer in the next Section.

Soils which were autoclaved before irradiation and addition of nitrite gave negligible evolution of nitrogen dioxide and nitric oxide, not exceeding 82 µg N (equivalent to 2.7 p.p.m. N on a soil dry weight basis) over 14 days: after this period, the residual nitrite-N in the three soils was within the range 86 - 112 p.p.m., being lowest for Icknield series. These observations proved that biological activity rather than chemical decomposition was mainly responsible for production of the two gases, in agreement with results from parallel experiments on the release of nitrous oxide.

Losses of 29.6 - 31.1 p.p.m. N over 14 days as nitrogen dioxide and nitric oxide from Broad and Icknield series by biological reduction of nitrite can be compared with losses in soils of lower pH, where chemical decomposition of nitrite would be the predominant mechanism of reduction. For example, Wagner and Smith (1958) reported

2.3 p.p.m. nitrogen dioxide + nitric oxide-N lost over 14 days from a soil of pH 5.6 with 500 p.p.m. of urea-N added, and Reuss and Smith (1965) found that 90 p.p.m. nitrogen dioxide + nitric oxide-N was released over 6 days from soil at pH 4.6 which had received 400 p.p.m. nitrite-N.

Comparison of nitrite deficits in the closed nitrous oxide system with the present continuous flow method showed good agreement apart from Broadmoor series (Table 8-2), which was incubated for a longer period of 21 days: the lowering of oxygen concentration to 13.57% by volume may have stimulated greater reduction of nitrite in the closed apparatus. However, owing to the different techniques employed, it is impossible to closely relate the preceding results for nitrous oxide with the findings for nitrogen dioxide and nitric oxide. The intermittent circulation of the atmosphere in the closed system used for nitrous oxide studies would permit better absorption, re-oxidation, or reduction of nitrogen dioxide and nitric oxide by the soil, and this limitation was recognised. Nevertheless, the experiments made with the continuous flow method have successfully demonstrated that there is very great stimulation of nitrogen dioxide and nitric oxide production in soils following treatment with 0.75 Mrad at medium moisture, with normal atmospheric concentrations of carbon dioxide and oxygen always present in the soil air.

Table 8-2 **Residual nitrite concentrations after incubation of soils treated with 0.75 Mrad and 100 p.p.m. nitrite-N**

Soil series	p.p.m. nitrite-N			p.p.m. N lost	
	expected 3 days after 0.75 Mrad	14* or 21+ days after 0.75 Mrad & NO_2 addition	Deficit	as $NO_2^{\ddagger}$+NO	as $NO_2^{\ddagger}$+NO+N_2O
Broad	41.6	69.5*	72.1	29.6	50.8
Broadmoor	53.2	83.1+	70.1	30.1	57.2
Icknield	44.6	13.5*	131.1	31.1	46.2

$NO_2^{\ddagger}$ = nitrogen dioxide

NO_2 = nitrite

9 Fate of ^{15}N-labelled nitrite in Icknield soil following application of 0.75 Mrad

The particularly extensive fall in nitrite concentration in irradiated (0.75 Mrad) Icknield soil, firstly during incubation in closed and flow-through systems in which air was passed through the soil (Tables 7-2.2 and 8-2), and secondly under normal conditions of incubation (Figures 10-2.1.2 and 10-2.1.3), prompted further study of the pathways concerned in nitrite removal.

Previous experiments had indicated that the release of nitrous oxide, nitrogen dioxide and nitric oxide only partly accounted for the total nitrite deficit observed, and it was decided to utilise ^{15}N-labelled nitrite to investigate the extent of oxidation to nitrate, loss as gaseous products and fixation to organic matter; the soil would be incubated in a normal manner, without disturbing the soil air, therefore only small losses of nitrogen dioxide and nitric oxide would be expected. The high organic content of Icknield series would favour fixation reactions, but the rapid nitrification recorded after treatment in the 0.75 Mrad region (Section 4-1) suggested that extensive oxidation could well be mainly responsible for removal of nitrite.

Materials and Methods:

Four samples of 75 g soil at 40.7% moisture were treated with 0.75 Mrad, and after allowing 24 hr for the nitrite concentration to rise (shown by preliminary tests to reach 29.8 p.p.m. nitrite-N in this time), approximately 1 mg of nitrite-N was added from ^{15}N-labelled sodium nitrite solution (92.81 atom % ^{15}N excess) with sufficient water to raise the soil moisture to 60% MWHC. Later, the nitrite-N added was accurately determined as 1.029 mg N. After incubation for 14 days at 25°C, the organic-N and mineral-N fractions were separated for

determination of their ^{15}N content.

For separation of organic-N, 5 g was removed from the main sample and weighed accurately. It was shaken four times for 20 minutes on each occasion with 25 ml of N potassium chloride, centrifuging at 30,000 G for 40 minutes after each shake. The supernatant fractions were rejected, and the precipitate was transferred to a Kjeldahl flask for acid digestion by the macro-method including glucose (Section 2-3.1); ^{15}N was determined in the digest (Section 2-3.2). Organic-N was analysed in incubated control and irradiated samples to detect any differences in the solubility of organic matter.

For separation of mineral-N, 70 g of soil was shaken for 1 hr with 150 ml of N potassium chloride, and filtered. Nitrite was analysed in 0.2 ml of filtrate, and 25 ml aliquots were steam-distilled to obtain four fractions:

(i) ammonium-N by distillation with magnesium oxide.

(ii) nitrate-N by destruction of nitrite with sulphamic acid, followed by distillation with Devarda's alloy.

(iii) nitrate + nitrite-N by distillation with Devarda's alloy after distillation (i) to remove ammonium-N.

(iv) total mineral-N by distillation with magnesium oxide and Devarda's alloy, and combination of the distillates.

Determination of the ^{15}N recovery in (iv) permitted an additional check on the recovery obtained in fractions (i) and (iii). It was impossible to separately determine the ^{15}N ratio in the nitrite pool because practically all of it was removed during incubation. The residues in the distillation flasks were retained for micro-Kjeldahl analysis, but it was found that less than 2 p.p.m. N on a soil dry weight basis remained.

After quantitative determination of nitrogen present in the distillates from each fraction, the ^{15}N-enrichment was found by mass spectrometry of nitrogen gas and the recovery of tracer in the respective pools was calculated.

Separate tests with 10 g samples showed that 38.6 p.p.m. nitrite-N had been formed by 3 days after 0.75 Mrad, without tracer being added; when 19.5 p.p.m. of tracer nitrite-N was incorporated at 24 hr after irradiation, the level at the end of 3 days only amounted to 45.5 p.p.m. nitrite-N. This implied that some nitrite had been removed between 1 and 3 days of incubation.

Results:

The inorganic- and organic-N present before and after incubation is shown in Table 9-1, and the gross size of the nitrogen pools together with their atom % ^{15}N excess appear in Table 9-2. Recoveries of tracer were calculated as percentages of the 955 µg ^{15}nitrite-N initially added, and there was good agreement between the recovery of ^{15}N calculated from analysis of the total mineral-N fraction and the sum of ^{15}N found in separate ammonium and nitrate + nitrite pools.

The large amount of nitrite recovered in the nitrate pool proved that there had been very extensive oxidation after irradiation which led to the removal of just over half of the added nitrite, no doubt by the continued metabolism of Nitrobacter cells.

The next important pathway of nitrite removal was volatilisation, with 28% of tracer unaccounted for in the nitrogen pools. The total decrease in nitrite in the sample between 3 and 14 days of incubation amounted to 2047 µg N (38.4 p.p.m. X 53.3 g dry weight of soil), and making the assumption that $^{14}NO_2$ and $^{15}NO_2$ were both lost at the same rate, the loss by volatilisation (28%) was 573 µg nitrite-N or 10.8 p.p.m.N

Table 9-1 Mineral-N and organic-N in Icknield soil after 0.75 Mrad

Form of N (± S.E. of mean)	p.p.m. organic-N or mineral-N				
		3 days incubn. after irradn.		14 days incubn. after irradn.	
	before irradn.	without tracer	with tracer*	control	0.75 Mrad + tracer†
NH_4 ± 0.4	2.5			2.8	82.5
NO_2 ± 0.6	0.5	38.6	45.5	0.7	7.1
NO_3 ± 3.7	95.3			111.6	199.7
Organic ± 46.2	10,536			10,572	10,513

* 19.5 p.p.m. nitrite-N added 24 hr after irradiation

† 955 µg $^{15}NO_2$-N added to 53.3 g dry weight of soil

Table 9-2 Recovery of $^{15}NO_2$ tracer in Icknield soil 14 days after 0.75 Mrad

N pool in which recovered	atom % ^{15}N excess in duplicate samples*	mean	µg ^{15}N present	% recovery of ^{15}N
NH_4	0.231 ± 0.001 0.290 ± 0.001	0.261	11.5	1.2
NO_3	5.29 ± 0.01 4.99 ± 0.01	5.14	547.1	57.3
NO_3 + NO_2	5.38 ± 0.01 5.04 ± 0.01	5.21	574.3	60.1
Total mineral-N	3.87 ± 0.01 3.83 ± 0.01	3.85	593.7	62.2
Organic-N	0.020 ± 0.001 0.018 ± 0.001	0.019	106.5	11.2

* ± standard deviation of each ^{15}N analysis from 10 determinations per individual gas sample

on a soil dry weight basis. This is in reasonable agreement with a nitrous oxide-N release of 8.1 p.p.m. and 15.1 p.p.m. N, with and without added nitrite respectively (Section 7), if it is assumed that the evolution of other oxides of nitrogen was negligible under the present conditions of incubation. Previous experiments in Sections 7 and 8 had shown that autoclaving before irradiation practically eliminated the release of oxides of nitrogen from soil to which nitrite was added, therefore volatilisation must have occurred mainly by microbial action.

Investigators who have estimated the recovery of nitrite applied to soil have tended to concentrate on types of lower pH, where chemical decomposition is likely to intensify the loss: some mechanisms which may be involved are discussed in Section 11-2. However, Clark et al. (1960) found that in soils of pH 7.3 - 8.3, 7.5% of added nitrite was removed after 4 weeks at 50% MWHC, but the method of analysis used meant that fixation to organic matter as well as volatilisation could have been responsible. With regard to loss by volatilisation, Ekpete and Cornfield (1964) reported that a soil of pH 6.5 kept at 50% MWHC lost 22% of its nitrate-N over 12 weeks at 28°C. It was clear that in the present tracer / irradiation experiment, the extent of volatilisation was high for a soil at medium moisture (with extensive release of nitrous oxide as supporting evidence), even though the nitrite pool was depleted to a greater extent by microbial oxidation to nitrate.

Fixation of nitrite to organic matter accounted for 11.2% of the total decrease observed, and the possible mechanism of fixation is mentioned later (Section 11-2). Comparison of the loss by fixation with work on unirradiated soils (Bremner and Nelson, 1968; Nelson and

Bremner, 1969) is difficult owing to the high pH and organic matter content of Icknield soil and a longer incubation time. For example, Nelson and Bremner (1969) found that soils in the pH range 7.1 - 7.8 only fixed 1% of added nitrite in 4 days, but none of them possessed more than 2.9% of organic carbon.

Finally, a negligible amount of ^{15}N was recovered in the ammonium pool, and probably originated from microbial assimilation followed by lysis of some cells during the incubation period, as noticed by Overrein (1967) with ^{15}N-labelled nitrate distribution in soil which had been incubated for 10 days.

10 Radiolytic reduction of nitrate in aqueous solution and in soils over an extended dose range, up to 12 Mrad

10-1 Reduction of nitrate by radiolysis in aqueous solution

There have been several studies on the effects of gamma radiation on aqueous solutions of nitrate salts and nitrate ices as part of basic research in radiation chemistry (Mahlman and Schweitzer, 1958; Kevan, 1966; Daniels and Wigg, 1967), but it has been pointed out that many aspects of the radiolysis mechanism remain uncertain (Pikaev, 1967).

Daniels and Wigg (1967) proposed a scheme whereby capture of the solvated electron by nitrate leads to formation of nitrite, with H atoms acting similarly to electrons, competing for NO_3^- :

$$e^- + NO_3^- = NO_3^{2-}$$
$$NO_3^{2-} + H^+ = HNO_3^-$$
$$H^\circ + NO_3^- = HNO_3^-$$
$$HNO_3^- = OH^- + NO_2^\circ$$

and in the presence of oxygen, the following reaction can compete for solvated electrons and hydrogen atoms:

$$e^- + O_2 = O_2^-$$
$$H^\circ + O_2 = HO_2^\circ$$

Finally, there is a hydrolytic dismutation process:

$$2\,NO_2^\circ + H_2O = NO_3^- + 2\,H^+ + NO_2^-$$

but nitrite ions can be partially oxidised by OH° radicals produced by the radiolysis of water:

$$OH^\circ + NO_2^- = OH^- + NO_2^\circ$$

To assess the extent of nitrite formation by radiolysis in

irradiated soils, soil solutions were first extracted for irradiation with various doses, and their response was later compared with pure solutions of nitrate salts. It was thought that if enzymes contributed to nitrite formation, their effect in soil solution would be negligible owing to adsorption on clay particles.

Materials and Methods:

Broad, Icknield, Fyfield and Grove soils were adjusted to 70, 70, 30 and 45% moisture respectively. The first two series were chosen as very positive "nitrite-formers" after irradiation, while the Fyfield and Grove types were negative. The soil solutions were removed by a centrifugation technique described by Bowen and Cawse (1964b) and were analysed for nitrate; the pH's were also measured.

First, solutions from all series were treated with 0.5, 1.0, 2.0 and 4.0 Mrad for analysis of nitrite immediately after irradiation (within 10 minutes); solutions from Broad and Icknield series were also given 0.8 and 0.6 Mrad respectively for analysis of nitrite after 64 hr incubation, because these were the radiation doses that gave maximum accumulation of nitrite in the fresh soils, and a direct comparison of soil and solution response was required.

Secondly, potassium nitrate solutions containing 100, 200 and 400 p.p.m. nitrate-N were adjusted to pH 8.0 with 0.1 N potassium hydroxide, and analysed for nitrite immediately after treatment over the range 0.5 - 12 Mrad. Effects from the associated cations Ca, K, Mg and Na were examined by irradiating 400 p.p.m. nitrate-N solutions with 4 Mrad, and analysing for nitrite immediately afterwards.

Thirdly, to confirm that nitrate reduction was taking place on irradiation, both soil solutions and potassium nitrate solution were analysed for nitrate before and after treatment with 4 Mrad, so that the

loss in nitrate could be compared with nitrite increases observed in the first experiment in this Section.

The temperature of the solutions during irradiation was 22°C, and in this connection, Daniels and Wigg (1967) found that between 25 - 60°C there was little change in nitrate reduction in an irradiated 1.6×10^{-3} M solution of sodium nitrate. All solutions in the present tests were irradiated in glass tubes which were not air-tight.

Results:

Table 10-1.1 shows that nitrite accumulated in irradiated soil solutions, almost certainly by radiolysis, but in the case of Broad and Icknield series the amounts formed were not so great as in irradiated soils at the same moisture. Furthermore, nitrite failed to accumulate rapidly with time in the solutions, whereas it did in irradiated soils:

Soil and treatment	p.p.m. NO_2-N 64 hr after irradn.	
	in soil	in soil solution
Broad, 70% moisture, 0.8 Mrad	35.6	7.3*
Icknield " " , 0.6 Mrad	35.5	6.0*

* calculated as p.p.m. dry weight soil

The above observation suggested that the accumulation of nitrite in irradiated soils was the net result of two components: a microbial / enzymic one, and radiolysis. The contribution of radiolysis would be smaller up to a dose of 2 Mrad, but above this it would be expected to increasingly exceed microbial / enzymic reduction as the latter becomes inhibited by radiation and the radiolytic yield of nitrite rises with dose. The production of more nitrite in Broad and Icknield series compared with the Fyfield and Grove types was no doubt due to differences in nitrate concentration at the time of irradiation.

Table 10-1.1 Effect of radiation dose on nitrite formation in soil solutions immediately after treatment

Dose (Mrad)	p.p.m. nitrite-N in soil solution* Broad (pH 7.3)	Fyfield (pH 7.3)	Grove (pH 6.8)	Icknield (pH 7.5)
0	<0.05	0.1	0.1	<0.05
	(<0.03)	(0.03)	(0.05)	(<0.03)
0.5	5.4	4.6	4.3	6.8
	(3.8)	(1.4)	(1.9)	(4.8)
1.0	9.7	7.2	7.1	11.4
	(6.8)	(2.2)	(3.2)	(8.0)
2.0	13.5	8.7	8.7	14.2
	(9.5)	(2.6)	(3.9)	(9.9)
4.0	23.8	8.8	10.2	23.2
	(16.7)	(2.6)	(4.6)	(16.2)
	p.p.m. nitrate-N in soil solution at irradiation			
	272.0	29.6	43.2	185.2

L.S.D. nitrite-N p 0.05 = 1.0, p 0.01 = 2.4

* figures in parentheses represent nitrite in solution calculated on a p.p.m. dry weight soil basis

Table 10-1.2 Effect of radiation dose on nitrite formation in potassium nitrate solution at different concentrations

Dose (Mrad)	p.p.m nitrite-N immediately after irradiation 100 p.p.m. NO_3-N	200 p.p.m. NO_3-N	400 p.p.m. NO_3-N
0	<0.05	<0.05	<0.05
0.5	2.2	2.7	3.0
1.0	3.2	4.7	5.6
2.0	3.2	6.9	10.1
4.0	2.1	6.7	16.2
8.0	1.6	4.2	17.6
12.0	1.6	4.1	15.9

L.S.D. p 0.05 = 0.5, p 0.01 = 1.2

Table 10-1.2 shows that at all doses, increasing the nitrate concentration raised nitrite yield, or "G NO_2^-" : the "G" value used in radiation chemistry represents molecules of product formed per 100 eV of energy absorbed. The results also establish that greater G NO_2^- values are obtained with increasing dose (at least up to 8 Mrad for 400 p.p.m. nitrate-N solution), although with 100 and 200 p.p.m. nitrate-N a rise from 2 to 4 Mrad gave no extra yield, and with 400 p.p.m. nitrate-N there was less nitrite present after 12 Mrad than 8 Mrad. Such behaviour was confirmed by repeated tests, and suggested that nitrate concentration and instability of nitrite could be affecting G NO_2^-. A similar effect of nitrate concentration was also evident with Fyfield and Grove soil solutions which were low in nitrate, and gave little extra nitrite yield between 2 and 4 Mrad compared with Broad and Icknield solutions having high nitrate concentrations (Table 10-1.1).

The G value can be calculated from the present data: for example, a 400 p.p.m. nitrate-N solution treated with 2 Mrad yields 10.1 p.p.m. nitrite-N, or 33.2 p.p.m. nitrite, and it follows that:

$$\text{Weight of one molecule } NO_2 = \frac{46}{6.023 \times 10^{23}} = 7.64 \times 10^{-23} \text{ g}$$

$$\text{or } 7.64 \times 10^{-17} \mu\text{g}$$

$$\therefore \text{ molecules formed / g} = \frac{33.2}{7.64 \times 10^{-17}} = 4.35 \times 10^{17}$$

$$1 \text{ rad} = 6.24 \times 10^{13} \text{ eV / g}$$

$$\therefore \text{ 2 Mrad} = 2 \times 6.24 \times 10^{19} \text{ eV / g}$$

$$\text{and molecules formed / g / 100 eV} = \frac{4.35 \times 10^{17} \times 10^{2}}{2 \times 6.24 \times 10^{19}}$$

$$\text{i.e. } G = 0.35$$

Table 10-1.3 **Effect of associated cation on nitrite formation in irradiated nitrate (400 p.p.m. NO_3-N) solutions**

Cation	p.p.m. nitrite-N immediately after treatment with 4 Mrad
Ca	15.5
K	14.9
Mg	14.6
Na	15.4
L.S.D. p 0.05 = 0.8, p 0.01 = 1.8	

Table 10-1.4 **Reduction of nitrate to nitrite in irradiated soil solutions**

Soil	p.p.m nitrate-N		
	Control	immediately after 4 Mrad	Loss
Broad	272.0	252.0	20.0
Fyfield	29.6	18.4	11.2
Grove	43.2	31.5	11.7
Icknield	185.2	161.6	23.6
KNO_3 solution	392.0	377.0	15.0
L.S.D. p 0.05 = 7.8, p 0.01 = 18.0			

For a 200 p.p.m. nitrate-N solution given 2 Mrad the G value was 0.24, and soil solution from Broad series treated with 2 and 4 Mrad (Table 10-1.1) gave G 0.46 and 0.41 respectively. These values compare with G 0.35 quoted by Daniels and Wigg (1967) for aerated neutral 140 p.p.m. nitrate-N solution (from sodium nitrate).

There was little effect of cation on nitrite yield (Table 10-1.3) in agreement with Mahlman and Schweitzer (1958) who reported that alkali cations had no influence in 0.1 - 0.5 M nitrate solutions, although Kevan (1966) found that 0.2 M nitrate ices gave a decided effect after 0.11 Mrad: for example, G NO_2^- was 1.2 for sodium nitrate but 0.5 for potassium nitrate.

The nitrate analyses on soil solutions and potassium nitrate treated with 4 Mrad (Table 10-1.4) indicate that losses occur by reduction to nitrite. The extent of these losses is in close agreement with the increases in nitrite after 4 Mrad (Table 10-1.1), and confirms a reduction pathway. However, more nitrite was formed per µg of nitrate present in soil solution than in pure solution, and may have arisen from enzymic reduction in the former; when soil solution from Broad series was autoclaved at 15 lbs / sq. in. for 30 minutes before receiving 8 Mrad it was found that the accumulation of nitrite was not decreased, suggesting the absence of an enzyme factor. An alternative possibility is that the presence of organic compounds in the soil solution favoured free radical reactions leading to reduction of nitrate.

10-2 Production and behaviour of nitrite over a wide dose range, 0.5 - 12 Mrad

Experiments were designed to provide further evidence for the suggestion that nitrite can accumulate in irradiated soils by enzymic action up to a dose in the region of 2 Mrad, and by radiolytic reduction of nitrate at higher doses; the persistence of nitrite formed by both pathways was also of interest. All irradiations required for experiments described in Section 10-2 were carried out at dose rates between 0.616 - 0.660 Mrad / hr.

10-2.1 Production of nitrite in four soils between 0.5 - 12 Mrad

Materials and Methods:

Broad, Icknield, Fyfield and Grove soils were analysed for nitrate, and adjusted to 200 p.p.m. nitrate-N by adding a solution of potassium nitrate (3 mg nitrate-N / ml) to 800 g samples from each soil, together with sufficient water to raise their moistures to 70, 70, 30 and 45% respectively. It was necessary to provide a good concentration of nitrate to ensure that radiolytic reduction to nitrite at high dose levels would not be limited. As preliminary tests at 12 Mrad had indicated the presence of about 100 p.p.m. nitrite-N immediately after irradiation, it was decided to set the nitrate concentration well above this, at 200 p.p.m. nitrate-N on a dry weight basis for all soils.

After equilibration, samples of 10 g were treated with 0, 0.5, 0.75, 1, 2, 4, 6, 8, 10 and 12 Mrad: one set of samples was analysed for nitrite immediately afterwards, and the remainder were analysed after incubation for 7, 14 and 21 days. The polystyrene containers in which soils were irradiated and incubated permitted gaseous diffusion

through their perforated caps, and loosely fitting polythene bags were inverted over sets of samples to avoid microbial contamination during incubation. Tests on all soil types showed that when they were exposed to the air (not inoculated) after 12 Mrad, grey mould fungi became visible on the surface after only 3 - 4 days of incubation and were thus good indicators of chance contamination. The fungal growth did not appear under the conditions of the main experiment described here, even after 21 days of incubation.

To demonstrate the limiting effect of nitrate concentration on nitrite levels in irradiated soil, extra samples from Fyfield and Grove series were raised to only 40 p.p.m. nitrate-N before treatment with 2 - 12 Mrad, and were analysed immediately afterwards.

Results:

The amounts of nitrite detected at different times are shown in Figures 10-2.1.1 to 10-2.1.4. Immediately after irradiation, there was an almost linear response from all soils with a maximum of 106.6 p.p.m. nitrite-N present at 12 Mrad (G value 0.61); comparison with results from irradiation of soil solutions up to 4 Mrad (Table 10-1.1) showed that a considerably greater yield of nitrite was obtained on irradiation of the complete soil system, and this aspect was examined later. Figure 10-2.1.1 also includes the result from Fyfield and Grove soils at 40 p.p.m. nitrate-N, and demonstrates the limiting effect of initial nitrate level on nitrite formation.

The situation after 7 days had altered significantly, and Figure 10-2.1.2 indicates two major changes. Firstly, there was the expected increase in nitrite over the range 0.5 - 1 Mrad in Broad and Icknield soils (thought to occur by microbial enzyme activity) without any corresponding increase in Fyfield and Grove series. Such behaviour was

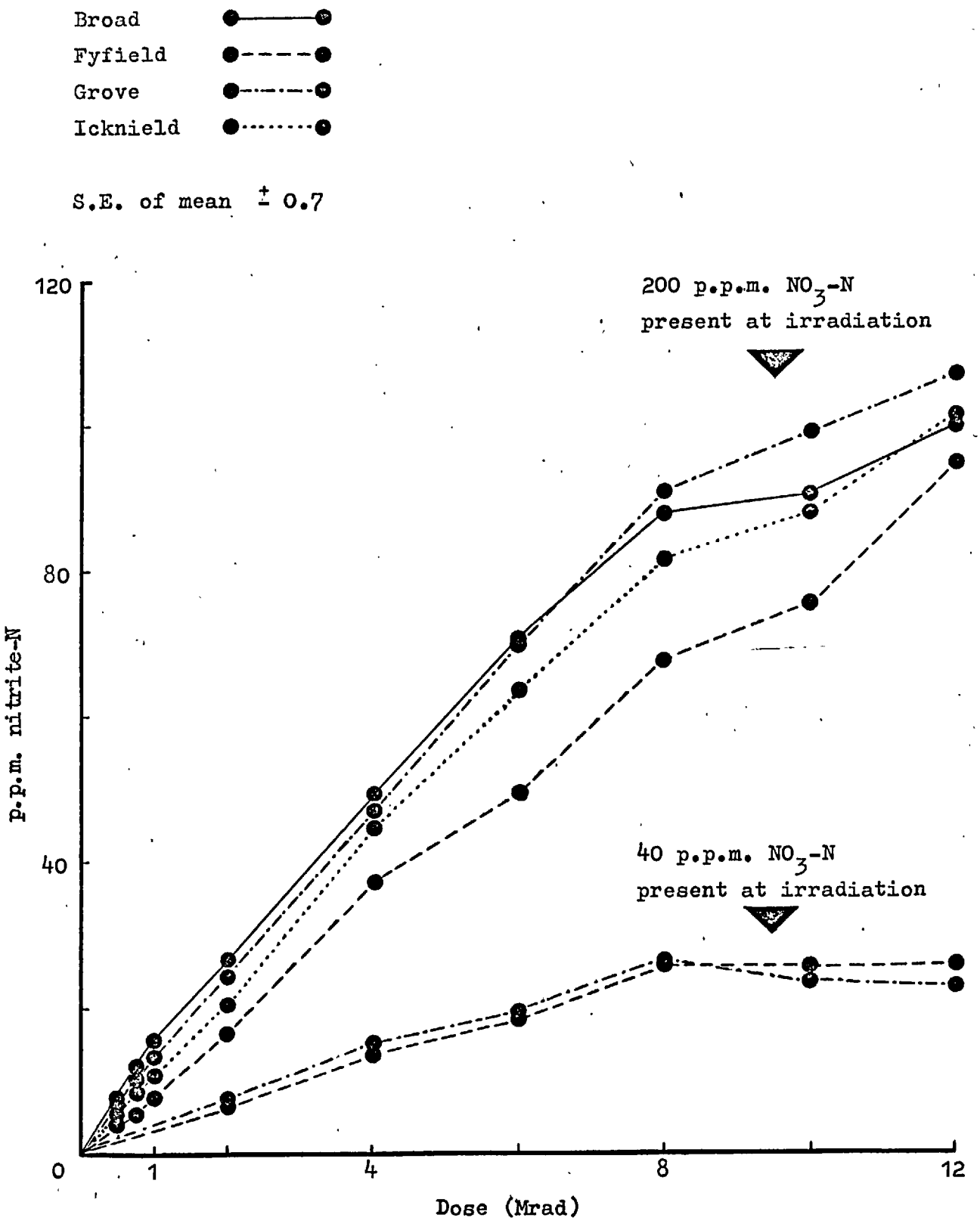


Figure 10-2.1.1 Amounts of nitrite present in soils immediately after irradiation

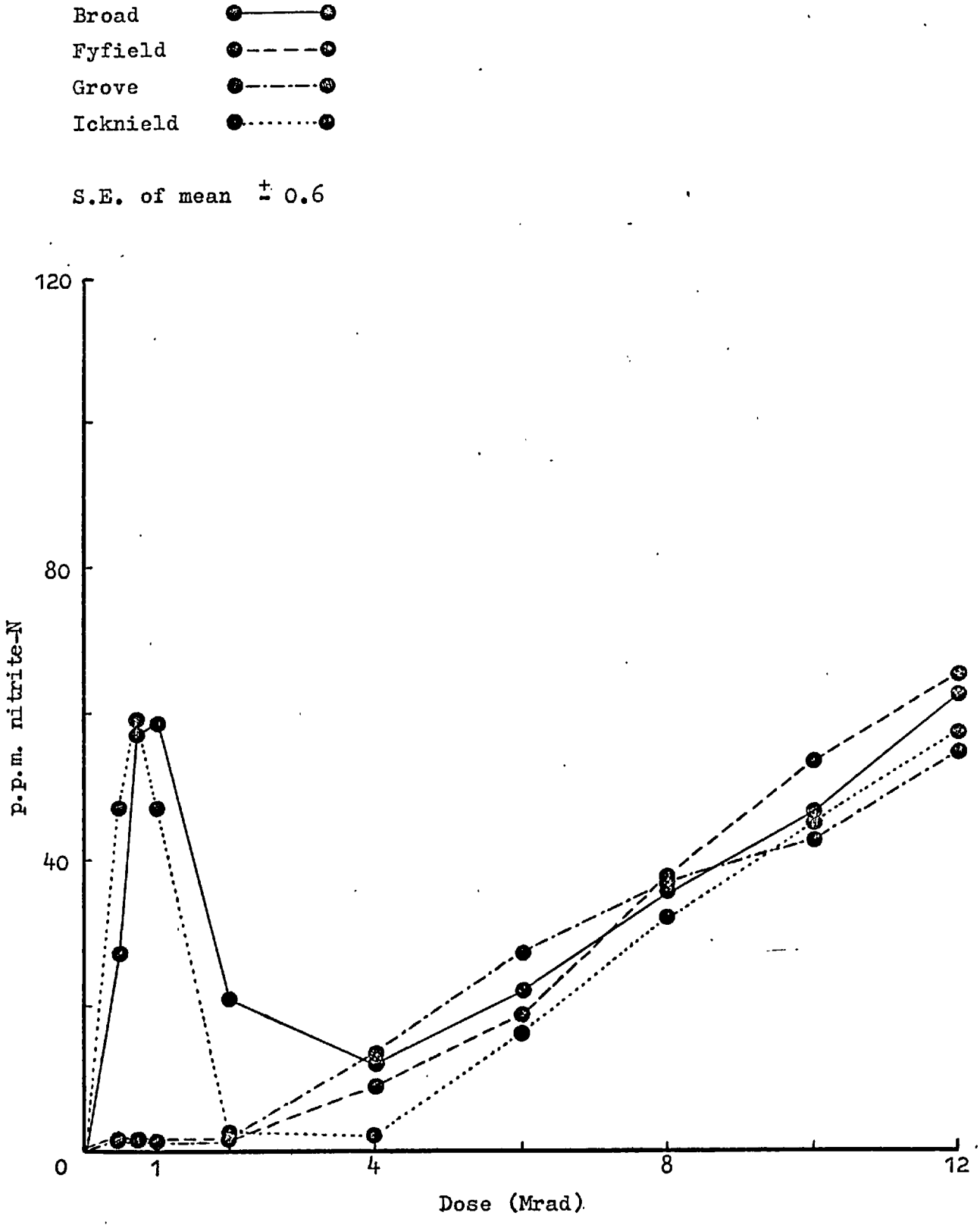


Figure 10-2.1.2 Amounts of nitrite present in soils at 7 days after irradiation

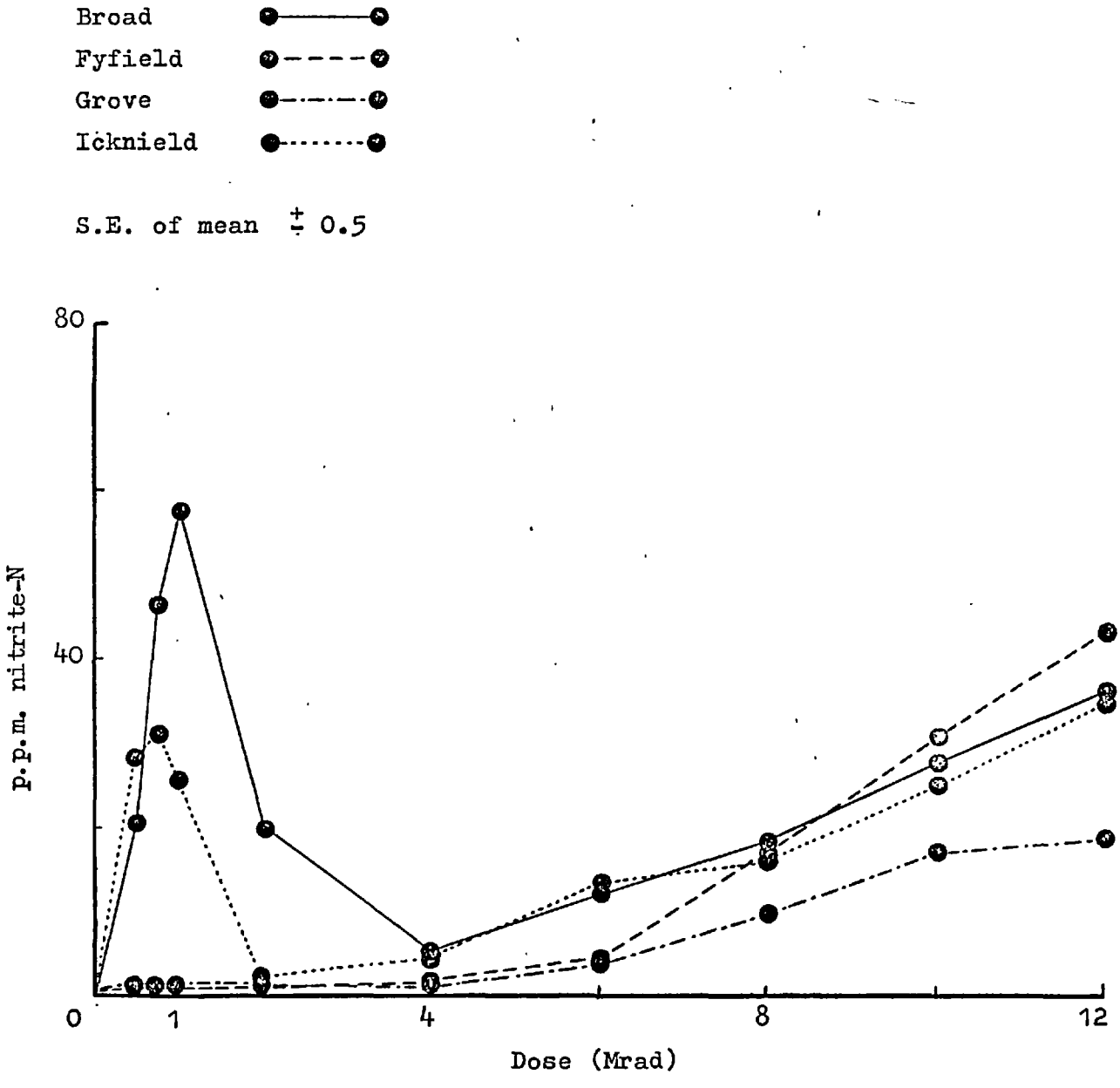


Figure 10-2.1.3 Amounts of nitrite present in soils at 14 days after irradiation

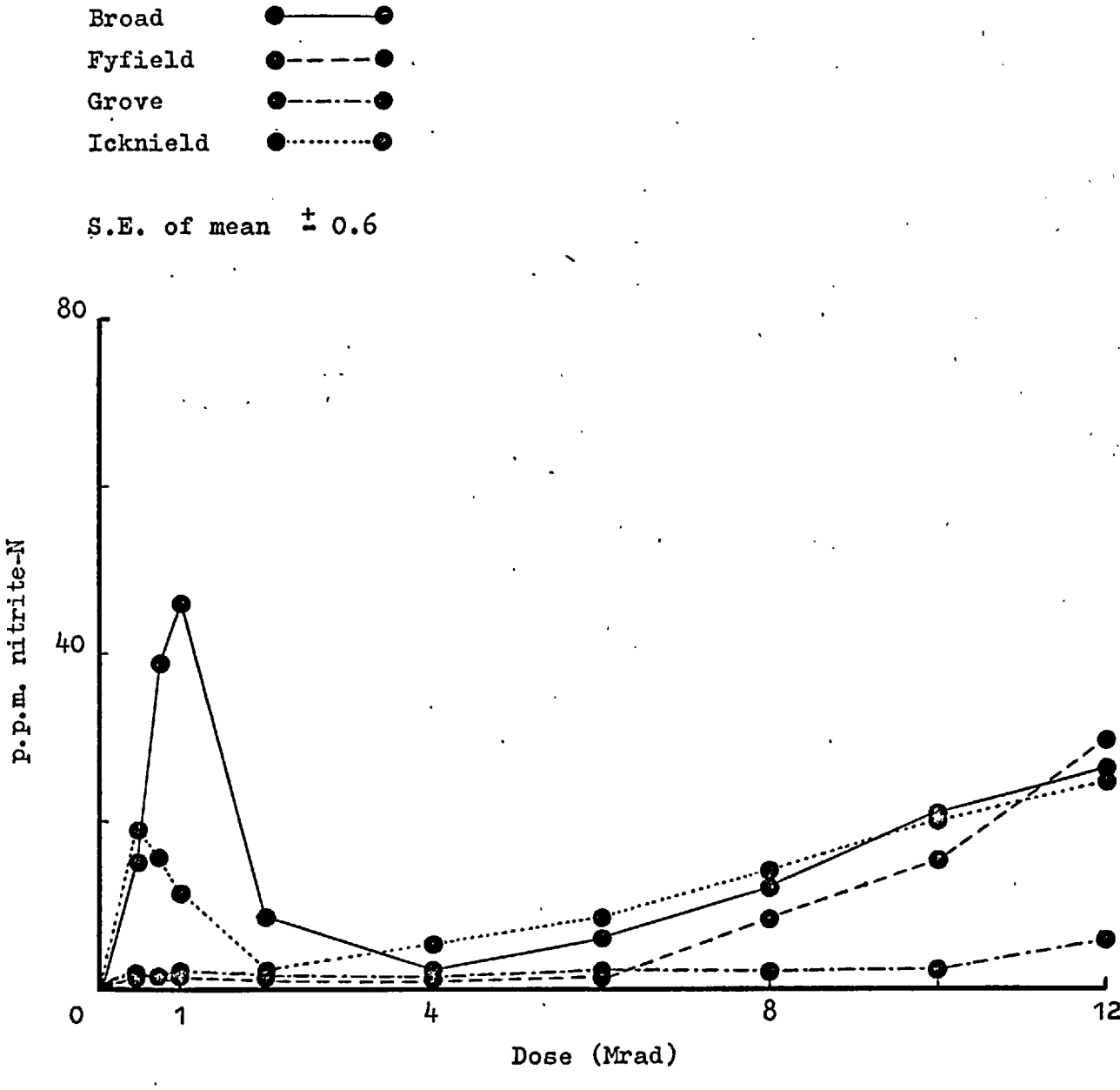


Figure 10-2.1.4 Amounts of nitrite present in soils at 21 days after irradiation

satisfactory because the latter types were selected for their inability to form nitrite in this dose region. Nevertheless, from the observation that all soils yielded similar concentrations of nitrite above 4 Mrad, it appeared that a common radiolytic pathway was responsible.

The second major change after 7 days was a sharp fall in nitrite levels in all soils between 4 and 12 Mrad. Figures 10-2.1.3 and 10-2.1.4 show that further loss in extractable nitrite occurred in the same dose region after 14 and 21 days of incubation, being most marked at 12 Mrad. The fate of this nitrite was the subject of later investigations using ^{15}N-labelled nitrite tracer (Section 11).

The concentration of nitrite which was present at 7 days after 0.75 Mrad fell by 18.6 p.p.m. in Broad soil and by 42.9 p.p.m. in Icknield over the next 14 days. In this connection, some gaseous products of nitrite decomposition at a dose of 0.75 Mrad have been reported in Sections 7 and 8.

10-2.2 Elimination of enzyme activity by autoclaving

More definite evidence was required to support the idea that both microbial enzyme activity and radiolytic reduction of nitrate could contribute to nitrite formation at different points within the dose range 0.5 - 12 Mrad. Autoclaving was used to inhibit the enzyme component and allow the importance of radiolytic action to be assessed.

Materials and Methods:

Broad and Icknield soils were raised to 200 p.p.m. nitrate-N and 70% moisture. After equilibration, three sets of samples weighing 10 g were taken from each soil and autoclaved for 1 hr at 15 lbs / sq. in. When the soils were cool, two batches received 1 Mrad followed by analysis immediately or after incubation for 7 days: the nitrite formed

on incubation would indicate if enzymic activity was checked. The third set of autoclaved samples was given 12 Mrad and analysed for nitrite immediately, to examine whether the proposed radiolytic reduction of nitrate had been affected.

Results:

The amounts of nitrite formed appear in Table 10-2.2, and for intercomparison, the previous results without autoclaving have been included in parentheses.

Table 10-2.2 Nitrite formation in autoclaved soils

Soil	p.p.m. nitrite-N*		
	1 Mrad, instantaneous	1 Mrad & 7 days' incubn.	12 Mrad, instantaneous
Broad	17.2 (15.9)	11.3 (58.5)	116.3 (99.2)
Icknield	16.9 (10.7)	10.3 (46.9)	114.7 (100.6)
L.S.D. p 0.05 = 7.0, p 0.01 = 16.1			

* figures in parentheses denote nitrite formed without autoclaving. Autoclaving alone gave 0.2 p.p.m. nitrite-N (instantaneous) in both soils.

Autoclaved soils receiving 1 Mrad produced smaller rather than larger concentrations of nitrite on incubation for 7 days, and it seemed safe to conclude that enzymic processes were largely eliminated; the 10 - 11 p.p.m. nitrite-N that was present after 7 days most probably originated from radiolytic reduction, as did the nitrite formed immediately after the combined autoclaving and 1 Mrad treatment.

It was apparent that autoclaving soil before the application of

1 or 12 Mrad increased the yield of nitrite, perhaps by the influence of soluble organic compounds on radical reactions. However, the most important finding was that autoclaved soils treated with 12 Mrad gave as much nitrite as non-autoclaved samples, which confirmed the radiolytic nature of nitrate reduction at high doses.

10-2.3 Nitrite production in soil solutions after 12 Mrad

The influence of the solid phase on radiolytic formation and subsequent loss of nitrite at a high dose was examined by extraction and irradiation of the soil solution.

Materials and Methods:

Samples of Broad and Icknield soils weighing 250 g were raised to 200 p.p.m. nitrate-N and 70% moisture. After equilibration, the soil solutions were extracted by centrifugation and were further centrifuged at 30,000 G for 40 minutes to ensure the removal of clay particles: 20 ml of solution from each soil was treated with 12 Mrad, then analysed for nitrite and nitrate immediately before and after irradiation and after 7 days at 25°C, to examine whether the loss of nitrite which accumulated initially took place at a similar rate to that observed in the complete soil system. The pH values of solutions were recorded before and after irradiation, in case the absence of a buffering effect from the solid phase led to a lower pH and greater instability of nitrite. Nitrite was also determined immediately after treatment of the solutions with 4 Mrad, for comparison of the yield with that given by 12 Mrad.

Results:

The analytical data in Table 10-2.3 shows that the formation of nitrite became severely inhibited in the absence of the solid phase.

Table 10-2.3 Nitrite production in soil solutions after 12 Mrad

Soil series	p.p.m. mineral-N in soil solution* and pH						
	before γ			immediately after γ			7 days after γ
	NO_2	NO_3	pH	NO_2	NO_3	pH	NO_2
Broad	0.1	339.5	7.8	23.8 (16.7)	314.0 (219.8)	6.6	23.9
Icknield	0.1	323.7	7.6	20.5 (14.4)	294.6 (206.2)	6.5	21.6
Broad				20.4			
Icknield				21.4			
				immediately after 4 Mrad			
L.S.D. nitrite-N p 0.05 = 0.4, p 0.01 = 1.0							
L.S.D. nitrate-N " = 14.3, " = 33.1							

* soil solution results expressed as p.p.m. soil are in parentheses

Although the pH decreased on irradiation, the good recovery from the sum of nitrite + nitrate analyses proved that the radiolytic reduction of nitrate to nitrite which did occur was not followed by rapid decomposition and volatilisation from the solutions. In addition, the small amount of nitrite formed in the solutions was stable over 7 days, which closely connects the solid phase with loss from soil during incubation.

Comparison of the results obtained at 12 Mrad with those at 4 Mrad (Table 10-2.3) revealed that the amount of nitrite produced was almost identical. A decreased yield of free radicals with increasing

dose has been reported for carbohydrates by Ehrenberg (1961), faster recombination between more densely produced radicals being proposed as a main reason for such behaviour; presumably the presence of clay and organic compounds introduces alternative radical reactions which favour the formation of nitrite from nitrate, thus avoiding inter-radical combination and lower yield of nitrite.

Alternatively, it could be proposed that nitrite was being produced by a radiolytic reaction other than nitrate reduction, since it has been reported that an aqueous solution of ammonia can be oxidised during irradiation to yield nitrite (Rigg et al. 1952); two observations discount such a possibility and indicate that reduction is predominant. Firstly, the formation of nitrite is severely restricted by the amount of nitrate present in the irradiated soil sample (Figure 10-2.1.1), and secondly, Table 10-2.4 shows that the deficiency of nitrate found in the next experiment with soil irradiated (12 Mrad) at 50% moisture is only just accounted for by the gain in nitrite: however, the nitrite level should more than compensate for any decline in nitrate if oxidation of ammonium was taking place, assuming that the whole 86.2 p.p.m. nitrite-N in the soil originated from reduction of nitrate.

10-2.4 Effect of moisture on nitrite yield after 12 Mrad

It was expected that the yield of nitrite on irradiation could be greatly influenced by the moisture percentage owing to the effect on migration of radiolytic products and thus their rates of reaction. The relationship was examined at a high dose which was known to give extensive accumulation of nitrite.

Materials and Methods:

Broad soil at 30.0% moisture was adjusted to 10, 20, 30, 40, 50, 60, 70 and 80% moisture, and to 200 p.p.m. nitrate-N on a dry weight basis. After equilibration, samples of 10 g were treated with 12 Mrad and analysed for nitrite immediately, together with unirradiated controls. It was found that the nitrite concentration decreased between 70 and 80% moisture, and this was verified by repeat irradiations. To establish whether nitrite was in fact being formed but then rapidly decomposing, or if the radiolytic yield had been reduced, further samples were analysed for nitrate as well as nitrite immediately before and after irradiation at 50 - 80% moisture. Once again, a similar pattern of nitrite behaviour was detected.

Results:

The yield of nitrite obtained over the entire moisture range appears in Figure 10-2.4, and shows a linear response only up to 50% moisture. The recovery figures for nitrate (Table 10-2.4) proved that progressively less was present after irradiation as the moisture content increased from 50 - 80%, the deficiency presumably being caused by gradually improved reduction to nitrite: however, a low recovery of nitrate + nitrite was obtained immediately after irradiation, and suggested that nitrite formed during gamma treatment must have been unstable. Such instability became more severe as the moisture level rose from 60 - 80%, and could result from nitrite fixation to organic matter as well as immediate decomposition; it accounted for the non-linear nature of the nitrite yield above 50% moisture in Figure 10-2.4. It is reasonable to expect that the total irradiation time of 19 hr 28 minutes would be sufficient for extensive nitrite reactions to occur, leading to fixation and volatilisation. The

S.E. of mean ± 0.7

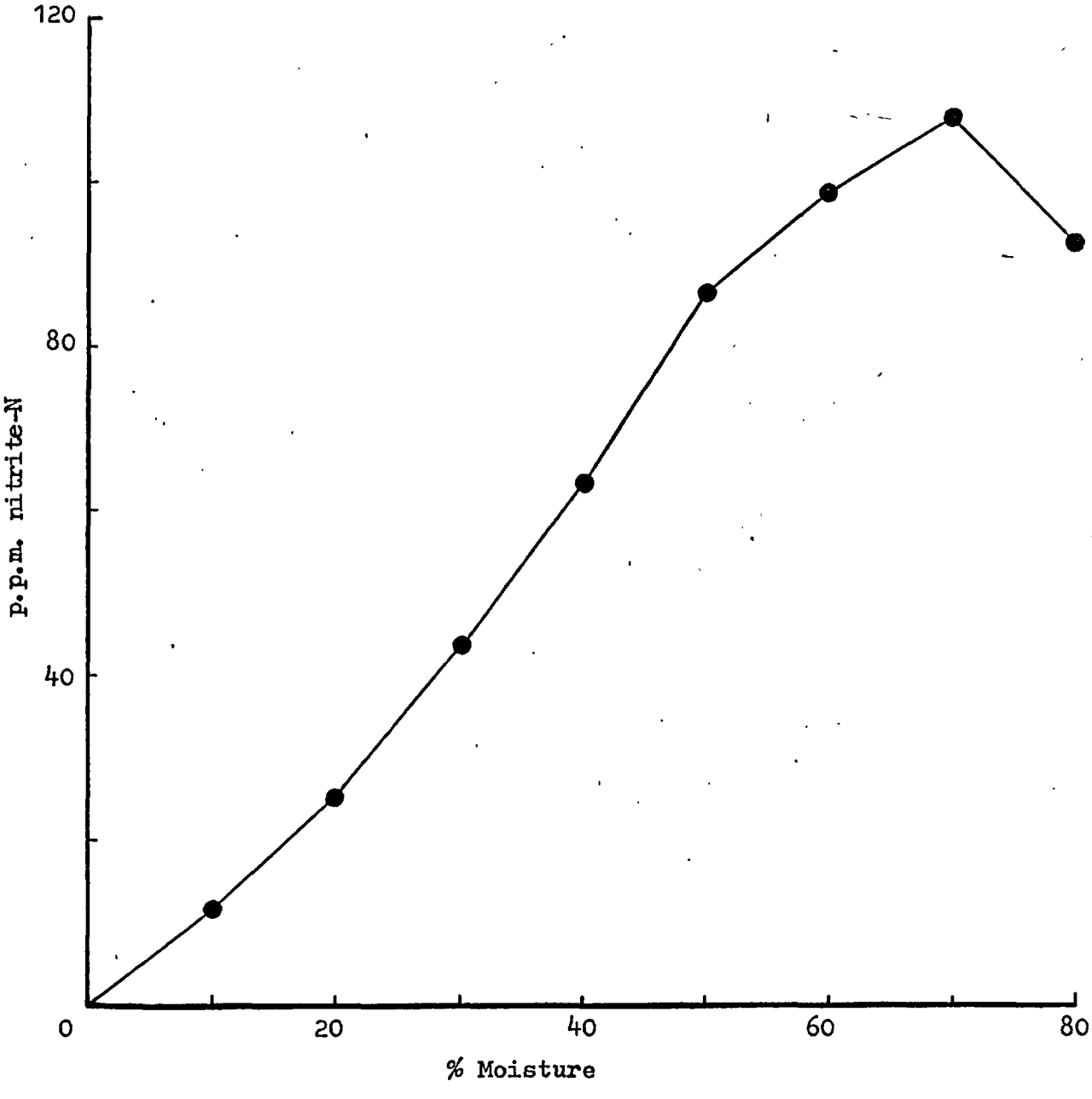


Figure 10-2.4 Effect of moisture level on yield of nitrite in Broad soil immediately after 12 Mrad

Table 10-2.4 Effect of moisture level on recovery of nitrate and nitrite from Broad soil after 12 Mrad

Moisture %	p.p.m. mineral-N in soil: nitrate before γ	nitrate after γ	nitrite after γ	nitrate + nitrite recovered	nitrate + nitrite gain or loss
50	221.2	137.4	86.2	223.6	+ 2.4
60	222.2	113.5	98.6	212.1	- 10.1
70	214.4	73.0	107.7	180.7	- 33.7
80	216.3	53.9	92.2	146.1	- 70.2
L.S.D. nitrite-N	p 0.05 = 4.9,	p 0.01 = 11.3			
L.S.D. nitrate-N	" = 12.6,	" = 29.1			

unirradiated control soil did not contain >1.2 p.p.m. nitrite-N at any moisture.

The conclusion was drawn that reduction of nitrate to nitrite by radiation increases in a linear manner with soil moisture level to a certain point, but there are factors which affect the stability of nitrite produced at high moisture percentages. The irradiation of seeds at different moistures has shown that equal amounts of free radicals are generated by a given dose, irrespective of water content (Ehrenberg, 1961); if this response is similar in soil, the extra mobility and reactivity of radiolytic products between the solid and liquid phases at very high water content may be responsible for the greater reduction of nitrate to nitrite.

11 Loss of nitrite formed by treatment of Fyfield soil with 12 Mrad

11-1 Recovery of $^{15}NO_2$ tracer from Fyfield soil after 12 Mrad

It was reasonable to suppose that the decline in extractable nitrite found in soils up to 3 weeks after treatment with large doses of radiation (Section 10-2.1) resulted from decomposition to nitrous oxide, nitrogen dioxide and nitrogen, or from fixation to organic matter, since these mechanisms have been detected in autoclaved soil to which nitrite tracer was added (Bremner and Nelson, 1968).

The behaviour of nitrite in heavily irradiated soil was examined by the addition of ^{15}N-labelled sodium nitrite immediately after irradiation, followed by 14 days of incubation. Finally, the ^{15}N which remained in either the soil organic matter or mineral-N fractions (ammonium-, nitrite- and nitrate-N) would be recovered.

Materials and Methods:

To select one of the four soils originally examined in Section 10-2.1, extractable nitrite losses were compared after 14 days (using data in Section 10-2.1) and are presented in Table 11-1.1. It is necessary to refer to the decline in nitrite with time after irradiation more specifically as "extractable nitrite" loss, the extractant being water, because it does not represent a total loss of nitrite-N from the soil system if fixation reactions take place.

In the first instance, the series with the greatest nitrite deficit (Grove) appeared to be the most suitable choice. However, if any ^{15}N-labelled nitrite was eventually recovered in the organic pool it would become extensively diluted by the total organic-N present, the latter varying in amount between soils. For the accuracy of analysis by mass spectrometry the highest atom % ^{15}N was desirable, therefore

Table 11-1.1 Extractable nitrite loss in irradiated soils relative to their organic-N content

Soil series	pH in water	total N %	extractable nitrite-N p.p.m. loss 14 days after 12 Mrad	extractable nitrite-N loss as % of organic-N
Broad	8.0	0.78	63.6	0.82
Fyfield	7.6	0.24	51.2	2.23
Grove	7.4	0.39	88.0	2.26
Icknield	8.0	1.20	65.8	0.55

the extractable nitrite loss was expressed as a percentage of the organic-N present in each soil series to obtain some idea of the relative dilution of ^{15}N which might occur. In making this assessment, one is forced to assume that from one soil to another, the proportion of extractable nitrite lost which becomes fixed is similar. The indication was that either Fyfield or Grove soil would be most suitable for tracer work, and as the calculated difference in dilution of tracer appeared to be small, Fyfield was chosen because the lower percentage of clay would permit easier extraction of ammonium and better purification by centrifugation than Grove soil.

Three sets of samples equivalent to 10 g dry weight were taken from Fyfield soil previously air-dried to 8.40% moisture. The nitrate-N concentration was found to be 58.4 p.p.m., and a solution of potassium nitrate (1.5 mg nitrate-N / ml) was added to each sample to raise the level to 200 p.p.m. nitrate-N, thus providing sufficient for unlimited

reduction on irradiation. At the same time, water was added to obtain 24% moisture in the samples, which were then allowed to equilibrate for 2 days before treatment with radiation.

Immediately before irradiation, the first pair of samples was shaken with 100 ml of N potassium chloride for 60 minutes, and ammonium-, nitrite- and nitrate-N were determined in the filtered extracts. The two remaining pairs of samples were treated with 12 Mrad at a dose rate of 0.638 Mrad / hr. Immediately after irradiation, the second set of samples was analysed in a similar manner to the first, while the third pair had 0.6 ml of ^{15}N enriched sodium nitrite (92.81 atom % ^{15}N excess) added: this brought the moisture content to 30%, and these samples were incubated for 14 days at 25°C before analysis. The 0.6 ml of added tracer contained 518.4 µg total nitrite-N, or 481 µg ^{15}N excess, and a previous test at 12 Mrad showed that approximately five-fold dilution would occur in the mineral-N pool, giving about 19 atom % ^{15}N excess enrichment. Such an amount was considered to be sufficiently high to allow detection of fixation in the organic-N pool without greatly disturbing the rate of nitrite loss in irradiated soil. Preliminary trials indicated that this assumption was correct, and it was subsequently confirmed by the main experiment.

For separation of organic-N from mineral-N in incubated samples only, the soil was transferred to a 40 ml centrifuge tube, and shaken three times with 25 ml of N potassium chloride to remove the ammonium-, nitrite- and nitrate-N. After each shaking period the tubes were centrifuged at 30,000 G for 40 minutes to clarify the extract. The whole supernatant fraction was retained for steam-distillation to determine ammonium- and nitrate-N, then both distillates were combined for evaporation prior to mass spectrometry.

Organic-N in the residue from the centrifugation process was determined by the macro-Kjeldahl method (Section 2-3.1). The digest was diluted to 500 ml in a graduated flask, and an aliquot which contained 1 - 1.5 mg N was steam-distilled with 5 ml of 40% w/v sodium hydroxide. The distillate was titrated, re-acidified and evaporated down for mass spectrometry.

Finally, samples of 11 g fresh weight were analysed for organic-N before irradiation, using the separation procedure described above. The object was to compare with analysis after irradiation and assess whether the nitrogen content of the organic fraction was less, since fragmentation of protein molecules and partial deamination is well recognised at such high doses (Kuzin, 1964). A difference of 170 p.p.m. organic-N was found between the analyses, or 7% deficit in the organic-N fraction. Because it represents a relatively small loss, and analysis of flask residues after steam-distillation of mineral-N extracts showed that only 59 p.p.m. organic-N (35%) remained behind, the overall effect on the fixation and recovery pattern would be extremely small. However, a very slight reduction in ^{15}N bound to the organic fraction could be expected.

Results:

The organic-N and mineral-N analyses and determinations of ^{14}N / ^{15}N isotope ratios are presented in Table 11-1.2. Nitrate analysis before irradiation ensured that adjustment to the level of 200 p.p.m. nitrate-N had been correct. Analysis immediately after irradiation detected a severe loss of nitrate with virtually a corresponding increase in nitrite, comparable with earlier results from Broad soil (Section 10-2.4). Following the addition of sodium nitrite tracer, there was a loss of 571 µg nitrite-N over 14 days: this is 10% higher

Table 11-1.2 Recovery of $^{15}NO_2$ tracer from Fyfield soil after 12 Mrad

Time of analysis	p.p.m. mineral-N ‡			total µg N in pools			atom % ^{15}N excess in pools ‡	
	NH_4 (± 0.6)	NO_2 (± 0.5)	NO_3 (± 2.7)	total mineral	NO_2	organic	total mineral	organic
before γ	2.1	0.4	195.2	2006	4.1	24,689	-	-
immediately after γ	16.9*	72.4*	115.8*	2081* 2599+	735* 1253+	-	18.50 (theoretical)	-
14 days after γ	45.2+	67.2+	122.7+	2386+	682+	22,964+	15.99 ± 0.05	0.192 ± 0.003
Recovery of ^{15}N in µg							381.5 (79.3%)	44.1 (9.2%)

* nitrite tracer not added, to calculate dilution of $^{15}NO_2$-N applied after irradiation.

\+ 481 µg $^{15}NO_2$-N tracer added (518 µg ^{14} + $^{15}NO_2$-N).

‡ ± Standard error of mean.

than the amount reported lost without extra nitrite being added (Section 10-2.1), and indicates only a slight disturbance of the loss mechanisms by the presence of extra tracer nitrite.

The main observation was that according to isotope ratio analysis, recovery of ^{15}N in the mineral-N pool only reached 79.3%, with removal of 9.2% by fixation to organic matter, and the difference of 11.5% presumably lost to the atmosphere. The combined loss of 20.7% is equivalent to 100 µg ^{15}N, which compares very well with a figure of 106 µg ^{15}N obtained from separate data, i.e. 18.50 atom % of 571 µg extractable nitrite-N lost. The decline in nitrogen in the total mineral-N pool from 2599 to 2386 µg by 14 days after irradiation is less than the loss of extractable nitrite, mainly owing to the rise in ammonia; deamination of organic matter is the most likely cause.

The positive influence of organic matter on the loss of nitrite from soil was indicated by the early studies of Robinson (1923), and later by Clark and Beard (1960). The advent of ^{15}N techniques permitted further investigation (Fuhr and Bremner, 1964a, 1964b), which showed fixation of nitrite to be most severe when the soil carbon level was high and pH was low. Bremner and Fuhr (1966) subsequently reported that the lignin-derived fraction of organic matter was mainly responsible for nitrite fixation.

More recently, Nelson and Bremner (1969) examined the response given by 30 soils having pH values from 4.8 - 7.8 : when 5 soils in the pH range 7.1 - 7.8 (to which Fyfield series would belong) were air-dried, autoclaved and treated with 200 p.p.m. nitrite-N, the average loss of extractable nitrite following 4 days' incubation at 25°C and 33% moisture was 5% by volatilisation and 1% as fixed nitrogen. In comparison, the total loss of extractable nitrite in Fyfield soil was 13% over 4 days,

making the approximate assumption that the decline was linear over 14 days of incubation.

A supplementary experiment was made on the effect of autoclaving soil before irradiation for two reasons. Firstly, to ensure that insterility and residual enzyme activity did not contribute to both formation of nitrite and its removal, and secondly, to examine whether ammonia release and degradation of organic matter by heat influenced the rate of nitrite loss. Fyfield soil was autoclaved (15 lbs / sq. in. for 1 hr) before receiving 12 Mrad, and other conditions were identical to the main tracer experiment except that ^{15}N-labelled nitrite was not added. Table 11-1.3 shows that autoclaving did not prevent the formation of nitrite, or seriously affect the rate at which it was lost in comparison with either of the previous tests.

Table 11-1.3 Effect of autoclaving on loss of extractable nitrite in Fyfield soil subsequently treated with 12 Mrad

Time after irradiation	p.p.m. nitrite-N	p.p.m. nitrite-N loss		
		autoclaved+γ present expt.	γ only tracer expt. (11-1.2)	γ only initial expt. (10-2.1)
Immediately	80.6	-	-	-
7 days	49.4	31.2	-	29.5
14 days	24.2	56.4	56.3	51.2

L.S.D. p 0.05 = 6.5, p 0.01 = 15.0

11-2 Decomposition of nitrite to oxides of nitrogen after 12 Mrad

Following the observation that 11.5% of the nitrite initially present in heavily irradiated Fyfield soil was lost by volatilisation over 14 days, it is appropriate to consider some chemical reactions which could be responsible: various mechanisms leading to decomposition and volatilisation of nitrite have been proposed by several authors.

In the first instance, Bremner and Nelson (1968) have described the self-decomposition of nitrous acid, especially in soils of low pH; they found that it was best represented by the equation:

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

rather than the reaction generally described:

$$3\ HNO_2 \longrightarrow HNO_3 + 2\ NO + H_2O$$

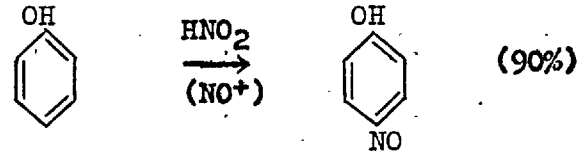
Nitrous acid is stable above pH 7, but evolution of small but significant amounts of nitrogen dioxide from soils with pH 7.1 - 7.8, led Bremner and Nelson (1968) to conclude that it is difficult to assess the extent to which self-decomposition contributes to production of the gas at such high pH. It was suggested that the existence of regions of lower pH, perhaps at surfaces of clay particles, may encourage the reaction to a limited extent.

Secondly, Bremner (1957), Stevenson and Swaby (1964), Bremner and Nelson (1968) and Nelson and Bremner (1969) have established that humic acids, lignins and low molecular weight model phenolic compounds can promote fixation of nitrite, and they propose that nitrosation takes place to form nitrosophenols which then react with nitrous acid to yield nitrous oxide and gaseous nitrogen; soil inorganic compounds did not participate in these processes, and the majority of their experiments were made in solution at pH 5. Furthermore, methyl nitrite has been identified as a product of the reaction between nitrous acid

and aromatic methoxyl groups in lignin (Stevenson and Swaby, 1964; Bremner and Fuhr, 1966):

$$R\text{-}OCH_3 + O{=}N\text{-}OH \longrightarrow R\text{-}OH + O{=}N\text{-}OCH_3$$

but the occurrence of this reaction with soil organic matter has not yet been established. Nitrosation is the process of replacing a hydrogen atom in a molecule by the nitroso (-N=O) group, and when phenol for example is treated with nitrous acid, it forms mainly p-nitrosophenol and a small amount of the σ-compound:



Thirdly, Porter (1969) recently studied the reactions of synthesised soil organic matter oxime compounds with nitrous acid, because it was thought that they may decompose according to the reaction:

$$R_2C{=}NOH + HNO_2 \longrightarrow R_2C{=}O + N_2O + H_2O$$

Oximation was presumed to occur primarily by reaction of carbonyl groups (>C=O) from humic acids with hydroxylamine to form >C=NOH. However, the synthesised organic matter oximes released greater amounts of nitric oxide than nitrous oxide, whereas the reverse was generally true for model oxime compounds; it was pointed out that the mechanisms involved in the formation of nitric oxide in these circumstances are not well understood.

Fourthly, it has been stated that oxides of nitrogen and gaseous nitrogen can be formed from reduction of nitrite in acidic and basic solution by ferrous iron (Chao and Kroontje, 1966; Wullstein and Gilmour, 1966), and also by sulphur compounds such as sodium hydrosulphide (Chao, 1967).

Finally, according to Allison (1963), nitrite can react spontaneously with ammonia, especially in acid soils:

$$NH_3 + HNO_2 \longrightarrow NH_4NO_2 \longrightarrow N_2 + 2\,H_2O$$

but in alkaline soils the reaction is dependent on very high concentrations of reactants (Ewing and Bauer, 1966). The true Van Slyke reaction, which is used for the quantitative assay of alpha-amino acids by reaction with nitrous acid in an atmosphere of nitric oxide, proceeds according to the equation:

$$RNH_2 + HNO_2 \longrightarrow ROH + H_2O + N_2$$

but it is not thought to be important in acid soils owing to the instability of nitrous acid in the presence of air (Allison, 1963). The addition of amino acids, amides and proteins had no effect on decomposition of nitrite in solution at pH 5 (Bremner and Nelson, 1968).

Having considered some opinions and findings on processes which may lead to decomposition and volatilisation of nitrite in soil, it was decided to test for the presence of nitrous oxide, nitrogen dioxide and nitric oxide in the atmosphere above Fyfield soil in which the nitrite concentration was falling as incubation progressed.

Materials and Methods:

Three sets of duplicate samples each containing Fyfield soil equivalent to 30 g dry weight were adjusted to 200 p.p.m. nitrate-N and 30% moisture. After equilibration they were treated with 12 Mrad at a dose rate of 0.608 Mrad / hr, and the first set was analysed immediately for nitrite. The second set of samples was connected to the infra-red apparatus for detection of nitrous oxide, and the third set was used for measurement of nitrogen dioxide and nitric oxide by means of the flow-through technique (Section 2-3.4). The evolution of oxides of nitrogen was recorded at frequent (24 - 48 hr) intervals over a total

period of 3 weeks at 20°C, and after this time the residual nitrite was determined.

Results:

Nitrous oxide did not accumulate above the detection limit of 20 µg nitrous oxide-N over the whole incubation time, and infra-red absorption peaks for methyl nitrite failed to appear. Nitrogen dioxide and nitric oxide were detected, and the amounts are shown in Table 11-2. However, when the total amount of nitrogen released in the form of these gases over 14 days was compared with the loss in extractable nitrite-N by volatilisation (from Section 11-1.2), it only amounted to 18% : thus the formation and evolution of gaseous nitrogen was probably responsible for 82% of the loss by volatilisation.

Analysis for nitrite after 21 days revealed that there had been a decrease of 59.0 p.p.m. extractable nitrite-N from the amount present immediately after irradiation, caused by fixation and volatilisation; this figure agreed fairly well with 65.2 p.p.m. decrease which was found in Section 10-2.1. The fact that the incubation temperature in the present study was 5°C lower than in previous work made little difference to the rate of nitrite loss.

11-3 Decomposition of nitrite in the presence of lignin and catechol

The effect of compounds known to promote the decomposition of nitrite in pure solution (Stevenson and Swaby, 1964; Bremner and Nelson, 1969) was studied in heavily irradiated Fyfield soil, which was already found to lose a large amount of nitrite during subsequent incubation.

Materials and Methods:

Moist samples containing 10 g dry weight of soil were raised to

Table 11-2 Evolution of nitrogen dioxide and nitric oxide from Fyfield soil after 12 Mrad

Days of incubation	cumulative total of nitrogen dioxide + nitric oxide-N* released from 30 g dry soil
1	26.2
2	60.3
3	74.8
4	87.6
7	109.3
9	138.7
11	162.5
14	171.5
16	185.7
18	195.2
21	201.9

L.S.D. p 0.05 = 23.2, p 0.01 = 53.4

* 17.2% of N from nitrogen dioxide, 82.8% from nitric oxide

Table 11-3 Decomposition of nitrite in the presence of indulin and catechol

Treatment applied to Fyfield soil	loss in p.p.m. nitrite-N after: 7 days	14 days
12 Mrad + 0.5 ml water	23.1	42.8
12 Mrad + 0.1 g indulin + 0.5 ml water	41.0	64.2
12 Mrad + 0.5 ml Molar catechol	69.0	69.0

L.S.D. p 0.05 = 3.8, p 0.01 = 8.9

200 p.p.m. nitrate-N and treated with 12 Mrad. Immediately afterwards, 0.1 g of lignin ("Indulin A") + 0.5 ml of water, or 0.5 ml of molar catechol solution, $C_6H_4(OH)_2$, was mixed with each sample and increased the moisture level to 30%. "Indulin A" is the trade name given to a pine wood lignin preparation sold by the West Virginia Pulp and Paper Company ; it has a methoxyl content of 14.0% and 0.08% nitrogen.

Nitrite was determined after incubation for 7 and 14 days, and the concentrations found were deducted from nitrite present immediately after 12 Mrad. The losses were then compared with the nitrite deficit in an irradiated but unamended sample.

The ratio of added indulin and catechol to nitrite present immediately after irradiation was approximately the same as that used by Bremner and Nelson (1968) for their studies with pure solutions at pH 5.

Results:

There was a significant increase in the loss of nitrite in the presence of lignin and especially catechol, exceeding the loss detected after irradiation alone (Table 11-3). The results indicated that nitrite can be removed by chemical decomposition in heavily irradiated soil.

12 DISCUSSION

12-1 Biological formation and decomposition of nitrite in soil after irradiation in the region of 0.75 Mrad

The reduction of nitrate to nitrite in irradiated soil maintained under conditions of medium moisture has been shown to be favoured by factors which encourage microbial activity in the sample, and further reduction of the nitrite formed appears to be primarily the result of biological mechanisms rather than chemical decomposition. A point of major interest is that extensive biological reduction of nitrate to nitrite and oxides of nitrogen occurs at an oxygen concentration of 16%, in an atmosphere which is circulated through the soil every 24 hours.

It is difficult to explain why radiation should stimulate reduction mechanisms under apparently aerobic conditions, although several authors have presented evidence for the occurrence of denitrification at medium to low soil moisture levels. Corbet and Wooldridge (1940) concluded that a biological system exists which is capable of causing aerobic losses of nitrate and nitrite in the presence of glucose, and Meiklejohn (1940) found that two species of Pseudomonas denitrified 75% of added nitrate in aerobic culture. Broadbent (1951) reported a loss of 76 p.p.m. nitrate-N when a soil of pH 7 was aerated with 21% oxygen and incubated with clover tissue for 9 days, and experiments by Broadbent and Stojanovic (1952) with another soil proved that 16% of ^{15}N-labelled nitrate was lost over 10 days at 19% oxygen, even without addition of organic-C. Ekpete and Cornfield (1964) found nitrate losses at 20% MWHC, a moisture level which should certainly have allowed good diffusion of oxygen.

To discuss some mechanisms which may account for the

accumulation and decomposition of nitrite in irradiated soils at high concentrations of oxygen, there are several aspects of the soil response to radiation which require consideration, as well as studies made by other workers on enzyme systems of denitrifying bacteria.

It was originally suggested (Cawse and Crawford, 1967) that extension of the radiation dose to the region 0.6 - 0.8 Mrad selectively inhibited nitrification to a greater extent than nitrate reduction, with the latter process becoming dominant and leading to formation of nitrite. Incorporation of ^{15}N-labelled nitrite with irradiated Icknield soil has now shown (Section 9) that nitrification removes 57% of the nitrite during incubation for 14 days, compared with a loss of 28% by volatilisation; it is therefore reasonable to expect that increased radiation damage to nitrifying organisms will upset the balance between ammonium oxidation to nitrate and the reduction of nitrate. A high initial population of nitrifying organisms in a soil (and therefore survival of more bacteria after 0.75 Mrad) would lessen the amount of nitrite available for reduction and organic matter fixation pathways, and may partly explain why Icknield soil produced fewer oxides of nitrogen than other series. However, it must be borne in mind that less intensive reduction of nitrate by an irradiated soil would also lower the size of the nitrite pool relative to other soil types, and produce a similar effect with regard to the yield of nitrogenous gases.

The stimulation of nitrate and nitrite reduction under apparently aerobic conditions may result from improved accessibility of reducing enzymes within cells of denitrifying bacteria to nitrate and nitrite. A stimulatory effect of radiation on soil urease activity has already been reported (Skujins and McLaren, 1969), and it was proposed that either release of urease from damaged cells or faster

diffusion of substrate into cells could have been responsible. The fact that decreasing the moisture content of irradiated soil gradually limits reduction of nitrite to nitrous oxide (Section 7-2.3) is in agreement with restriction of enzyme and substrate mobility, and furthermore, improved diffusion of oxygen at low soil moisture may also inhibit evolution of nitrous oxide by decreasing the number of anaerobic microzones in which reducing enzymes are thought to operate (Hauck, 1968). An increase in soil respiration rate after irradiation (Cawse, 1967a) may encourage anaerobic conditions, but it is known that saturation with carbon dioxide stimulates reduction so much that the nitrite concentration falls (Section 4-6).

Irradiation of waterlogged soil greatly stimulates nitrate and nitrite loss, and the production of nitrous oxide exceeds that detected under aerobic conditions (Section 4-5). With an overall deficiency of oxygen under waterlogging, the sites available for reduction to proceed are unrestricted and radiation-released carbon compounds could diffuse readily; reduction would therefore be so rapid that nitrite has little chance to accumulate during incubation, as observed in Section 4-1. However, when the irradiated soil is at medium moisture, improved diffusion of oxygen would restrict the extent of anaerobic microzones (Hauck, 1968) available for reduction of nitrate, thus an increase from 57 - 72% MWHC in Broad soil increases nitrite accumulation (Table 4-3). Poorer diffusion of carbon compounds could also cause nitrate reduction to be slower than in waterlogged soil.

It is interesting to note the findings of Jordan et al. (1967) in relation to the accumulation of nitrite; they reported that Arthrobacter simplex reduced all nitrate to nitrite before reduction of nitrite began, and that Bacillus polymyxa showed a lag in reduction

of nitrite but not nitrate. Their observations support the results obtained from increasing the moisture of irradiated soil (Figures 4-1.1 and 4-1.2), where nitrate was reduced before nitrite as the moisture level rose above 75% MWHC.

Campbell and Lees (1967) discussed the reduction of nitrate under aerobic and anaerobic conditions in relation to the behaviour of enzyme systems possessed by denitrifying organisms. Nitrate reductase is a complex enzyme system involving flavin adenine dinucleotide (FAD), cytochrome c and molybdenum, and it is thought that it competes with the cytochrome oxidase $\longrightarrow$ oxygen reduction system characteristic of aerobic respiration for electrons passed to cytochrome c during metabolism, according to the relative concentrations of oxygen and nitrate in the media. It is known that certain denitrifying organisms possess an especially active nitrate reductase system (Campbell and Lees, 1967), but it is not yet possible to specify the precise oxygen concentration or oxygen : nitrate ratio at which significant reduction of nitrate occurs. The large amount of nitrate usually produced by calcareous soils after 0.75 Mrad (Cawse and White, 1969a) may stimulate the nitrate reductase system. Apart from the possibility of anaerobic microzones in soils, McKee (1962) remarked that there may be regions within bacterial cells where less oxygen is present compared with the main volume of the cell.

Rapid proliferation of denitrifying bacteria which survive irradiation might be considered responsible for increasing nitrate reduction and accumulation of nitrite. As far as nitrifying bacteria are concerned, experiments have shown that they do not proliferate until 25 days after 0.2 Mrad (Cawse, 1968), therefore rapid division of denitrifying cells seems to be very unlikely within 3 days after

0.75 Mrad, which is the average time / dose relationship for maximum nitrite accumulation.

Reports on the emission of nitrogen dioxide and nitric oxide from soil appear to have been solely concerned with the formation of these gases by chemical decomposition of nitrous acid at low pH (Cady and Bartholomew, 1960; Reuss and Smith, 1965; Makarov, 1969), and Alexander (1961) claims that nitric oxide is only released in acid surroundings. In alkaline soils treated with 0.75 Mrad, extensive formation of nitrogen dioxide and especially nitric oxide appears to take place by biological mechanisms, but it should be stressed that the continuous flow of air through the soil no doubt helped to prevent re-absorption of the gases. If the soil atmosphere was undisturbed, nitrogen dioxide would be better absorbed in the soil water, and nitric oxide could be removed by oxidation to nitrogen dioxide and nitrate or by nitration reactions with lignin (Mortland and Wolcott, 1965).

Reduction of nitrite to nitrogen by cell free extracts of Bacillus subtilis gave nitric oxide as the first major product (Najjar and Allen, 1954), and work with pure cultures of Thiobacillus denitrificans has shown that nitric oxide appears during the reduction of nitrite (McKee, 1962). It is therefore not surprising that biological mechanisms for production of nitric oxide should exist in soil, and the radiation-stimulation of nitrite formation by rapid nitrate reductase activity may in turn encourage nitrite reductase to produce nitric oxide; it was found that the yield of this gas could be increased by addition of extra nitrite to irradiated soil, and that a biological mechanism was involved (Section 8).

The production of nitric oxide by nitrite reductase in cell-free preparations of denitrifying bacteria has been discussed by

Nason (1962), as well as the existence of nitric oxide reductase which yields gaseous nitrogen. The exact position of nitrous oxide in the pathway of denitrification to gaseous nitrogen is controversial, and some work with cell-free extracts indicates that it is not an obligate intermediate in the formation of nitrogen gas (Nason, 1962). However, measurement of the sequence of gaseous products during denitrification in soil closely correlates a decline in concentration of nitrous oxide with an increase in gaseous nitrogen (Cady and Bartholomew, 1960; Cooper and Smith, 1963).

The reduction of nitrate and nitrite when radiation causes death and lysis of cells in an aerobic soil system suggests that climatic factors such as freezing or drought, which adversely affect microorganisms, could have a similar but perhaps far less obvious action when the soil thaws or is re-wetted. McGarity (1962) reported that denitrification in waterlogged soil was increased by prior freezing, but other investigators who have studied freezing and drying effects under more aerobic conditions do not appear to have measured the evolution of nitrous oxide or nitrogen (Stevenson, 1956; Gasser, 1958; Allen and Grimshaw, 1962).

12-2 Non-biological (radiolytic) formation and decomposition of nitrite in soil irradiated with high doses, up to 12 Mrad

The relative unimportance of nitrous oxide in the process of nitrite volatilisation from sterile soil agrees with the studies made by Bremner and Nelson (1968); they recovered as much as 53% of volatilised nitrite in the form of nitrogen dioxide (45% recovered as nitrogen and 2% as nitrous oxide) from soil at pH 7, using 2 days' incubation in a helium / oxygen atmosphere. The corresponding figure for Fyfield

soil is 18% when calculated over the first 14 days, but it does possess a higher pH of 7.6 which is known to decrease the formation of nitrogen dioxide (Bremner and Nelson, 1968).

There is a considerable difference in the ratio of 1 : 5 for fixed : volatilised N in American soils (Nelson and Bremner, 1969) and that of 1 : 1.3 observed for Fyfield series (Table 11-1.2). This discrepancy might have arisen from the use of autoclaving for sterilisation by Nelson and Bremner (1969), because it would encourage greater release of ammonia than irradiation, and provide more favourable conditions for volatilisation by decomposition of ammonium nitrite (Allison, 1963; Ewing and Bauer, 1966). In addition, less extensive degradation of organic matter in Fyfield soil following the use of radiation as a sterilising agent could have permitted better fixation of nitrite, but the fact that none of the American soils with pH 7.1 - 7.8 was incubated for longer than 4 days allows only a tentative forecast that they would produce a similar ratio of nitrite fixation : volatilisation over a longer period, which has greater practical bearing.

Results obtained by Nelson and Bremner (1969) from autoclaved alkaline soils over 24 hours only, indicate that the influence of ammonia and organic matter on transformation of nitrite is unimportant, but their behaviour beyond this time is unknown; an increase in ammonia after sterilisation would be certain. Another limitation is the inadequacy of information concerning the effect of organic matter on nitrite removal in soils above pH 7. Investigators have concentrated on acid soils in which nitrite disappears rapidly, and where loss is encouraged by the presence of organic matter. However, as far as the current irradiation experiments are concerned, the decline in extractable nitrite in four soils of pH 7.4 - 8.0 was not highest in

the two most organic soils (Table 11-1.1), which also happen to be the most calcareous. This behaviour is partly supported by Wullstein (1969), who reduced soil nitrite deficits by adding alkaline metal carbonates, but did not evaluate the role of organic matter.

12-3 Possibilities for future research

Research on pathways of nitrate reduction to gaseous nitrogen in irradiated soil and the fixation of nitrite to organic matter, is justified on the grounds that such mechanisms are little understood, and are of economic importance to World agriculture (Wullstein, 1967; Hauck, 1968). They are responsible for severe losses of fertiliser nitrogen, certainly under anaerobic conditions, and possibly at medium levels of soil moisture as well. The Appendix to this thesis discusses the application of radiation as a tool in agricultural research, and to enable the use to be entirely successful, one must be fully aware of the response which soils give to various levels of radiation.

Application of 0.75 Mrad to certain soils appears to be a convenient pre-treatment for detailed study of nitrate and nitrite reduction mechanisms operating at medium soil moisture. More exact information on the activity of reducing enzymes over a wide range of oxygen concentration could be obtained by the continuous passage of known gas mixtures through the soil during irradiation, and in the course of subsequent incubation. To examine whether high nitrate concentration affects competition between cytochrome oxidase and nitrate reductase at medium moisture, increasing amounts of nitrate could be added to irradiated (0.75 Mrad) soil, followed by measurement of nitrite and formation of oxides of nitrogen. Further experiments with ^{15}N-labelled nitrite are needed to ascertain if some irradiated soils

produce fewer oxides of nitrogen than others owing to more extensive re-oxidation of nitrite by nitrifying organisms.

The successful colonisation of radiation-sterilised soil by nitrifying bacteria (Section 6) suggests that it may also be possible to inoculate with pure cultures of denitrifying bacteria, and then study their relative efficiency in reducing nitrate and nitrite, and perhaps nitrous oxide as well, in a soil environment.

Although a good correlation was obtained between nitrite accumulation after 0.75 Mrad and the denitrifying potential for some British soils (Section 5), it has not yet been established to what extent soils from other Continents accumulate nitrite and oxides of nitrogen on irradiation, and if biological reduction of nitrite is more prevalent than chemical decomposition pathways in neutral to alkaline series.

It has not been possible to account for the smaller yield of nitrite obtained from application of 12 Mrad to soil solutions and pure solutions of nitrate salts relative to similarly irradiated soil, except that experiments closely connect the solid phase of soil with the stimulatory effect on yield of nitrite (Section 10-2.3). This problem could be investigated by mixing pure clay minerals and extracted soil organic matter separately with nitrate solutions, and measuring the effect on nitrite yield after 12 Mrad.

The application of high gamma doses in the region of 12 Mrad would seem to be useful for studies on nitrite fixation to organic matter, and volatilisation of nitrite (Section 11); radiation is likely to cause less damage to soil physical and chemical properties compared with sterilisation by autoclaving, and the radiolytic production of nitrite avoids the danger of microbial contamination when

nitrite is added after heat treatment.

To study the release of gaseous nitrogen in addition to nitrogen dioxide and nitric oxide, soil treated with 12 Mrad could be immediately flushed through with an atmosphere of pure helium, or perhaps 80% helium / 20% oxygen mixture, provided that the subsequent loss of nitrite was undisturbed; there appears to be no major problem in this respect, because Bulla et al. (1970) recently reported the release of nitric oxide and nitrogen in large amounts when autoclaved soils were incubated with nitrite in an atmosphere of pure helium. Samples of the atmosphere above irradiated soil could be removed after different periods of incubation, and analysed for nitrogen by gas chromatography.

13 BIBLIOGRAPHY

Aleem, M.I.H., and Alexander, M. (1960) Nutrition and physiology of *Nitrobacter agilis*. Appl. Microbiol. 8, 80-84.

Alexander, M. (1961) Introduction to Soil Microbiology, J. Wiley and Sons, New York.

Alexander, P., Hamilton, L.D.G., and Stacey, K.A. (1959) Disorganisation of the secondary structure in proteins exposed to ionising radiations in the solid state. Nature, Lond. 184, 226-228.

Allen, S.E., and Grimshaw, H.M. (1962) Effect of low-temperature storage on the extraotable nutrient ions in soils. J. Sci. Fd Agric. 13, 525-529.

Allison, F.E. (1963) Losses of gaseous nitrogen from soils by chemical mechanisms involving nitrous acid and nitrites. Soil Sci. 96, 404-409.

Allison, F.E., Carter, J.N., and Sterling, L.D. (1960) The effect of partial pressure of oxygen on denitrification in soil. Proc. Soil Sci. Soc. Am. 24, 283-285.

Allison, L.E. (1960) Wet-combustion apparatus and procedure for organic and inorganic carbon in soil. Proc. Soil Sci. Soc. Am. 24, 36-40.

Arnold, P.W. (1954) Losses of nitrous oxide from soil. J. Soil Sci. 5, 116-128.

Avery, B.W. (1964) The soils and land use of the district around Aylesbury and Hemel Hempstead (Sheet 238), Memoirs of the Soil Survey of England and Wales, A.R.C. Harpenden.

Azim, M.A., and Saraf, S.D. (1956) Effect of *Azotobacter* on fixed nitrogen. Biochim. biophys. Acta 21, 321-323.

Baker, P.R.W. (1955) The micro Kjeldahl determination of nitro nitrogen. Analyst, Lond. 80, 481-482.

Bernard, V.V., and Geller, I.T. (1962) [Effect of gamma rays on some groups of the soil microflora]. Agrobiologiya No.4, 610-616.

Berry, R.J., Hills, P.R., and Trillwood, W. (1965) Demonstration of a cytotoxic agent in gamma-irradiated carbohydrate solutions. Int. J. Radiat. Biol. 9, 559-572.

Birch, H.F. (1958) The effect of soil drying on humus decomposition and availability. Pl. Soil 10, 9-31.

Blasco, M.L., and Cornfield, A.H. (1967) Effect of soil moisture content during incubation on the nitrogen-mineralising characteristics of the soils of Colombia (South America). Geoderma 1, 19-25.

Bovard, P., and Grauby, A. (1963) Essais d'irradiation de sols dans le réacteur-piscine Triton. Énergie nucl. 5, 149-153.

Bowen, H.J.M. (1960) Biological fractionation of isotopes. Int. J. appl. Radiat. Isotopes 7, 261-272.

Bowen, H.J.M., and Cawse, P.A. (1962) The effect of ionising radiation on the availability of inorganic ions in soils, 17-30, in Radioisotopes in Soil-Plant Nutrition Studies, International Atomic Energy Agency, Vienna.

Bowen, H.J.M., and Cawse, P.A. (1964a) Effects of ionising radiation on soils and subsequent crop growth. Soil Sci. 97, 252-259.

Bowen, H.J.M., and Cawse, P.A. (1964b) Some effects of gamma radiation on the composition of the soil solution and soil organic matter. Soil Sci. 98, 358-361.

Boyer, J., Combeau, A., Grauby, A., and Thomas, A. (1966) Évolution des sols sous l'effet d'une irradiation gamma. Bull. Infs. scient. tech. Commt Énerg. atom. No.106, 39-55.

Bremner, J.M. (1957) Studies on soil humic acids. II. Observations on the estimation of free amino groups. Reactions of humic acid and lignin preparations with nitrous acid. J. agric. Sci., Camb. 48, 352-360.

Bremner, J.M. (1965a) Total nitrogen, 1149-1178, in Methods of Soil Analysis, edit. Black, C.A., American Society of Agronomy, Madison, Wisconsin.

Bremner, J.M. (1965b) Isotope-ratio analysis of nitrogen in nitrogen-15 tracer investigations, 1256-1286, in Methods of Soil Analysis, edit. Black, C.A., American Society of Agronomy, Madison, Wisconsin.

Bremner, J.M., and Edwards, A.P. (1965) Determination and isotope-ratio analysis of different forms of nitrogen in soils - 1. Apparatus and procedure for distillation and determination of ammonium. Proc. Soil Sci. Soc. Am. 29, 504-507.

Bremner, J.M., and Fuhr, F. (1966) Tracer studies of the reaction of soil organic matter with nitrite, 337-346, in Use of Isotopes in Soil Organic Matter Studies, Pergamon Press, Oxford.

Bremner, J.M., and Jenkinson, D.S. (1960) Determination of organic carbon in soil. I. Oxidation by dichromate of organic matter in soil and plant materials. J. Soil Sci. 11, 394-402.

Bremner, J.M., and Nelson, D.W. (1968) Chemical decomposition of nitrite in soils, 495-503, in Transactions of the Ninth International Congress of Soil Science, Vol.2, Adelaide.

Bremner, J.M., and Shaw, K. (1958a) Denitrification in soil. I. Methods of investigation. J. agric. Sci., Camb. 51, 22-39.

Bremner, J.M., and Shaw, K. (1958b) Denitrification in soil. II. Factors affecting denitrification. J. agric. Sci., Camb. 51, 40-42.

Broadbent, F.E. (1951) Denitrification in some California soils. Soil Sci. 72, 129-137.

Broadbent, F.E., and Stojanovic, B.F. (1952) The effect of partial pressure of oxygen on some soil nitrogen transformations. Proc. Soil Sci. Soc. Am. 16, 359-363.

Bulla, L.A. Jun., Gilmour, C.M., and Bollen, W.B. (1970) Non-biological reduction of nitrite in soil. Nature, Lond. 225, p.664.

Burdick, C.L. (1922) Analytical determination of oxides of nitrogen in gas mixtures. J. ind. Engng. Chem. 14, 308-310.

Burford, J.R., and Millington, R.J. (1968) Nitrous oxide in the atmosphere of a red-brown earth, 505-511, in Transactions of the Ninth International Congress of Soil Science, Vol.2, Adelaide.

Cady, F.B., and Bartholomew, W.V. (1960) Sequential products of anaerobic denitrification in Norfolk soil material. Proc. Soil Sci. Soc. Am. 24, 477-482.

Campbell, N.E.R., and Lees, H. (1967) The nitrogen cycle, 194-215, in Soil Biochemistry, edit. McLaren, A.D., and Peterson, G.H., Marcel Dekker Inc., New York.

Casarett, A.P. (1968) Effects of radiation on the cell, 90-117, in Radiation Biology, edit. Swanson, C.P., Prentice-Hall Inc., New Jersey.

Cawse, P.A. (1967a) Effects of low sub-sterilising doses of gamma radiation on carbon, nitrogen and phosphorus in fresh soils. J. Sci. Fd Agric. 18, 388-391.

Cawse, P.A. (1967b) The effects of gamma radiation on the mineralisation of soil nitrogen. M.Sc. thesis, University of Nottingham.

Cawse, P.A. (1967c) The determination of nitrate in soil solutions by ultraviolet spectrophotometry. Analyst, Lond. 92, 311-315.

Cawse, P.A. (1968) Effects of gamma radiation on accumulation of mineral nitrogen in fresh soils. J. Sci. Fd Agric. 19, 395-398.

Cawse, P.A., and Crawford, D.V. (1967) Accumulation of nitrite in fresh soils after gamma irradiation. Nature, Lond. 216, 1142-1143.

Cawse, P.A., and White, T. (1969a) Accumulation of nitrate in fresh soils after gamma irradiation. J. agric. Sci., Camb. 72, 331-333.

Cawse, P.A., and White, T. (1969b) Rapid changes in nitrite after gamma irradiation of fresh soils. J. agric. Sci., Camb. 73, 113-118.

Chao, T. (1967) Inorganic nitrogen transformations through the oxidation and reduction of sulphur compounds. Proc. Soil Sci. Soc. Am. 31, 485-490.

Chao, T., and Kroontje, W. (1966) Inorganic nitrogen transformations through the oxidation and reduction of iron. Proc. Soil Sci. Soc. Am. 30, 193-196.

Chapman, H.D., and Liebig, G.F. Jun. (1952) Field and laboratory studies of nitrite accumulation in soils. Proc. Soil Sci. Soc. Am. 16, 276-282.

Cheng, H.H., and Bremner, J.M. (1965) Gaseous forms of nitrogen, 1287-1323, in Methods of Soil Analysis, edit. Black, C.A., American Society of Agronomy, Madison, Wisconsin.

Clark, F.E., and Beard, W.E. (1960) Influence of organic matter on volatile losses of nitrogen from soil, 501-508, in Transactions of the Seventh International Congress of Soil Science, Vol.3, Madison, Wisconsin.

Clark, F.E., Beard, W.E., and Smith, D.H. (1960) Dissimilar nitrifying capacities of soils in relation to losses of applied nitrogen. Proc. Soil Sci. Soc. Am. 24, 50-54.

Coleman, D.C., and Macfadyen, A. (1966) The recolonisation of gamma-irradiated soil by small arthropods. Oikos 17, 62-70.

Cooper, G.S., and Smith, R.L. (1963) Sequence of products formed during denitrification in some diverse Western soils. Proc. Soil Sci. Soc. Am. 27, 659-662.

Corbet, A.S., and Wooldridge, W.R. (1940) The nitrogen cycle in biological syetems. 3. Aerobic denitrification in soils. Biochem. J. 34, 1036-1040.

Crosby, N.T. (1967) The determination of nitrite in water using Cleve's acid, 1-napthylamine-7-sulphonic acid. Proc. Soc. Wat. Treat. Exam. 10, 51-55.

Cummins, D.G., and McCreery, R.A. (1964) The effect of gamma and neutron irradiation of soil on the yield and nutrient uptake of rye. Proc. Soil Sci. Soc. Am. 28, 390-393.

Currie, J.A. (1961) Gaseous diffusion in the aeration of aggregated soils. Soil Sci. 90, 40-45.

Daniels, M., and Wigg, E.E. (1967) Radiation chemistry of the aqueous nitrate system. I. γ radiolysis of dilute solutions. J. Phys. Chem. 71, 1024-1033.

Edwards, C.A. (1967) Sterilisation of soil by gamma radiation, 196-197, in Rep. Rothamsted exp. Sta. for 1966.

Ehrenberg, A. (1961) Research on free radicals in enzyme chemistry and in radiation biology, 337-350, in Free Radicals in Biological Systems, edit. Blois, M.S., Brown, H.W., Lemmon, R.M., Lindblom, R.O., and Weissbluth, M., Academic Press, New York.

Ekpete, D.M., and Cornfield, A.H. (1964) Losses, through denitrification, from soil of applied inorganic nitrogen even at low moisture contents. Nature, Lond. 201, 322-323.

Ekpete, D.M., and Cornfield, A.H. (1965) Effect of varying static and changing moisture levels during incubation on the mineralisation of carbon in soil. J. agric. Sci., Camb. 64, 205-209.

Ekpete, D.M., and Cornfield, A.H. (1966) Effects of varying static and changing moisture contents during incubation on ammonia and nitrate levels in soil. J. agric. Sci., Camb. 66, 125-129.

Eno, C.F., and Popenoe, H. (1964) Gamma radiation compared with steam and methyl bromide as a soil sterilising agent. Proc. Soil Sci. Soc. Am. 28, 533-535.

Ewing, G.J., and Bauer, N. (1966) An evaluation of nitrogen losses from the soil due to the reaction of ammonium ions with nitrous acid. Soil Sci. 102, 64-69.

Fricke, H., and Morse, S. (1927) The chemical action of roentgen rays on dilute ferrous sulphate solutions as a measure of dose. Am. J. Roentg. 18, 430-432.

Fuhr, F., and Bremner, J.M. (1964a) Beeinflussende Faktoren in der Fixierung des Nitrit-Stickstoffs durch die organische Masse des Bodens. Atompraxis 10, 109-113.

Fuhr, F., and Bremner, J.M. (1964b) Untersuchungen zur Fixierung des Nitrit-Stickstoffs durch die organische Masse des Bodens. Landw. Forsch. 11, 43-51.

Gasser, J.K.R. (1958) Use of deep-freezing in the preservation and preparation of fresh soil samples. Nature, Lond. 181, 1334-1335.

Ghormley, J.A. (1956) Lifetime of intermediates in water subjected to electron irradiation. Radiat. Res. 5, 247-251.

Gibson, E.J. (1959) The breakdown of peat by gamma radiation. Report No. RR 1859, Warren Spring Laboratory, Stevenage.

Gilmour, C.M., Damsky, L., and Bollen, W.B. (1958) Manometric gas analysis as an index of microbial oxidations and reductions in soil. Can. J. Microbiol. 4, 287-293.

Gottlieb, I., and Markakis, P. (1968) Radiation-induced glyoxal formation in sugar solutions. Radiat. Res. 36, 55-58.

Greenland, D.J. (1962) Denitrification in some tropical soils. J. agric. Sci., Camb. 58, 227-233.

Greenwood, D.J. (1962) Nitrification and nitrate dissimilation in soil. II. Effect of oxygen concentration. Pl. Soil 17, 378-391.

Greenwood, D.J. (1967) Direct measurements of the distribution of oxygen in soil aggregates and in columns of fine soil crumbs. J. Soil Sci. 18, 182-196.

Greenwood, D.J. (1968) Measurement of microbial metabolism in soil, 138-157, in The Ecology of Soil Bacteria, edit. Gray, T.R.G., and Parkinson, D., Liverpool University Press.

Greenwood, D.J., and Goodman, D. (1964) Oxygen diffusion and aerobic respiration in soil spheres. J. Sci. Fd Agric. 15, 579-588.

Grossbard, E., and Hall, D.M. (1964) An investigation into possible changes in the microbial population of soils stored at -15°C. Pl. Soil 21, 317-332.

Harada, T., and Hayashi, R. (1968) Studies on the organic nitrogen becoming decomposable through the effect of drying a soil. Soil Sci. Pl. Nutr., Tokyo 14, 13-19.

Hauck, R.D. (1968) Soil and fertiliser nitrogen - a review of recent work and commentary, 475-486, in Transactions of the Ninth International Congress of Soil Science, Vol.2, Adelaide.

Higashimura, T., Ichikawa, Y., and Sidei, T. (1963) Dosimetry of atomic bomb radiation in Hiroshima by thermoluminescence of roof tiles. Science 139, 1284-1285.

Jackson, N.E., Corey, J.C., Frederick, L.R., and Picken, J.C. Jun. (1967) Gamma irradiation and the microbial population of soils at two water contents. Proc. Soil Sci. Soc. Am. 31, 491-494.

Jansson, S.L. (1958) Tracer studies on nitrogen transformations in soil with special attention to mineralisation-immobilisation relationships. K. LantbrHögsk. Annlr. 24, 101-361.

Jarvis, M.G. (in the press) The soils of the Wantage district, Memoirs of the Soil Survey of Great Britain, A.R.C. Harpenden.

Jarvis, R.A. (1968) The soils of the Reading district (Sheet 268), Memoirs of the Soil Survey of Great Britain, A.R.C. Harpenden.

Jefferson, S., and Rogers, F. (1964) Radiation sources, 271-304, in Massive Radiation Techniques, edit. Jefferson, S., George Newnes, London.

Jenkinson, D.S. (1966) Studies on the decomposition of plant material in soil. II. Partial sterilisation of soil and the soil biomass. J. Soil Sci. 17, 280-302.

Jenkinson, D.S., Nowakowski, T.Z., and Mitchell, J.D.D. (1968) Release of nitrogen by partially sterilised soil, 77-78, in Rep. Rothamsted exp. Sta. for 1967.

Jenkinson, D.S., and Powlson, D.S. (1970) Residual effects of soil fumigation on soil respiration and mineralisation. Soil Biol. Biochem. 2, 99-108.

Johnson, L.F., and Osborne, T.S. (1964) Survival of fungi in soil exposed to gamma radiation. Can. J. Bot. 42, 105-113.

Jones, E.J. (1951) Loss of elemental nitrogen from soils under anaerobic conditions. Soil Sci. 71, 193-196.

Jordan, J.H. Jun., Patrick, W.H. Jun., and Willis, W.H. (1967) Nitrate reduction by bacteria isolated from waterlogged Crowley soil. Soil Sci. 104, 129-133.

Justice, J.K., and Smith, R.L. (1962) Nitrification of ammonium sulphate in a calcareous soil as influenced by combinations of moisture, temperature, and levels of added nitrogen. Proc. Soil Sci. Soc. Am. 26, 246-250.

Kashkina, G.B., and Abaturov, Yu. D. (1967) [The survival of soil microorganisms upon gamma irradiation of the soil] . Radiobiologiya 7, 263-266.

Kay, F.F. (1934) A soil survey of the Eastern portion of the Vale of the White Horse, Bulletin No.48, University of Reading.

Kay, F.F. (1936) A soil survey of the University farm, Sonning, Berks., Bulletin No.49, University of Reading.

Kayser, E. (1921) Influence des sels d'urane sur le fixateur d'azote. C. r. hebd. Séanc. Acad. Sci., Paris 172, 1133-1134.

Keeney, D.R., and Bremner, J.M. (1966) Comparison and evaluation of laboratory methods of obtaining an index of soil nitrogen availability. Agron. J. 58, 498-503.

Kertesz, Z.I., and Parsons, G.F. (1963) Ozone formation in air exposed to cobalt-60 gamma radiation. Science 142, 1289-1290.

Kevan, L. (1966) Radiolysis of frozen solutions. VI. Electron and excited water reactions in nitrate ices. J. Phys. Chem. 70, 2529-2534.

Kononova, M.M. (1961) Soil Organic Matter, Its Nature, Its Role in Soil Formation and in Soil Fertility, Pergamon Press, London.

Kuzin, A.M. (1964) Effect of ionising radiations on simple proteins and their decomposition products, 30-49, in Radiation Biochemistry, edit. Quastel, M.R., Israel Programme for Scientific Translations, Jerusalem.

Lees, H. (1963) Inhibitors of nitrification, 615-629, in Metabolic Inhibitors, Vol.2, edit. Hochster, R.M., and Quastel, J.H., Academic Press, New York.

Lees, H., and Quastel, J.H. (1946) Biochemistry of nitrification in soil. Part 2. The site of soil nitrification. Biochem. J. 40, 815-823.

Mahendrappa, M.K., and Smith, R.L. (1967) Some effects of moisture on denitrification in acid and alkaline soils. Proc. Soil Sci. Soc. Am. 31, 212-215.

Mahlman, H.A., and Schweitzer, G.K. (1958) Radiation-induced nitrite formation from concentrated nitrate solutions. J. inorg. nucl. Chem. 5, 213-218.

Makarov, B.N. (1969) [Liberation of nitrogen dioxide from soil]. Pochvovedeniye 1, 49-53.

Marshall, K.C. (1968) Interaction between colloidal montmorillonite and cells of Rhizobium species with different ionogenic surfaces. Biochim. biophys. Acta 156, 179-186.

McGarity, J.W. (1961) Denitrification studies on some South Australian soils. Pl. Soil 14, 1-21.

McGarity, J.W. (1962) Effect of freezing soil on denitrification. Nature, Lond. 196, 1342-1343.

McKee, H.S. (1962) Denitrification, 116-125, in Nitrogen Metabolism in Plants, Clarendon Press, Oxford.

McLaren, A.D. (1969) Radiation as a technique in soil biology and biochemistry. Soil Biol. Biochem. 1, 63-73.

McLaren, A.D., Reshetko, L., and Huber, W. (1957) Sterilisation of soil with an electron beam, and some observations on soil enzyme activity. Soil Sci. 83, 497-502.

Mebius, L.J. (1960) A rapid method for the determination of organic carbon in soil. Analytica chim. Acta 22, 120-124.

Meek, B.D., and MacKenzie, A.J. (1965) The effect of nitrite and organic matter on aerobic gaseous losses of nitrogen from a calcareous soil. Proc. Soil Sci. Soc. Am. 29, 176-178.

Meiklejohn, J. (1940) Aerobic denitrification. Ann. appl. Biol. 27, 558-573.

Meiklejohn, J. (1951) The effects of glucose on impure cultures of nitrifying bacteria. Pl. Soil 3, 88-93.

Merritt, C. Jun. (1966) Chemical changes induced by irradiation in meats and meat components, 197-210, in Food Irradiation, F.A.O./ I.A.E.A., Vienna.

Merzari, A.H., and Broeshart, H. (1968) The utilisation by rice of nitrogen from ammonium fertilisers as affected by fertiliser placement and microbiological activity, 79-87, in Isotope Studies on the Nitrogen Chain, F.A.O./ I.A.E.A., Vienna.

Miège, E., and Coupé, H. (1914) De l'influence des rayons X sur la végétation. C. r. hebd. Séanc. Acad. Sci., Paris 159, 338-340.

Molin, N., and Ehrenberg, L. (1964) Anti-bacterial action of irradiated glucose. Int. J. Radiat. Biol. 8, 223-231.

Monib, M., and Zayed, M.N. (1963) The effect of gamma radiation on some of the organisms of soil. J. appl. Bact. 26, 35-42.

Mortland, M.M., and Wolcott, A.R. (1965) Sorption of inorganic nitrogen compounds by soil materials, 150-197, in Soil Nitrogen, edit. Bartholomew, W.V., and Clark, F.E., American Society of Agronomy, Madison, Wisconsin.

Muller, H.P. (1968) Bodensterilisation mit ionisierenden strahlen. Atompraxis 14, 480-484.

Munro, D.C., and MacKay, D.C. (1964) Effect of incubation method and storage conditions on nitrate production of incubated soil samples. Proc. Soil Sci. Soc. Am. 28, 778-781.

Najjar, V.A., and Allen, M.B. (1954) Formation of nitrogen, nitrous oxide, and nitric oxide by extracts of denitrifying bacteria. J. Biol. Chem. 206, 209-214.

Nason, A. (1962) Symposium on Metabolism of Inorganic Compounds. II. Enzymatic pathways of nitrate, nitrite, and hydroxylamine metabolisms. Bact. Rev. 26, 16-41.

Nelson, D.W., and Bremner, J.M. (1969) Factors affecting chemical transformations of nitrite in soils. Soil Biol. Biochem. 1, 229-239.

Newman, A.C.D. (1966) The distillation of ammonia for isotopic analysis. Chemy Ind. 115-116.

Nommik, H. (1956) Investigations on denitrification in soil. Acta Agric. scand. 6, 195-228.

Nishita, H., and Hamilton, M. (1968) Some thermoluminescent characteristics of gamma irradiated soils. Soil Sci. 106, 76-84.

Nishita, H., and Hamilton, M. (1969) Heating effects on the thermo-luminescence of gamma irradiated soils. Soil Sci. 108, 1-10.

Ojima, T., Toratani, H., and Fujimoto, H. (1963) Gamma and electron irradiation of clays and soils. A. Rep. Radiat. Cent. Osaka 4, 107-111.

Okada, S. (1958) Formation of hydroperoxides from certain amino acids and peptides in aqueous solutions by irradiation in the presence of oxygen, 46-48, in Organic Peroxides in Radiobiology, edit. Haissinsky, M., Pergamon Press, London.

Osmond, D.A., Swarbrick, T., Thompson, C.R., and Wallace, T. (1949) A survey of the soil and fruit in the Vale of Evesham, Min. of Agric. Bulletin No.116, H.M.S.O., London.

Overrein, L.N. (1967) Immobilisation and mineralisation of tracer nitrogen in forest raw humus. I. Effect of temperature on the interchange of nitrogen after addition of urea-, ammonium-, and nitrate-N^{15}. Pl. Soil 27, 1-19.

Overrein, L.N. (1969) Lysimeter studies on tracer nitrogen in forest soil. 2. Comparative losses of nitrogen through leaching and volatilisation after the addition of urea-, ammonium-, and nitrate-N^{15}. Soil Sci. 107, 149-159.

Peterson, G.H. (1962a) Respiration of soil sterilised by ionising radiations. Soil Sci. 94, 71-74.

Peterson, G.H. (1962b) Microbial activity in heat- and electron-sterilised soil seeded with microorganisms. Can. J. Microbiol. 8, 519-524.

Pikaev, A.K. (1967) The radiolysis of aqueous nitrates, 146-153, in Pulse Radiolysis of Water and Aqueous Solutions, Indiana University Press, Bloomington, U.S.A.

Piper, C.S. (1950) Soil and Plant Analysis, 2nd Ed, The University Press, Adelaide.

Popenoe, H., and Eno, C.F. (1962) The effect of gamma radiation on the microbial population of the soil. Proc. Soil Sci. Soc. Am. 26, 164-167.

Porter, L.K. (1965) Enzymes, 1536-1549, in Methods of Soil Analysis, edit. Black, C.A., American Society of Agronomy, Madison, Wisconsin.

Porter, L.K. (1969) Gaseous products produced by anaerobic reaction of sodium nitrite with oxime compounds and oximes synthesised from organic matter. Proc. Soil Sci. Soc. Am. 33, 696-702.

Promsy, G., and Drevon, M. (1912) Influence des rayons X sur la germination. Revue gén. Bot. 24, 177-197.

Reuss, J.O., and Smith, R.L. (1965) Chemical reactions of nitrites in acid soils. Proc. Soil Sci. Soc. Am. 29, 267-270.

Rigg, T., Scholes, G., and Weiss, J. (1952) Chemical actions of ionising radiations in solutions. Part X. The action of X-rays on ammonia in aqueous solution. J. chem. Soc. 3034-3038.

Roberge, M.R., and Knowles, R. (1968) Factors affecting urease activity in a black spruce humus sterilised by gamma radiation. Can. J. Soil Sci. 48, 355-361.

Robinson, R.H. (1923) Action of sodium nitrite in soil. J. agric. Res. 26, 1-7.

Rovira, A.D., and McDougall, B.M. (1967) Microbiological and biochemical aspects of the rhizosphere, 417-463, in Soil Biochemistry, edit. McLaren, A.D., and Peterson, G.H., Marcel Dekker Inc., New York.

Sacks, L.E., and Barker, H.A. (1952) Substrate oxidation and nitrous oxide utilisation in denitrification. J. Bact. 64, 247-252.

Salonius, P.O., Robinson, J.B., and Chase, F.E. (1967) A comparison of autoclaved and gamma-irradiated soils as media for microbial colonisation experiments. Pl. Soil 27, 239-248.

Saltzmann, B.E. (1965) Determination of nitrogen dioxide and nitric oxide: Saltzmann method, C1-C7, in Selected Methods for the Measurement of Air Pollution, U.S. Dept. of Health, Education and Welfare, Public Health Service Publication No.999-AP-11.

Scholes, G., and Weiss, J. (1960) Organic hydroxy-hydroperoxides: a class of hydroperoxides formed under the influence of ionising radiations. Nature, Lond. 185, 305-306.

Scholes, G., and Weiss, J. (1962) Effects of ionising radiations on nucleic acids and nucleoproteins, 127-136, in Biological Effects of Ionising Radiation at the Molecular Level, International Atomic Energy Agency, Vienna.

Schubert, J., Watson, J.A., and White, E.R. (1967) Hydroxyalkyl peroxides and the toxicity of irradiated sucrose. Int. J. Radiat. Biol. 13, 485-489.

Sims, A.P., and Cocking, E.C. (1958) Assay of isotopic nitrogen by mass spectrometer. Nature, Lond. 181, p.474.

Sims, C.M., and Collins, F.M. (1958) A modified design of the Audus soil perfusion apparatus. Analyst, Lond. 83, 699-701.

Singh, B.R., Agarwal, A.S., and Kanehiro, Y. (1969) Recovery of added nitrogen from gamma irradiated soils. Soil Sci. 108, 85-88.

Singh, B.R., and Kanehiro, Y. (1970) Effects of gamma irradiation on the available nitrogen status of soils. J. Sci. Fd Agric. 21, 61-64.

Skujins, J.J., Braal, L., and McLaren, A.D. (1962) Characterisation of phosphatase in a terrestrial soil sterilised with an electron beam. Enzymologia 25, 125-133.

Skujins, J.J., and McLaren, A.D. (1969) Assay of urease activity using ^{14}C-urea in stored, geologically preserved, and in irradiated soils. Soil Biol. Biochem. 1, 89-99.

Skyring, G., and Thompson, J.P. (1966) The availability of organic matter in dried and undried soil, estimated by an anaerobic respiration technique. Pl. Soil 24, 289-298.

Smith, D.M., and Mixer, R.Y. (1959) The effects of lignin on the degradation of wood by gamma irradiation. Radiat. Res. 11, 776-780.

Sorensen, L.H. (1969) Fixation of enzyme protein in soil by the clay mineral montmorillonite. Experientia 25, 20-21.

Soulides, D.A. (1969) Antibiotic tolerance of the soil microflora in relation to type of clay minerals. Soil Sci. 107, 105-107.

Soulides, D.A., and Allison, F.E. (1961) Effect of drying and freezing soils on carbon dioxide production, available mineral nutrients, aggregation, and bacterial population. Soil Sci. 91, 291-298.

Splittstoesser, D.F., and Massey, L.M. Jun. (1967) Irradiation of composts used in propagation of Agaricus bisporus. Appl. Microbiol. 15, 646-649.

Sprinson, D.B., and Rittenberg, D. (1949) The rate of utilisation of ammonia for protein synthesis. J. Biol. Chem. 180, 707-714.

Stanier, R.Y. (1942) Are there obligate cellulose-decomposing bacteria? Soil Sci. 53, 479-480.

Stanovick, R., Giddens, J., and McCreery, R.A. (1961) Effect of ionising radiation on soil microorganisms. Soil Sci. 92, 183-187.

Stevenson, F.J., and Swaby, R.J. (1964) Nitrosation of soil organic matter: I. Nature of gases evolved during nitrous acid treatment of lignins and humic substances. Proc. Soil Sci. Soc. Am. 28, 773-778.

Stevenson, I.L. (1956) Some observations on the microbial activity in remoistened air-dried soils. Pl. Soil 8, 170-182.

Stocklasa, M.J. (1913a) De l'influence de l'uranium et du plomb sur la végétation. C. r. hebd. Séanc. Acad. Sci., Paris 156, 153-155.

Stocklasa, M.J. (1913b) Influence de la radioactivité sur les microorganisms fixateurs d'azote ou transformateurs de matières azotées. C. r. hebd. Séanc. Acad. Sci., Paris 157, 879-882.

Stotzky, G., and Mortensen, J.L. (1959) Effect of gamma radiation on the growth and metabolism of microorganisms in an organic soil. Proc. Soil Sci. Soc. Am. 23, 125-127.

Stotzky, G. (1967) Clay minerals and microbial ecology. Trans. N.Y. Acad. Sci. 30, 11-21.

Syrokomsky, W.S., and Stiepin, V.V. (1936) New oxidation-reduction indicators. I. Phenylanthranilic acid (σ-diphenylamine carbonic acid). J. Am. chem. Soc. 58, 928-929.

Tanada, T. (1969) An early lesion from low doses of X-irradiation in plant cells. Radiat. Res. 37, 103-107.

Theron, J.J. (1951) The influence of plants on the mineralisation of nitrogen and the maintenance of organic matter in the soil. J. agric. Sci., Camb. 41, 289-296.

Thomas, M.D., MacLeod, J.A., Robbins, R.C., Goettelman, R.C., Eldridge, R.W., and Rogers, L.H. (1956) Automatic apparatus for determination of nitric oxide and nitrogen dioxide in the atmosphere. Analyt. Chem. 28, 1810-1816.

Turner, F.T., and Patrick, W.H. Jun. (1968) Chemical changes in waterlogged soils as a result of oxygen depletion, 53-65, in Transactions of the Ninth International Congress of Soil Science, Vol.4, Adelaide.

Tyler, K.B., and Broadbent, F.E. (1960) Nitrite transformations in California soils. Proc. Soil Sci. Soc. Am. 24, 279-282.

Valera, C.L. (1959) Investigations on the denitrifying flora of the soil. Diss. Abstr. 20, 1929-1930.

Valera, C.L., and Alexander, M. (1961) Nutrition and physiology of denitrifying bacteria. Pl. Soil 15, 268-280.

Vela, G.R. (1969) The effects of ionising radiation on nitrification in the soil. Tex. J. Sci. 20, 315-322.

Vela, G.R., and Wyss, O. (1965) Radiation resistance of soil Azotobacter. J. Bact. 89, 1280-1285.

Verhoeven, W. (1956) Some remarks on nitrate and nitrite metabolism in microorganisms, 61-86, in Inorganic Nitrogen Metabolism, edit. McElroy, W.D., and Glass, B., John Hopkins Press, Baltimore.

Wagner, G.H., and Smith, G.E. (1958) Nitrogen losses from soils fertilised with different nitrogen carriers. Soil Sci. 85, 125-129.

Wahzab, A., and Uddin, F. (1954) Loss of nitrogen through reaction of ammonium and nitrite ions. Soil Sci. 78, 119-126.

Waring, S.A., and Bremner, J.M. (1964) Ammonium production in soil under waterlogged conditions as an index of nitrogen availability. Nature, Lond. 201, 951-952.

Wellman, R.P., Cook, F.D., and Krouse, H.R. (1968) Nitrogen-15: microbiological alteration of abundance. Science 161, 269-270.

Whelden, R.M., Enzmann, E.V., and Haskins, C.P. (1941) The influence of Roentgen rays upon the nitrogen fixation by Azotobacter. J. gen. Physiol. 24, 789-796.

Whittaker, B. (1964) Radiation dosimetry technique using commercial red "Perspex". Report No. R 3360, Atomic Energy Research Establishment, Harwell.

Wills, E.D., and Wilkinson, A.E. (1967) The effect of irradiation on lipid peroxide formation in subcellular fractions. Radiat. Res. 31, 732-747.

Woldendorp, J.W. (1962) The quantitative influence of the rhizosphere on denitrification. Pl. Soil 17, 267-270.

Woldendorp, J.W. (1963) The influence of living plants on denitrification. Mededel. Landbouwhogeschool Wageningen 63, 1-100.

Wood, R.T., and Casida, L.E. (1967) Isolation and characterisation of a highly pleomorphic soil Bacillus. Bact. Proc. A 13, 2-3.

Wullstein, L.H. (1967) Soil nitrogen volatilisation. Agric. Sci. Rev. 5, 8-13.

Wullstein, L.H. (1969) Reduction of nitrite deficits by alkaline metal carbonates. Soil Sci. 108, 222-226.

Wullstein, L.H., and Gilmour, C.M. (1966) Non-enzymatic formation of nitrogen gas. Nature, Lond. 210, 1150-1151.

Zobell, C.E. (1943) The effect of solid surfaces upon bacterial activity. J. Bact. 46, 39-56.

14 APPENDIX

"The Use of Gamma Radiation in Soil Research"

The following essay-review was undertaken as approved course work, and published during the period of study for Ph.D.

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RESEARCH GROUP

Report

THE USE OF GAMMA RADIATION IN SOIL RESEARCH

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1969

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AERE-R 6061

THE USE OF GAMMA RADIATION IN SOIL RESEARCH

by

P. A. Cawse

ABSTRACT

In agricultural research practice it has been found that there are several clear advantages in using radiation rather than heat or chemicals to sterilise or partially sterilise soil. Radiation has already been applied to many delicate experiments on such topics as enzyme behaviour, degradation and adsorption of organic compounds by soil, colonisation of selected microorganisms in sterile soil, nitrogen mineralisation and nitrate reduction, microfauna activities, uptake of nutrients by sterile plants, and soil aggregation. Irradiation of plants in gamma fields has prompted several studies on the overall response of the soil ecosystem.

At present, radiation is a relatively new tool for soil research workers, but by virtue of its successful application to a wide variety of problems the technique should find increasing application to many future programmes. To this end, some suggestions have been put forward in the present review.

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1. INTRODUCTION

The historical background to studies concerning the effects of gamma radiation on soils is frequently neglected and little appreciated, no doubt because of the association of radiation with atomic energy production which is a relatively new technology. However, Atkinson (1897) carried out brief experiments to determine the effects of radiation on plants only two years after the discovery of X-rays by W. C. Roentgen.

Stocklasa (1913a) reported increases in the yield of plants when radioactive materials were mixed with soil, and subsequently (Stocklasa 1913b) he stated that nitrogen fixation could be increased by passing radioactive air containing the alpha-emitter Radon-222 over the soil. When pure cultures of Azotobacter chroococcum, nitrogen mineralising bacteria and denitrifying bacteria were treated with alpha, beta and gamma radiations from pitchblende (uraninite), nitrogen fixation was increased by alpha particles but beta and gamma radiations were found to exert a harmful effect on all three processes. Stimulation of Azotobacter agile was later confirmed by Kayser (1921), and for three separate species of the bacteria by Kayser and Delaval (1924).

Although the discovery of artificial induction of radioactivity in 1934 by Joliot-Curie had resulted in the use of particle accelerators for production of over 200 radioisotopes by 1939, there was little further investigation of the response of soil to radiations emitted from these isotopes. With the construction of prototype power reactors, cobalt-60 could be produced at far less cost and in sufficient quantity to permit the development of large gamma sources of the type described by Jefferson and Rogers (1964). Such installations have been used for a wide range of biological and chemical research, and various aspects of soil irradiation have been presented by approximately sixty papers in the world literature since 1950.

Cobalt-60 emits two gamma quanta at 1.17 and 1.33 MeV, and has a half-

life of 5.6 years; for sterilisation purposes it is preferable to the longer lived caesium-137 because the radiation is of higher energy and thus more penetrating. At these gamma energies there is no induction of radioactivity in the irradiated sample, which is absolutely safe to handle.

Some workers have sterilised thin layers of soil in an electron beam, but the penetration of 5 MeV electrons is very poor if the soil thickness is greater than 1.2 cm (McLaren et al., 1962) and gamma radiation is far more satisfactory. The present review is not concerned with the research value of radiation measurements to trace radioisotope behaviour in soils.

RELATIVE MERITS OF STERILISATION BY RADIATION

Radiation can be used to sterilise or partially sterilise soil as a preliminary treatment to a wide range of soil research experiments which will be mentioned later. It is preferred to heat or chemical treatments such as 'D-D' (dichloropropene/propane mixture), methyl bromide, chloropicrin or formalin for several reasons.

First, the degree of sterility for experiments that require only partial sterilisation can be very accurately controlled by varying the radiation dose. Because different soil organisms show a wide variation in radiosensitivity, the selective nature of the process also has considerable significance, and several workers have emphasised this point (Stotzky and Mortensen, 1959; Popenoe and Eno, 1962; Monib and Zayed, 1963; Kashkina and Abaturov, 1967).

In general, an increase in gamma dose will affect the major groups of soil organisms in the order microfauna, fungi, bacteria, viruses and enzymes. One would not expect many invertebrates to survive 0.2 Mrad (Megarads, where rad = "radiation absorbed dose", equivalent to deposition of 100 ergs per gram of absorbing material), and 0.2 - 0.5 Mrad would inactivate the majority of fungi. While 1-2 Mrad should inactivate practically all soil bacteria, enzymes are extremely radioresistant and can easily tolerate 5 Mrad. However,

variation in the numbers of organisms present from one soil to another and differenoes in radiosensitivity make over-generalisation dangerous.

The effectiveness of any sterilisation procedure depends upon the numbers of organisms that are initially present (Ley and Tallentire, 1965; Jackson et al., 1967), and it is impossible to guarantee that a soil sample will be sterile after 2.5 Mrad, which is the accepted dose for sterilisation of medical goods in the U.K. As manufactured articles, the latter obviously support a very small microbial population compared with soil, where 10^8 bacteria per gram may have to be eliminated. From the experience gained in sterilisation of soils at Wantage for outside research institutes, it can be said that 2.5 Mrad has rarely resulted in unsatisfactory sterilisation; it is recommended that sterility is always tested separately for each soil type in order to allow for high population densities.

Use of the term "inactivation" may lead to confusion unless it is realised that it refers to prevention of cell proliferation and not to the complete cessation of metabolic activity within the cell. This situation can be misleading in the case of chemoautotrophic organisms whose activity is most conveniently measured by biochemical means (Starkey, 1962). For example, Nitrosomonas and Nitrobacter cells can still carry out extensive oxidation of ammonium to nitrate after 1 Mrad, even though proliferation of the bacteria is prevented by 0.2 Mrad (Cawse, 1968); analysis for nitrate would not reflect inactivation but only total inhibition, which most probably occurs above 1 Mrad. A similar result would be expected for sulphur oxidising bacteria of the genus Thiobacillus, and these organisms were almost certainly present in experiments described by Popenoe and Eno (1962), where sulphate production from elemental sulphur was measured after irradiation. Furthermore, it is known that doses sufficient to inactivate Azotobacter fail to prevent nitrogen fixation (Sokurova, 1956), and that the microbial reduction of nitrate to nitrite proceeds rapidly at 0.8 Mrad (Cawse and Crawford, 1967;

Cawse and White, 1969b); this is a dose well above the region of 0.1 - 0.2 Mrad required to prevent proliferation.

Secondly, radiation may be preferred if there is any danger that the phytotoxicity of chemical residues could prevent any useful experimental conclusions from being reached, with wasted research effort. At the outset there must be a minimum delay of 30 days for chloropiorin and 10 days for formalin before experimental work can begin, whereas irradiated soil can be used at once. Jenkinson et al. (1968) compared the growth of wheat and ryegrass in soil treated with either 2.5 Mrad, or methyl bromide, or formalin. The irradiated treatment produced most dry matter with or without added nitrogen fertiliser, and at the highest level of fertilisation both chemical treatments depressed wheat growth below the control. Analysis revealed that the soils treated with 1000 p.p.m. methyl bromide still contained 121 p.p.m. bromine at harvest, and it was concluded that non-volatile degradation products were almost certainly responsible for the depression of growth. Aldrich and Martin (1952) previously reported that 'D-D' and chloropicrin can decompose to give chlorides, and plant damage is liable to follow.

The presence of phytotoxic substances in some heat-sterilised soils has long been recognised (Johnson, 1919), and in a comparison of heat, propylene oxide and radiation sterilisation of red-brown earth and sand soils by Bowen and Rovira (1961), subterranean clover (Trifolium subterraneum L.) was severely retarded by both heat and chemical methods. Hervey and Williams (1963) preferred gamma sterilisation because autoclaved soils used as growth media for cotton root rot fungus (Phymatotrichum omnivorum) showed toxicity to the organism.

Bowen and Cawse (1962) found that two soils treated with 2 Mrad gave a superior yield of lettuce (Lactuva sativa) compared with steam sterilisation, the increases being 1.5 and 3 times greater. Analysis of eight sterilised soils for exchangeable Cu, Fe, Mn and Zn showed that manganese was increased

by seven-fold to 121 p.p.m. after steaming, but only two-fold after 2 Mrad. The other trace elements were little affected. Presumably, so much manganese was reduced to available valency states by steaming that it had a harmful effect on plant growth. Lapensee (1959) found that more manganese was released by steaming than by chloropicrin or D-D, and on two soils growth of soybean (Soja hispida) was less than control; 10-30 p.p.m. Mn was toxic to this plant in culture solution, and it was known that the concentration in heat-treated soils could reach 155 p.p.m. Mn.

Thirdly, the choice of radiation may depend upon the relatively slight effect it has on humus. Any sterilisation technique will cause extra nitrogen mineralisation following death and lysis of the susceptible microbial population, and to a lesser extent from damage to humus. One would expect nitrogen release from the biomass to be greatest with the most effective sterilisation procedures, i.e. radiation or heat rather than chemicals, and damage to the humus fraction would be most severe after heating.

The work of Bowen and Cawse (1962) and Eno and Popenoe (1964) has shown that steaming gives greater ammonium release than irradiation, especially in very organic soils. Salonius et al. (1967) reported that an autoclaved clay soil contained more soluble organic matter, soluble carbohydrate, and water-extractable electrolyte than did soil treated with 5 Mrad, and it was toxic to microorganisms. These toxic substances were not identified, but it is known that normal soil breakdown of organic matter can produce growth inhibitors such as vanillin and benzoic acid (McCalla and Haskins, 1964).

Radiation damage to humus has certainly been detected, but there is very little release of nitrogen compared with the production from the biomass (Jenkinson, 1966; Cawse, 1968), and when Bowen and Cawse (1964b) irradiated soil organic matter obtained by sodium pyrophosphate extraction, it was calculated that only 4% of the increase in nitrogen after 4 Mrad originates from humus.

Finally, it is necessary to consider the effects of radiation, heat and chemicals on soil structure. Bowen and Cawse (1964a) observed that irradiated soils had a slower water percolation rate and dried out less rapidly than the controls, and Boyer et al. (1966) discussed a decrease in structural stability in several irradiated soils. Salonius et al. (1967) found that 3 Mrad reduced aggregate stability by 6%, but autoclaving increased the stability by 12%. On the other hand, Griffiths and Burns (1968) exposed natural aggregates to 2.5 Mrad without harmful effect. It seems that if physical damage does occur, it is very minor and less harmful to experimentation than chemical changes brought about by heat and chemical treatments, and this contention is supported by the continued success of microbial colonisation and plant growth experiments in irradiated soil. As far as chemical treatments are concerned, Aldrich and Martin (1952) found that D-D and chloropicrin did not affect aggregation.

A disadvantage of radiation treatment lies in the immobility of large sources that are required to deliver a high dose rate. The sample must always be transported to the source, and large scale field trials are impossible. It is quite feasible to treat 20-lb. packs of soil with 2.5 Mrad as a continuous process in a gamma-installation such as the Package Irradiation Plant at Wantage Laboratory. The cost is about 30/- per cwt. not including transport or packing costs estimated at £8 per ton, but it is very probable that a lower dose of 1 Mrad, which costs 12/- per cwt. plus handling charges, would be equally as effective as steam or chemicals. It is a dear process when compared with costs of about £600 per acre (7d per cwt*) for steaming, and £200 per acre (2d per cwt*) for chloropicrin or D-D, but the high cost of research experiments and the moderate quantities of soil usually required still make radiation an attractive proposition. With chemicals, hazard to the operator must also be considered. There is also the advantage that soil can be irradiated in the experimental pots, which reduces subsequent handling

*on the assumption that an acre to 5" depth = 2×10^6 lb.

and contamination to a minimum.

If only sub-sterilising doses of radiation were needed for certain types of investigation, a mobile gamma source could be employed. Dietz and Lafferty (1966) have described a mobile irradiator intended for fruit treatment in the U.S.A. which contains 100,000 curies of cobalt-60, weighs 59 tons complete, and can process 1000 lb. per hour with 0.2 Mrad. At present, irradiation facilities of this type are extremely rare, and a recent review of irradiation pilot plants (de Zeeuw, 1968) lists only two other mobile sources situated in Canada and France.

EXPERIMENTAL APPLICATION OF RADIATION

Owing to some clear advantages of radiation sterilisation which have just been mentioned, the technique has recently been recommended by Muller (1968) for application in soil research. There are already a considerable number of reports in the literature which mention the use of radiation in the following types of investigation:

(a) Soil Enzymes

The strong adsorption of soil enzymes to clay minerals makes extraction so difficult that their presence and behaviour is frequently investigated by addition of substrate to soil which has undergone some treatment to prevent microbial proliferation. In the past, toluene has often been employed for this purpose, but there are conflicting reports of its usefulness (Skujins, 1967). Certain bacteria, e.g. Streptomyces, are resistant, and up to 20% toluene is needed to sterilise some soils.

Radiation sterilisation has been successfully used for research into the activities of soil urease (McLaren et al., 1957) and phosphatase (Skujins et al., 1962; McLaren et al., 1962), but it is still impossible to say whether enzyme activity that occurs after irradiation proceeds within inactivated cells, or if released enzymes are operating extracellu-

larly, or if original naturally absorbed enzymes are responsible. Work by Ramirez-Martinez and McLaren (1966) on phosphatase activity in irradiated soils does indicate that there is a relatively stable extracellular enzyme component attached to humus. Furthermore, McLaren (1963) has suggested that urea is a sufficiently small molecule to diffuse into inactivated cells containing urease, and the reaction products, ammonia and carbon dioxide, diffuse out again. Stevenson (1964) has emphasised the findings of Peterson (1962a), that respiration continues in radiation-sterilised soil, and points out that the bactericidal effect of radiation is probably due to inhibition of enzyme synthesis by the cell rather than to inactivation of enzymes already within it; in this situation, diffusion of the substrate molecules to intracellular enzymes would be a critical factor controlling activity.

Voets et al. (1965) have used radiation to confirm results obtained from treatment with toluene. It was reported that amino acids were not attacked by free enzymes after either method of sterilisation, which implies that the direct conversion of these compounds is only accomplished by living micro-organisms. In addition it was shown that 2 Mrad did not affect the activity of urease, saccharase, acid phosphatase and proteolytic enzymes in an arable sandy loam.

Van Niekerk (1964) has developed an improved method for determining urease in soil by pre-treatment with 1 Mrad before addition of the urea-buffer. The radiation dose must ensure sterility and should be previously tested. Reproducibility was superior to existing methods which use toluene as a sterilising agent.

(b) Degradation and Adsorption of Compounds in Soil

Sterilisation by radiation has been utilised in a study on adsorption of 'disulfoton' (diethyl S - 2 - (ethylthio) ethyl phosphorothiolothionate) by soil (Graham-Bryce, 1967). Application of 2.5 Mrad permitted better

recovery of the insecticide, and indicated that microbial decomposition was taking place in the control soil, thus preventing correct interpretation of the adsorption phenomena.

Radiation could be particularly useful in pesticide and herbicide adsorption work because it has little effect on soil organic matter, which is itself closely related to adsorption. In work of this kind, it is first essential to examine the effect of radiation on the pure compound, unless it can be separately sterilised by membrane filtration and then added to gamma-sterilised soil.

Corey et al. (1967) have sterilised soil columns contained in acrylic plastic cylinders in order to study movement of nitrate. Normally, denitrification makes this experiment impossible so that chloride must be used to simulate nitrate, but in many other cases substitution will be impossible. The authors suggest a wide application for their technique in research on the movement of herbicides, fungicides and waste products through soils; carbon dioxide and oxygen flow could be examined in a more elaborate apparatus.

Bowman et al. (1967) have treated soil columns with 3 Mrad in experiments which show that there is little movement of added phytic acid; 92% of the organic phosphorus was recovered, and the slight deficit indicates that phosphatases were still active. The disadvantage of experiments of this type in unsterile soil is that organic phosphorus compounds may be synthesised by microorganisms in the lower horizons of a profile, making it impossible to assess the extent of downwards movement.

(c) Introduction of Selected Organisms into Sterile Soil

The successful colonisation of sterile soil with selected organisms is necessary for research into the inter-relationships of microbes, and some preliminary experiments have been made to assess the value of radiation. Peterson (1962b) proved that Arthrobacter sp., Bacillus megaterium, Pseudomonas sp., and Xanthomonas vesicatoria could easily be established in an

irradiated silty clay loam, but extended lag phases were frequently observed in autoclaved soils. One organism, Myxococcus fulvus, failed to grow in either heat- or radiation-sterilised soil.

Salonius et al. (1967) established that Pseudomonas fluorescens and Arthrobacter globiformis colonise gamma-irradiated better than autoclaved soil, but no explanations have been offered for the toxicity of the latter, although it was shown that radiation increased the conductivity of aqueous extracts by 16% compared with 62% after autoclaving. Van Groenewoud (1958) suggested that radiation sterilisation should be very suitable for examining the antagonism of microorganisms in natural media; he found that irradiated humus had practically no effect on the action of several antibiotics on Staphylococcus.

Skyring and Thompson (1966) set out to examine whether variation in the microbial population or organic matter release during air-drying was responsible for the well known "air-drying effect" (Birch, 1958), manifested by increased microbial activity in the re-moistened soil. Pseudomonas denitrificans was introduced into irradiated soil so that reduction of nitrate could be used as an index of organic matter availability.

Hering (1967) compared the decomposing abilities of several species of fungi on gamma-sterilised and autoclaved oak leaf litter. Three strains of Trichoderma viride tended to give higher rates of decomposition on the autoclaved material, probably because of nutrient release and breakage of cellulose fibres by the sterilisation.

Part of a study by Merzari and Broeshart (1968) on loss of nitrogen from N-15 labelled ammonium sulphate involved the inoculation of sterile irradiated soil with nitrifying organisms, a denitrifying organism, and the total flora. This was followed by addition of labelled fertiliser and culture of rice. Unfortunately, inoculation was unsuccessful and prevented the collection of some valuable data. Further effort seems to be required on the feasibility

of inoculating irradiated soil with economically important organisms, and elucidation of the reasons for failure to proliferate.

The inoculation of sterile soil with a selected organism for continuous flow investigations of the type described by Macura (1961) could be a useful technique to evaluate the contribution of various organisms towards mineralisation of carbon and nitrogen.

(d) Nitrogen Mineralisation and Denitrification

The nitrification of ammonium released by death, lysis and mineralisation of radiation-susceptible microorganisms occurs rapidly in many soils, and the nitrate concentration reaches a maximum between 0.2 and 0.8 Mrad (Cawse, 1968; Cawse and White, 1969a). A significant increase in nitrification has even been reported after 0.002 Mrad (Cawse, 1967). The oxidation process is thought to be carried out by non-proliferating cells of the nitrifying bacteria (Cawse, 1968), and the ammonium release is greatest under conditions of high soil moisture (Bowen and Cawse, 1964a).

Such radiobiological studies also provide information which may assist interpretation of the soil response to chemical sterilants and herbicides that may inactivate nitrifying bacteria without affecting their oxidation processes; in this case, measurement of nitrate concentration would not necessarily be proof of the long term survival of the organisms.

The release of total mineral nitrogen in an irradiated clay loam showed a sharp rise up to 0.6 Mrad, followed by a distinct plateau, which implies that there is no further damage to the biomass (Cawse, 1968); it may be possible to utilise this feature for estimating the relative size of microbial populations in different soils, otherwise a notoriously difficult task.

If soils are treated with 3 Mrad, nitrification is inhibited and ammonium will accumulate (Cawse, 1968). This situation is advantageous for experiments on the merits of ammonium or nitrate nutrition of crops, which must otherwise be confined to solution culture. Nitrification inhibitors such

as 2-chloro-6-(trichloromethyl) pyridine can be applied to soil, but in many cases problems of adsorption and volatilisation do not permit a satisfactory block to ammonium oxidation.

Rovira and Bowen (1969) have found it possible to study the ammonium nutrition of wheat in a soil treated with 2.5 Mrad, and then inoculated with soil suspension: heterotrophic organisms recolonised much faster than Nitrosomonas and Nitrobacter, and ammonium was not nitrified for at least 11 weeks after irradiation. Restoration of heterotrophic activity was required to approach conditions in the unsterilised soil, because these organisms can affect plant nutrient uptake by mineralisation of organic matter and development in the root rhizosphere.

Nitrite can accumulate under aerobic conditions in some soils treated over the range 0.6 - 2.5 Mrad, and the greatest concentrations have been detected in organic calcareous types, even up to 115 p.p.m. NO_2-N (Cawse and Crawford, 1967; Cawse and White, 1969b). It appears that a mechanism of microbial nitrate reduction is mainly responsible, and two ways in which this could be carried out are by non-proliferating bacteria, perhaps Bacillus and Pseudomonas spp., or by a residual enzyme fraction bound to clays. Further research into nitrite formation and decomposition to oxides of nitrogen after irradiation should add useful information to the scant knowledge of nitrate loss as gaseous nitrogen, and perhaps help to characterise the soil types that have a high denitrifying potential. It is also essential to obtain further information on the fluctuations in mineral nitrogen after irradiation to enable more accurate experimentation with this sterilisation technique. Problems of nitrite toxicity to plants could now be evaluated in soil which has been irradiated to prevent nitrification, whereas previous work on this subject was restricted to solution culture, and extrapolation to soil conditions is frequently criticised.

In experiments on nitrogen mineralisation by Overrein (1967), gamma-

sterilised humus has been used to investigate the rate of chemical fixation of fertiliser nitrogen; this was very rapid, and would be completed before biological assimilation of nitrogen could begin.

(e) Soil Irradiation and the Microfauna

Research on the litter-soil ecosystem by Coleman and MacFadyen (1966) involved the inoculation of gamma-sterilised cores of soil with selected fungi, and replacement in the field to assess recolonisation by microarthropods. Heat or chemical sterilants were considered unsuitable, and the authors concluded that their technique should be promising for future work of this kind.

Edwards (1969) investigated the effect of 0.01 - 0.2 Mrad on soil invertebrates, and has accumulated some useful basic information on the response of this section of the soil ecosystem. He remarked that radiation might be used to study ecological interactions by eradication of certain animal groups such as parasitic mites which are more sensitive than isotomid, onychiurid and podiurid Collembola; the latter group even increased in numbers after 0.01 Mrad. In addition, to assess the harmful effects of a soil inhabitant on plant growth it would be feasible to apply 0.2 Mrad, and then recolonise the soil with selected insects or nematodes; yields would be compared with an irradiated but uncolonised treatment.

(f) Sterile Plant Cultures and Fungal Effects on Plants

An attempt has been made to utilise irradiated soil for experiments on the uptake of large molecules, such as proteins, by plants (Ulrich et al., 1964). There was no evidence that protease activity remaining in the sterilised soil would change beta-lactoglobulin (M. Wt. 37,000) into compounds which could be taken up by tomato plants, but the extreme radioresistance of enzymes might well be a disadvantage in similar studies where different soils and/or other enzyme systems are involved. For example, the persistence of phosphatase in irradiated soil and its presence in sterile roots

frustrates experiments on the uptake of organophosphates by plants (McLaren, 1967).

The selective nature of radiation has allowed the removal of a plant pathogen, Plasmodiophora brassicae, by treatment of soil with 0.050 Mrad (Lur'e and Ter-Simonyan, 1959). Similarly, radiation could be applied to work on mycorrhizal relationships by removal of the specific fungus from soil; normally, the more radioresistant bacterial population would be relatively unharmed. As an alternative, soil could be treated with a sterilising dose before inoculation with selected fungi. The potential value of radiation in this field has been forecast by Barber (1968) in his review on microorganisms and the inorganic nutrition of higher plants.

(g) Response of the Ecosystem

There have been few reports on the effect of chronic irradiation on the soil microflora relative to those dealing with acute dosages, and experiments on the behaviour of plants in gamma fields have frequently ignored the soil reaction. It has been pointed out that some cases of plant stimulation in gamma fields could arise from the extra nitrogen mineralisation (Bowen and Cawse, 1964a). McCormick (1963) irradiated a natural plant community and noticed that the competitive vigour of Arenaria brevifolia was so increased between 0.008 and 0.035 Mrad that it became dominant; the soil nitrogen response may well have contributed to this observation.

Rambelli (1962) examined the microflora in a gamma field, and found increased nitrogen fixation and ammonifying capacity but reduced nitrification and denitrification. In a subsequent trial (Rambelli, 1963) rapid seasonal fluctuations in the microflora complicated interpretation of the exact influence of radiation,but it was clear that various physiological groups were greatly disturbed.

The possibility of environmental contamination by reactor waste has prompted some soil studies (Davis et al., 1956; Auerbach, 1958) which again

emphasise the differential response of various microbial groups. Blumenfeld (1966) has considered that a thermonuclear war would not serious affect the microflora in the long term, and two years after nuclear tests, Durell and Shields (1960) isolated 41 species of fungi from soils within 1 mile radius of ground zero. These types of study give little help to soil research programmes, except perhaps for rare cases where nuclear detonations are used for modifying the topography, as in river diversion, and an idea of soil behaviour is advisable.

(h) Miscellaneous Applications

Johnson and Osborne (1964) isolated certain species of fungi from soil treated with 0.25 - 1 Mrad, whereas the same organisms were absent on dilution or soil plates from normal soil. They suggest that radiation might be used to isolate certain rare fungi that occur in low numbers, if they are resistant to radiation. Bovard and Grauby (1963) stated that experiments were in progress on irradiation of soil to induce bacterial mutants which may be suitable for the production of antibiotics.

Griffiths and Burns (1968) established that although the stability of natural aggregates in four soils was unaffected by 2.5 Mrad, that of synthetic aggregates prepared with microbial polysaccharides was significantly reduced. This observation led to the suggestion that radiation may be of use in assessing the importance of microbial polysaccharides in soil aggregation.

Sterile kaolin pellets and aggregates containing soluble and insoluble organic substrates are valuable for investigation into localised microbial development and biochemical changes in soil. Sterilisation is best achieved by radiation since autoclaving is liable to permeate them with moisture (Webley and Jones, 1968).

Ojima et al. (1963) considered that if a toxic material was produced in irradiated soil it might be extracted and used for weed control, but a search for such compounds was negative.

REFERENCES

Aldrich, D. G., and Martin, J. P. (1952). Effect of fumigation on some chemical properties of soils. Soil Sci., 73, 149-159.

Atkinson, G. F. (1897). Report upon some preliminary experiments with the Röntgen rays on plants. Nature, Lond., 56, p.600.

Auerbach, S. I. (1958). The soil ecosystem and radioactive waste disposal to the ground. Ecology, 39, 522-529.

Barber, D. A. (1968). Microorganisms and the inorganic nutrition of higher plants. A. Rev. Plant Physiol., 19, 71-88.

Birch, H. F. (1958). The effect of soil drying on humus decomposition and nitrogen availability. Pl. Soil, 10, 9-31.

Blumenfeld, S. N. (1966). Nuclear war and soil microflora. USAEC Report RM-4827-TAB.

Bovard, P. and Grauby, A. (1963). Essais d'irradiation des sols dans le réacteur-piscine Triton. Énergie nucl., 5, 149-153.

Bowen, G. D. and Rovira, A. D. (1961). Plant growth in irradiated soil. Nature, Lond., 191, 936-937.

Bowen, H. J. M. and Cawse, P. A. (1962). The effect of ionising radiation on the availability of inorganic ions in soils, 17-30, in "Radioisotopes in Soil-Plant Nutrition Studies", International Atomic Energy Agency, Vienna.

Bowen, H. J. M. and Cawse, P. A. (1964a). Effects of ionising radiation on soils and subsequent crop growth. Soil Sci., 97, 252-259.

Bowen, H. J. M. and Cawse, P. A. (1964b). Some effects of gamma radiation on the composition of the soil solution and soil organic matter. Soil Sci., 98, 358-361.

Bowman, B. T., Thomas, R. L. and Elrick, D. E. (1967). The movement of phytic acid in soil cores. Proc. Soil Sci. Soc. Am., 31, 477-481.

Boyer, J., Combeau, A., Grauby, A. and Thomas A. (1966). Évolution des sols sous l'effet d'une irradiation gamma. Bull. Infs scient. tech. Commt Énerg. atom., No. 106, 39-55.

Cawse, P. A. (1967). Effects of low sub-sterilising doses of gamma radiation on carbon, nitrogen and phosphorus in fresh soils. J. Sci. Fd Agric., 18, 388-391.

Cawse, P. A. (1968). Effects of gamma radiation on accumulation of mineral nitrogen in fresh soils. J. Sci. Fd Agric., 19, 395-398.

Cawse, P. A. and Crawford, D. V. (1967). Accumulation of nitrite in fresh soils after gamma irradiation. Nature, Lond., 216, 1142-1143.

Cawse, P. A. and White, T. (1969a). Accumulation of nitrate in fresh soils after gamma irradiation. J. agric. Sci., Camb., in the press.

Cawse, P. A. and White T. (1969b). Rapid changes in nitrite after gamma irradiation of fresh soils. J. agric. Sci., Camb., in the press.

Coleman, D. C. and Macfadyen, A. (1966). The recolonisation of gamma-irradiated soil by small arthropods. Oikos, 17, 62-70.

Corey, J. C., Nielson, D. R. Picken, J. C. Jr., and Kirkham, D. (1967). Miscible displacement through gamma radiation-sterilised soil columns. Envir. Sci. Tech., 1, 144-147.

Davis, R. J., Sheldon, V. L. and Auerbach, S. I. (1956). Lethal effects of gamma radiation upon segments of a natural microbial population. J. Bact., 72, 505-510.

Dietz, G. R. and Lafferty, R. H. (1966). USAEC food irradiator program. Fd Irradiation, 6, A35-A49.

Durell, L. W. and Shields, L. M. (1960). Fungi isolated in culture from soils of the Nevada test site. Mycologia, 52, 636-641.

Edwards, C. A. (1969). The effects of gamma irradiation on populations of soil invertebrates. (in the press). Paper read at the 2nd National Symposium on Radioecology, Ann Arbor, Michigan, 1967.

Eno, C. F. and Popenoe, H. (1964). Gamma radiation compared with steam and methyl bromide as a soil sterilising agent. Proc. Soil Sci. Soc. Am., 28, 533-535.

Graham-Bryce, I. J. (1967). Adsorption of disulfoton by soil. J. Sci. Fd Agric., 18, 72-77.

Griffiths, E. and Burns, R. G. (1968). Effects of gamma irradiation on soil aggregate stability. Pl. Soil, 28, 169-172.

Groenewoud,H. van (1958). Humus sterilisation by gamma irradiation. Proc. Can. phytopath. Soc. No. 26, p.12.

Hering, T. F. (1967). Fungal decomposition of oak leaf litter. Trans. Br. mycol. Soc., 50, 267-273.

Hervey, R. J. and Williams, Z. M. (1963). Microbial survival in gamma irradiated soils. Tex. Rep. Biol. Med., 21, p.450.

Jackson, N. E., Corey, J. C., Frederick, L. R. and Picken, J. C. Jr. (1967). Gamma irradiation and the microbial population of soils at two water contents. Proc. Soil Sci. Soc. Am., 31, 491-494.

Jefferson, S. and Rogers, F. (1964). Radiation Sources, 271-304, in "Massive Radiation Techniques", edit. Jefferson, S., G. Newnes, London.

Jenkinson, D. S. (1966). Studies on the decomposition of plant material in soil. II. Partial sterilisation of soil and the soil biomass. J. Soil Sci., 17, 280-302.

Jenkinson, D. S., Nowakowski, T. Z. and Mitchell, J. D. D. (1968). Release of nitrogen by partially sterilised soil, 77-78, in Rep. Rothamsted exp. Sta. for 1967.

Johnson, J. (1919). The influence of heated soils on germination and growth. Soil Sci., 7, 1-87.

Johnson, L. F. and Osborne, T. S. (1964). Survival of fungi in soil exposed to gamma radiation. Can J. Bot., 42, 105-113.

Kashkina, G. B. and Abaturov, Yu. D. (1967). [The survival of soil microorganisms upon gamma irradiation of the soil]. Radiobiologiya, 7, 263-266.

Kayser, E. (1921). Influence des sels d'urane sur le fixateur d'azote. C. r. hebd. Séanc. Acad. Sci., Paris, 172, 1133-1134.

Kayser, E. and Delaval, H. (1924). Radioactivité et fixateurs d'azote. C. r. hebd. Séanc. Acad. Sci., Paris, 179, 110-112.

Lapensee, J. M. (1959). Chemical and biological changes effected in certain Ohio soils by partial sterilisation and plant growth relationships. Diss. Abstr., 20, 1918-1920.

Ley, F. J. and Tallentire, A. (1965). Radiation sterilisation - the choice of dose. Pharm. J., 195, 216-218.

Lur'e, L. S. and Ter-Simonyan, L. G. (1959). [On the possibility of using gamma-radiation for soil disinfection and control of club root of Cruciferae]. Proc. Lenin Acad. agric. Sci., 24, 28-29.

Macura, J. (1961). Continuous flow method in soil microbiology. I. Apparatus. Folia microbiol., Praha, 6, 328-334.

McCalla, T. M. and Haskins, F. A. (1964). Phytotoxic substances from soil microorganisms and crop residues. Bact. Rev., 28, 181-207.

McCormick, F. (1963). Changes in a herbaceous plant community during a three-year period following exposure to ionising radiation gradients, 271-276, in "Radioecology", edit. Schultz, V. and Klement, A. W., Chapman & Hall Ltd., London.

McLaren, A. D., Reshetko, L. and Huber, W. (1957). Sterilisation of soil with an electron beam, and some observations on soil enzyme activity. Soil Sci., 83, 497-502.

McLaren, A. D. (1963). Enzyme activity in soils sterilised by ionising radiation and some comments on micro-environments in nature. Recent Prog. Microbiol., 8, 221-229.

McLaren, A. D. (1967). An investigation into the utility of radiation sterilised soil as a medium for growth of plants and microorganisms. University of California Report No. UCB-34P50-3, Berkeley.

McLaren, A. D., Luse, R. A. and Skujins, J. J. (1962). Sterilisation of soil by irradiation and some further observations on soil enzyme activity. Proc. Soil Sci. Soc. Am., 26, 371-377.

Merzari, A. H. and Broeshart, H. (1968). The utilisation by rice of nitrogen from ammonium fertilisers as affected by fertiliser placement and microbiological activity, 79-87, in "Isotope Studies on the Nitrogen Chain", International Atomic Energy Agency, Vienna.

Monib, M. and Zayed, M. N. (1963). The effect of gamma radiation on some of the organisms of soil. J. appl. Bact., 26, 35-42.

Muller, H. P. (1968). Bodensterilisation mit ionisierenden strahlen. Atompraxis, 14, 480-484.

Ojima, T., Toratani, H. and Fujimoto, H. (1963). Gamma and electron irradiation of clays and soils. A. Rep. Radiat. Cent. Osaka, 4, 107-111.

Overrein, L. N. (1967). Immobilisation and mineralisation of tracer nitrogen in forest raw humus. I. Effect of temperature on the interchange of nitrogen after addition of urea-, ammonium-, and nitrate-N^{15}. Pl. Soil, 27, 1-19.

Peterson, G. H. (1962a). Respiration of soil sterilised by ionising radiations. Soil Sci., 94, 71-74.

Peterson, G. H. (1962b). Microbial activity in heat- and electron-sterilised soil seeded with microorganisms. Can. J. Microbiol., 8, 519-524.

Popenoe, H. and Eno, C. F. (1962). The effect of gamma radiation on the microbial population of the soil. Proc. Soil Sci. Soc. Am., 26, 164-167.

Rambelli, A. (1962). Ricerche preliminari sul comportamento di alcuni gruppi fisiologici di microrganismi del suoli all'irraggiamento da cobalto-60. Pubbl. Cent. Sper. agric. for., 6, 117-129.

Rambelli, A. (1963). Ulteriori ricerche sulla influenza delle radiazioni gamma da cobalto-60 sulla microflora del terreno. Pubbl. Cent. Sper. agric. for., 7, 127-146.

Ramirez-Martinez, J. R. and McLaren, A. D. (1966). Some factors influencing the determination of phosphatase activity in native soils sterilised by irradiation. Enzymologia, 31, 23-28.

Rovira, A. D. and Bowen, G. D. (1969). The use of radiation-sterilised soil to study the ammonium nutrition of wheat. Aust. J. Soil Res., 7, 57-65.

Salonius, P. O., Robinson, J. B. and Chase, F. E. (1967). A comparison of autoclaved and gamma-irradiated soils as media for microbial colonisation experiments. Pl. Soil, 27, 239-248.

Skujins, J. J. (1967). Enzymes in Soil, 371-414, in "Soil Biochemistry", edit. McLaren, A. D. and Peterson, G. H., Marcel Dekker Inc., New York.

Skujins, J. J., Braal, L. and McLaren, A. D. (1962). Characterisation of phosphatase in a terrestrial soil sterilised with an electron beam. Enzymologia, 25, 125-133.

Skyring, G. and Thompson, J. P. (1966). The availability of organic matter in dried and undried soil, estimated by an anaerobic respiration technique. Pl. Soil, 24, 289-298.

Sokurova, E. N. (1956). [On certain regularities in ionising radiation effects on microorganisms]. Izv. Akad. Nauk SSSR., Ser. Biol., 6, 35-53.

Starkey, R. L. (1962). Symposium on autotrophy, Part I: Introduction. Bact. Rev., 26, 142-144.

Stevenson, I. L. (1964). Biochemistry of soil, 242-291, in "Chemistry of the Soil", 2nd edn., edit. Bear, F. E., Reinhold Publishing Corporation, New York.

Stocklasa, M. J. (1913a). De l'influence de l'uranium et du plomb sur la végétation. C. r. hebd. Séanc. Acad. Sci., Paris, 156, 153-155.

Stocklasa, M. J. (1913b). Influence de la radioactivité sur les micro-organisms fixateurs d'azote ou transformateurs de matieres azotées. C. r. hebd. Séanc. Acad. Sci., Paris, 157, 879-882.

Stotzky, G. and Mortensen, J. L. (1959). Effect of gamma radiation on the growth and metabolism of microorganisms in an organic soil. Proc. Soil Sci. Soc. Am., 23, 125-127.

Ulrich, J. M., Luse, R. A. and McLaren, A. D. (1964). Growth of tomato plants in presence of proteins and amino acids. Physiol. Plant., 17, 683-696.

Van Niekerk, P. E. le R. (1964). A modified method for the determination of urease activity in the soil. S. Afr. J. Agric. Sci., 7, 131-134.

Voets, J. P., Dedeken, M. and Bessems, E. (1965). The behaviour of some amino acids in gamma irradiated soils. Naturwissenschaften, 16, p. 476.

Webley, D. M. and Jones, D. (1968). Techniques for the study of localised microbial activity in soil. Trans. 9th Int. Congr. Soil Sci., Adelaide, 3, 657-664.

de Zeeuw, D. (1968). Application of atomic energy in agriculture, 4-5, in Euratom report 4080 e for 1967.

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Summary—Increases in nitrite which were previously observed in four soils from different series after treatment with γ-radiation, particularly over the dose range 0·5–0·8 Mrad, have been further investigated by a ^{15}N tracer technique. It was thought that nitrate reduction to nitrite was taking place, and this mechanism was confirmed by reduction of $^{15}NO_3$ to $^{15}NO_2$ in all of the irradiated soils at medium moisture levels. However, three soils had a lower percentage of ^{15}N tracer than expected in the nitrite pool, and it is considered that direct oxidation of unlabelled ammonium to nitrite is the most likely explanation.

INTRODUCTION

RAPID accumulation of mineral nitrogen has been observed in many fresh soils after treatment with γ-radiation, and the stimulatory effect on plant growth is well established (Bowen and Cawse, 1964; Cummins and McCreery, 1964; Rovira and Bowen, 1969). The relative amounts of ammonium, nitrite and nitrate which are formed show great variation with the dose employed over the range 0·25–2·5 Mrad, and with soil properties. Maximum accumulation of nitrate occurs between 0·25–0·75 Mrad, but a further increase in dose inhibits the oxidation processes of nitrifying organisms and the ammonium concentration rises (Cawse and White, 1969a).

The formation of nitrite was first reported by Cawse and Crawford (1967) following the irradiation of two calcareous soils from Broad and Icknield series, and it was suggested that there might be a selective effect of radiation which results in greater damage to nitrification compared with nitrate reduction. Subsequent experiments have shown that nitrite fails to accumulate when the soils are waterlogged, probably because it undergoes more rapid microbial reduction. In a survey of the response of ten soil series to radiation, Cawse and White (1969b) found that 24 hr after γ-treatment under aerobic conditions, large accumulations of nitrite (26–60 ppm N) appeared only in soils containing carbonate. The optimum dose region for nitrite to accumulate was always between 0·5–0·8 Mrad, and when these soils were leached with water, or ammonium or nitrate solutions immediately after irradiation, only the last treatment allowed nitrite to form. This strongly suggested that microbial nitrate reduction was the principal cause of nitrite formation. The object of the present investigation was to obtain more substantial evidence for the occurrence of this mechanism by the use of $^{15}NO_3$, especially as the microbial reduction of nitrate to nitrite is the first major step in the true (dissimilatory) denitrification pathway to gaseous nitrogen.

MATERIALS AND METHODS

The soils were sampled from the top 6 in. of the Broad, Broadmoor, Colthrop and Icknield series located in the Reading district (Jarvis, 1968). The Broad series was under arable cultivation, the remainder being under grass. Some of their major properties are given in Table 1 together with the experimental conditions.

The fresh soils were passed through a 2 mm sieve, and distilled water was added with ^{15}N-enriched potassium nitrate solution (96·71 atom % ^{15}N excess) to samples of 50 g so that the final moisture levels were as indicated in Table 1. The aliquot of added nitrate contained 214·2 μg ^{15}N, and it was ascertained from previous nitrate analyses that this would give at least 4 atom % ^{15}N excess in the nitrate pools of all soils at irradiation.

Irradiation and extraction

After equilibration for two days at room temperature, one set of duplicate samples was irradiated with a source of ^{60}Co giving a dose rate of 0·82 Mrad/hr then incubated at 25°C. Finally, analysis was carried out for nitrate and nitrite and the distribution of ^{15}N tracer within these fractions. The doses and incubation times appear in Table 1, and were chosen separately for each soil to achieve maximum formation of nitrite in the shortest possible time. Another set of duplicate samples (without tracer or irradiation) was analysed for nitrate and nitrite immediately before and after incubation, the latter to ensure that nitrite did not accumulate without irradiation.

Nitrate and nitrite were extracted by shaking the soil three times with 60, 20 and 20 ml of water respectively in a 200 ml centrifuge bottle, and centrifuging for 10 min at 1000 g after each shake of 15 min. Clay particles in the supernatant fraction were removed by further centrifugation at 30,000 g for 40 min.

Steam distillation and evaporation of extracts

Total nitrate and isotope ratio analysis of nitrate was determined in a 25 ml aliquot of the extract by steam distillation in an apparatus similar to that described by Bremner and Edwards (1965). An exception was replacement of the glass condenser by a silver one, as recommended for ^{15}N work by Newman (1966) to avoid chemisorption of ammonia by the cold glass surface. After nitrite had been removed by mixing 1 ml of 2% (w/v) sulphamic acid with the aliquot, 0·4 g magnesium oxide was added for distillation of any ammonia present; the distillate was rejected. Nitrate was reduced to ammonia by a second distillation with 0·6 g Devarda's alloy (< 300 mesh), passing the distillate into 15 ml 0·01N sulphuric acid containing Tashiro's indicator [a 1:4 mixture of 0·1% (w/v) solutions of methylene blue and methyl red]. When the total volume reached 40 ml, nitrate was determined by back titration with sodium hydroxide. The titrate was re-acidified with 0·5 ml N sulphuric acid, and evaporated down to approximately 2 ml at 28–30°C for isotope ratio analysis.

Total nitrite was determined in a 0·1–0·5 ml aliquot of extract by the colorimetric method of Crosby (1967). For isotope ratio analysis of nitrite 60 ml of extract was required, and preliminary tests indicated that when evaporated extracts were reacted with sulphamic acid to release nitrogen, foaming proved very troublesome. This was avoided by mixing the extract with 2 g of alumina cream, which had been found effective for the removal of interfering organic substances from soil solutions required for nitrate analysis (Cawse, 1967). After centrifugation to remove the alumina cream and organic matter, the supernatant fraction was evaporated to approximately 2 ml at 28–30°C with a current of warm air passing over the surface; 0·1 ml N potassium hydroxide was added to ensure at least a

TABLE 1. DESCRIPTION OF SOIL SERIES USED FOR IRRADIATION, AND EXPERIMENTAL CONDITIONS

Soil series	pH in: water	pH in: $CaCl_2$	Total N (%)	Organic C (%)	Inorg. C (%)	Moisture at MWHC (%)	Experimental conditions: Moisture at irradiation (%)	Experimental conditions: Dose (Mrad)	Experimental conditions: Incubation time (hr)
Broad	8·0	7·4	0·78	6·6	1·2	97·3	70	0·8	64
Broadmoor	8·0	7·5	0·53	5·7	4·0	110·8	65	0·5	24
Colthrop	7·9	7·3	0·65	6·6	2·9	93·4	45	0·5	24
Icknield	8·0	7·4	1·20	10·2	0·7	89·0	70	0·6	64

neutral reaction. It was found that such conditions allowed 95 per cent recovery of nitrite, but evaporation at 50°C reduced this to 81 per cent.

Conversion of nitrate and nitrite to gaseous nitrogen

The evaporated extracts contained 0·7–1·5 mg of either nitrate- or nitrite-N, and nitrogen gas was produced from them essentially by the technique of Bremner (1965). However, difficulty was experienced with the gas sample tubes of inverted 'Y' shape; cracking often occurred during the freezing and thawing cycles which were needed to de-gas the extract placed in one arm of the tube before mixing with the reactant, which was either hypobromite or sulphamic acid, contained in the other arm. A gas sample tube was therefore designed (Fig. 1) to hold the solutions in a thin layer. It had a cross wall to prevent mixing of extract with reactant until de-gassing was finished.

Freezing and thawing could be performed more rapidly and with less danger of breakage; furthermore, the melting ice showed no tendency to jump into the opposite arm as entrapped air was released. De-gassing the solutions four times produced nitrogen samples with $< 0{\cdot}1\%$ air contamination, and no correction of mass spectrometer results was deemed necessary (Bremner, 1965).

Evaporated extracts containing ammonium were reacted with 2 ml sodium hypobromite solution to release nitrogen. The hypobromite contained 0·1% (w/v) potassium iodide to prevent oxygen formation (Sims and Cocking, 1958).

Extracts containing nitrite were reacted with 1 ml of 4% (w/v) sulphamic acid:

$$NH_2SO_3H + HNO_2 \longrightarrow N_2 + H_2SO_4 + H_2O.$$

As the evolved nitrogen would be derived in equimolar proportions from the sulphamic acid and nitrite, the spectrometer analyses for ^{15}N were adjusted accordingly.

The released nitrogen was passed through a liquid nitrogen trap to remove moisture, nitrous oxide, amines and carbon dioxide, and was transferred to 10 ml break-seal tubes to avoid leakage and air contamination before analysis on an Atlas CH4 mass spectrometer.

Miscellaneous methods of analysis

Total nitrogen was determined by a semi-micro method (Bremner, 1960). Organic carbon was analysed by a modified Tinsley wet oxidation procedure (Bremner and Jenkinson, 1960), and inorganic carbon was determined by acidification and gravimetric assay of carbon dioxide (Allison, 1960). pH was measured in both water and 0·01M calcium chloride mixtures which had been shaken for 30 min at a 1:2·5 soil:solution ratio. Maximum water holding capacity (MWHC) was determined by the technique of Ekpete and Cornfield (1965).

RESULTS

The isotopic composition of nitrate and nitrite in the irradiated soils is shown in Table 2. From the appearance of ^{15}N in the nitrite pools of all four series it was concluded that nitrate can be reduced to nitrite under aerobic conditions, thus confirming the indications of leaching tests (Cawse and Crawford, 1967; Cawse and White, 1969b).

The atom % excess ^{15}N in the nitrate pool before irradiation was calculated from the following relationship, After incubation for time t following irradiation, analysis showed the gross inorganic nitrate and nitrite pools represented as N_a and N_b μg N respectively, with C_a and C_b atom % ^{15}N excess. The recovery of ^{15}N was then calculated as

$$\frac{(C_a \times N_a) + (C_b \times N_b)}{100}$$

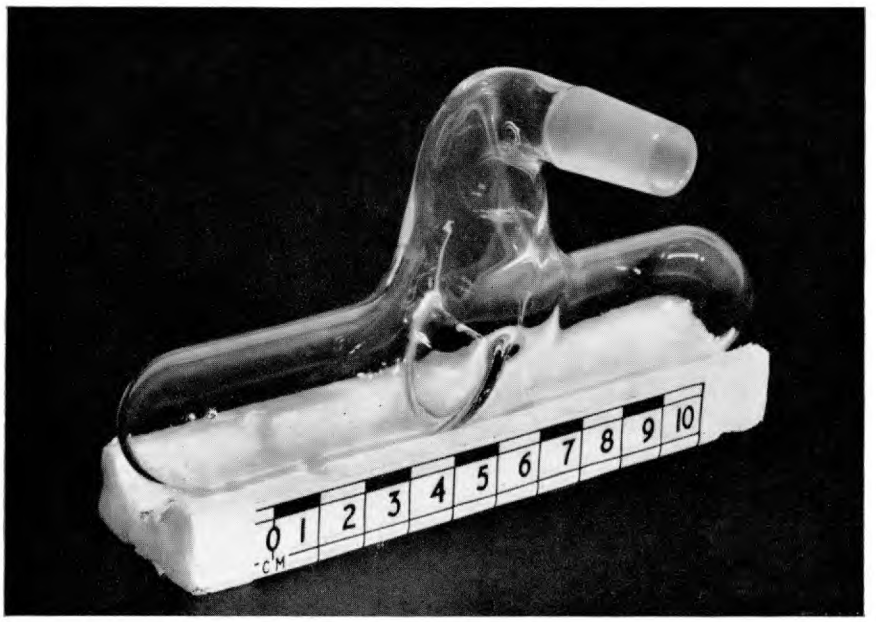


FIG. 1. Gas sample tube.

TABLE 2. THE ISOTOPIC COMPOSITION OF NITRATE AND NITRITE PRODUCED AFTER IRRADIATION OF SOILS CONTAINING $^{15}NO_3$ TRACER*

Soil Series	Total μg mineral-N in sample						Total μg ^{15}N excess† in sample				
	Nitrate pool			Nitrite pool			Nitrate pool			Nitrite pool	
	(i)	(ii)	(iii)	(i)	(ii)	(iii)	(i) ^{15}N added	(ii)	Lowest % expected after dilution	(ii)	% Recovery ^{15}N
Broad	4851	7572	4956	7·7	1429	68·9	214·2 (4·40)	186·6 (2·46)	(2·31)	23·0 (1·61)	97·9
Broadmoor	5340	6507	5424	11·0	1747	7·2	214·2 (3·99)	196·2 (3·02)	(2·52)	28·7 (1·64)	105·0
Colthrop	4783	5890	4677	35·6	1643	7·9	214·2 (4·46)	193·8 (3·29)	(2·75)	27·0 (1·64)	103·1
Icknield	2648	4492	2542	6·5	1381	22·8	214·2 (8·06)	169·5 (3·77)	(3·50)	75·4 (5·46)	114·3

Where (i) = before irradiation, with contribution from tracer-NO_3 added.
(ii) = after irradiation and incubation with tracer.
(iii) = after incubation only, without tracer.

* Figures in parentheses denote atom % ^{15}N excess.
† Natural abundance of ^{15}N = 0·366 atom %.
Coefficient of variation of ^{15}N analysis = 0·77%.

and was expressed as a percentage of the tracer initially added. These figures appear in the final column of Table 2, and the satisfactory recoveries obtained indicate that there was little decomposition of nitrite during incubation by chemical or microbial action.

It was thought that faster reduction of the nitrate pool, before it became diluted by nitrification, may have accounted for the higher percentage ^{15}N found in the nitrite pool of Icknield series compared with the Broad soil. To investigate this possibility, both series were treated with the same doses of radiation as before, and analysed after 3, 6, 12 and 24 hr. Very little difference in nitrite accumulation rates was apparent, and after 24 hr the Broad series contained 26·7 ppm against 23·6 ppm NO_2–N for Icknield.

Rapid nitrification of radiation-released ammonium would certainly dilute the tracer in the nitrate pool during incubation. The extent of such dilution and the lowest atom % ^{15}N excess expected in the nitrate pool was calculated on the assumption that all nitrite was produced by reduction of nitrate, thus nitrite was added to the total nitrate-N. The results show (Table 2) that nitrite pools in Broad, Broadmoor and Colthrop series have 0·7–1·1 atom % ^{15}N excess under these values. One explanation could be the direct contribution of ammonium oxidation to the nitrite pool. It was impossible to resolve this point with $^{15}NH_3$ because nitrification would occur and $^{15}NO_2$ could arise from nitrate reduction as well as ammonium oxidation.

However, it was noticed that when 100 ppm NH_4–N (soil dry wt. basis) was added to soils immediately before irradiation, under identical conditions to the main experiment, more nitrite accumulated than in irradiated soils alone. With Broadmoor and Colthrop series, nitrite even formed after ammonium was added to un-irradiated soil, probably by toxicity towards *Nitrobacter* (Stojanovic and Alexander, 1958). It was therefore very likely that direct ammonium oxidation to nitrite was partly responsible for lowering the atom % ^{15}N excess in the nitrite pools of these series.

DISCUSSION

To be strictly correct in the calculation of ^{15}N, the atom percentage figures should be transformed to weight percentage. However, Jansson (1958) points out that samples containing between 1 and 5 atom % ^{15}N only show a deviation between atom % and wt % of 6·8 and 7·1 relative per cent, and can be considered constant; therefore no transformations were made. Furthermore, the normal atomic weight of nitrogen was used for calculation of titration results, but at the levels of 1·5–3·0 atom % ^{15}N the error in nitrogen weight would only be 0·1–0·2% low. In the calculation of ^{15}N added and recovered, atom % has been used, and there would be little difference from using weight percentage figures (Jansson, 1958).

Apart from oxidation of ammonium to nitrite, two other processes may be considered responsible for lowering the percentage ^{15}N excess in the nitrite pools of three series. They are nitrate assimilation by microorganisms and isotopic discrimination, and all three pathways may operate either separately or in combination.

Nitrate assimilation was responsible for rapid immobilization of $^{15}NO_3$ in experiments by Overrein (1967), regardless of the presence of ammonium. This mechanism is not favoured in the present study owing to the high recoveries of tracer from the inorganic pools of nitrate and nitrite.

Wellman, Cook and Krouse (1968) have shown that there is discrimination between $^{14}NO_3$ and $^{15}NO_3$ in the reduction of nitrate by *Pseudomonas stutzeri*, *Bacillus* sp., and *Alcaligenes* sp., and $^{14}NO_3$ was utilized 2 per cent faster than the labelled anion. It is certainly well established that single cells can discriminate against other isotopes, and frac-

tionation has been reported between D_2O/H_2O, $^6Li/^7Li$, $^{13}C/^{14}C$, $^{16}O/^{18}O$ and $^{32}S/^{34}S$ (Bowen, 1960). Owing to the small percentage of the nitrate pool reduced to nitrite in the present work, slight discrimination effects are possible.

Previous reports suggest that nitrate reduction to nitrite after 0·5–0·8 Mrad occurs mainly by microbial action, and the accumulation of nitrite declines as the radiation dose is increased to 2·5 Mrad (Cawse and Crawford, 1967; Cawse and White, 1969b). The rapid nitrification which follows treatment of many soils with 0·2 Mrad appears to result from oxidation processes maintained by non-proliferating cells (Cawse, 1968), therefore it seems reasonable to propose that nitrate reduction is carried out by radio-resistant enzyme systems of denitrifying bacteria which are prevented from proliferation by 0·5–0·8 Mrad.

Bremner and Nelson (1968) have discussed the decomposition of nitrite in steam-sterilized soils, and have found that chemical decomposition ('chemodenitrification') can proceed by the reaction of nitrite with phenolic constituents of soil organic matter to form nitrosophenols. The latter are subsequently decomposed by nitrous acid to form nitrous oxide and nitrogen. Although the good recovery of ^{15}N tracer in the present investigation means that nitrite reduction has not occurred to a significant extent, it does not preclude the possibility that nitrite undergoes microbial or chemical reduction to oxides of nitrogen and finally gaseous nitrogen over longer periods of incubation lasting 7–14 days. The eventual fate of the nitrite which is formed in some irradiated soils is thus receiving further attention, together with experiments to determine how factors such as temperature, moisture, pH, aggregate size and available carbon can influence the extent of nitrite accumulation.

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REFERENCES

Allison L. E. (1960) Wet-combustion apparatus and procedure for organic and inorganic carbon in soil. *Proc. Soil Sci. Soc. Am.* **24**, 36–40.

Bowen H. J. M. (1960) Biological fractionation of isotopes. *Int. J. appl. Radiat. Isotopes* **7**, 261–272.

Bowen H. J. M. and Cawse P. A. (1964) Effects of ionising radiation on soils and subsequent crop growth. *Soil Sci.* **97**, 252–259.

Bremner J. M. (1960) Determination of nitrogen in soil by the Kjeldahl method. *J. agric. Sci., Camb.* **55**, 11–33.

Bremner J. M. (1965) Isotope-ratio analysis of nitrogen in nitrogen-15 tracer investigations. In *Methods of Soil Analysis* (C. A. Black, Ed.) pp. 1256–1286, American Society of Agronomy, Madison, Wisconsin.

Bremner J. M. and Edwards A. P. (1965) Determination and isotope-ratio analysis of different forms of nitrogen in soils—1. Apparatus and procedure for distillation and determination of ammonium. *Proc. Soil Sci. Soc. Am.* **29**, 504–507.

Bremner J. M. and Jenkinson D. S. (1960) Determination of organic carbon in soil. *J. Soil Sci.* **11**, 394–402.

Bremner J. M. and Nelson D. W. (1968) Chemical Decomposition of Nitrite in Soils, in *Transactions of the Ninth International Congress of Soil Science* Vol. 2, pp. 495–503 Adelaide.

Cawse P. A. (1967) The determination of nitrate in soil solutions by ultraviolet spectrophotometry. *Analyst, Lond.* **92**, 311–315.

Cawse P. A. (1968) Effects of gamma radiation on accumulation of mineral nitrogen in fresh soils. *J. Sci. Fd Agric.* **19**, 395–398.

Cawse P. A. and Crawford D. V. (1967) Accumulation of nitrite in fresh soils after gamma irradiation. *Nature, Lond.* **216**, 1142–1143.

Cawse P. A. and White T. (1969a) Accumulation of nitrate in fresh soils after gamma irradiation. *J. agric. Sci., Camb.* **72**, 331–333.

Cawse P. A. and White T. (1969b) Rapid changes in nitrite after gamma irradiation of fresh soils. *J. agric. Sci., Camb.* **73**, 113–118.

Crosby N. T. (1967) The determination of nitrite in water using Cleve's acid, 1-naphthylamine-7-sulphonic acid. *Proc. Soc. Wat. Treat. Exam.* **10**, 51–55.

Cummins D. G. and McCreery R. A. (1964) The effect of gamma and neutron irradiation of soil on the yield and nutrient uptake of rye. *Proc. Soil Sci. Soc. Am.* **28**, 390–393.

EKPETE D. M. and CORNFIELD A. H. (1965) Effect of varying static and changing moisture levels during incubation on the mineralisation of carbon in soil. *J. agric. Sci., Camb.* **64,** 205–209.

JANSSON S. L. (1958) Tracer studies on nitrogen transformations in soil with special attention to mineralisation–immobilisation relationships. *K. LantbrHögsk. Annlr* **24,** 101–361.

JARVIS R. A. (1968) *The Soils of the Reading District* (Sheet 268), Memoirs of the Soil Survey of England and Wales, A.R.C. Harpenden.

NEWMAN A. C. D. (1966) The distillation of ammonia for isotopic analysis. *Chemy Ind.* 115–116.

OVERREIN L. N. (1967) Immobilisation and mineralisation of tracer nitrogen in forest raw humus—1. Effect of temperature on the interchange of nitrogen after addition of urea-, ammonium- and nitrate-N^{15}. *Pl. Soil* **27,** 1–19.

ROVIRA A. D. and BOWEN G. D. (1969) The use of radiation-sterilized soil to study the ammonium nutrition of wheat. *Aust. J. Soil Res.* **7,** 57–65.

SIMS A. P. and COCKING E. C. (1958) Assay of isotopic nitrogen by mass spectrometer. *Nature, Lond.* **181,** 474.

STOJANOVIC B. J. and ALEXANDER M. (1958) Effect of inorganic nitrogen on nitrification. *Soil Sci.* **86,** 208–215.

WELLMAN R. P., COOK F. D. and KROUSE H. R. (1968) Nitrogen-15: microbiological alteration of abundance. *Science* **161,** 269–270.