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STUDIES ON THE MEASUREMENT OF EXTRACTABLE AND MINERALIZABLE NITROGEN IN SOIL

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the degree of
Doctor of Philosophy
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SUMMARY

The different potassium salts which are used for the extraction of inorganic nitrogen from soils contain variable and often high amounts of ammonium and nitrate nitrogen which could introduce an error in the analysis of low levels of soil inorganic nitrogen. The potassium salt solutions can be purified of ammonium nitrogen by increasing the pH to 11.0 with a solution of potassium hydroxide and then boiling and stirring for 15 minutes. The pH is then readjusted to pH 5.5 - 6.0 with a dilute acid. There is no simple method for the removal of nitrate nitrogen and therefore, it is suggested that a batch number with low nitrate nitrogen content be used for the extraction of soil.

Different grades and batches of filter papers also contain variable amounts of ammonium and nitrate nitrogen which needs removing before use for the filtration of soil extracts. Ammonium and nitrate nitrogen can be removed from the filter papers by washing with 50 cm^3 of 0.5 M potassium sulphate in 2 lots each of 25 cm^3, then rinsing twice with deionised water and drying for 4 hours at 70 ^oC. The results would be more uniform if the first 2 cm^3 of the filtrate were ignored.

Extraction of inorganic nitrogen from soil at room temperature leads to biological breakdown of soil organic matter and also transformations of inorganic nitrogen. Shaking of soil samples in 0.5 M potassium sulphate at 2 ^oC for a period of 2 hours seems to be the most suitable

method of extraction for soil inorganic nitrogen which would minimise changes. The method seems to be most suitable for soils which contain low initial inorganic nitrogen. If the filtered soil extracts cannot be analysed immediately, they can be stored safely in a 2 ^{o}C refrigerator for approximately one week. Incubation of air dried soils under aerobic conditions releases more carbon dioxide and nitrogen compared with fresh samples. The increase may be attributed to the microorganisms killed during the air drying treatment and to changes in the breakdown of soil organic matter. In the air dried samples at least three fractions contribute to the release of carbon dioxide and nitrogen.

(i) Resistant organic material which decomposes very slowly.

(ii) Readily decomposable organic material produced by air drying.

(iii) Autolysed microorganisms killed by air drying.

Incubation of fresh soil samples seems to be more reasonable for soil organic nitrogen mineralization studies under Scottish climatic conditions.

Zero order rate constants calculated from the slopes of the fitted regression lines were used as the basis for comparing mineralization rates in the fresh soils.First order mineralization rate constants and potentially mineralizable nitrogen in the air dried soils were calculated by the method of Stanford and Smith (1972) using their first approximation.

For most of the soils autoclaving in 0.01 M calcium

chloride solution released similar levels of ammonium nitrogen from the air dried and fresh samples.

Shaking of soil samples in a salt solution for 24 hours at 30 ^{o}C involves both chemical and biological processes for soil organic nitrogen mineralization. Cations like Na^{+} and to a lesser degree K^{+} help in the dispersion of the soil organic matter and thereby make it more susceptible to microbial decomposition. On the other hand cations like Ca^{2+} flocculate the soil organic matter and make it less susceptible to microbial decomposition.

Because of its quick time scale and ease of operation, the method could be further developed for routine use in soil testing laboratories as a method of measuring soil nitrogen availability by looking at:-

(1) Use of nitrification inhibitors which would stop nitrification and therefore, denitrification.

(2) Using different salt concentrations to find a suitable salt concentration.

(3) To compare different cations in releasing nitrogen from the soil.

The zero order nitrogen mineralization rate constant for fresh soils was poorly correlated with the N released by autoclaving in 0.01 M calcium chloride, biomass C, % total C, % total N and mineralization rate constant in the air dried soils. It was best correlated with the nitrogen released by shaking with the different salt solutions and with the total nitrogen mineralized by fresh soils.

The first order nitrogen mineralization rate constant and total nitrogen mineralized in the air dried soils were

correlated with % total C, % total N, Biomass C and nitrogen released by autoclaving in 0.01 M calcium chloride. However, the N rate constant in the air dried soils was poorly correlated with the N released by shaking of air dried or fresh soils with different salt solutions. Potentially mineralizable nitrogen was not correlated with the N released by autoclaving of soil in 0.01 M calcium chloride.

CHAPTER ONE

GENERAL INTRODUCTION

Nitrogen is the nutrient required in the largest amounts by most agricultural crops. This is due to the fact that it is the key building block of the protein molecule upon which all life is based and it is thus an indispensible component of the protoplasm of plants, animals and microorganisms. Therefore, large amounts of this element are required for optimal plant growth and maximum crop yields. Since declining amounts of available nitrogen give proportionally declining crop yields, it is evident that nitrogen supply of the plants is one of the fundamentals of agricultural production.

1.1. The Nitrogen Cycle in Soil

The ultimate source of nitrogen used by plants is the inert gas N_2 which constitutes about 78 percent of the earth´s atmosphere (Stevenson, 1982). Total nitrogen content of soil ranges from less than 0.02 percent in subsoils to 2.5 percent in peats. The surface layer of most cultivated soils contains between 0.06 and 0.5 percent (Bremner and Mulvaney, 1982).

Although in fertile soils containing adequate amounts of decomposable organic matter a considerable part of the nitrogen required for optimal yields is derived from the soil, a supplementary amount has often to be supplied in

the form of fertilizer. The lower the mineralization capacity, the higher the amount of nitrogenous fertilizers to be added. This does not apply, of course, to those plants which are able to fix atmospheric nitrogen and applies to a lesser extent to non-nitrogen fixing plants growing in association with nitrogen fixing plants.

Soils in Britain commonly contain 0.1 to 0.3 percent nitrogen in the top 15 cm, representing a total content throughout the soil profile of about 2000 to 10,000 kg/hectare. Although an annual crop takes up only about 100-200 kg N per hectare, because of the slow rate of mineralization of the organically combined soil N, it is usually necessary to add about 50 to 250 kg per hectare of fertilizer nitrogen to obtain high crop yields, depending on the crop, the soil and the farming system (Wild and Cameron, 1980).

Although higher plants take up nitrogen mostly in the inorganic form (ammonium or nitrate), certain amino acids can also readily be taken up. However, under natural conditions nitrate and ammonium are the common nitrogen sources. Since in most fertile soils of neutral or alkaline reaction, ammonia is readily converted to nitrate by the nitrifying bacteria, nitrate would be expected to be the normal nitrogen compound taken up by the plant roots from such soils. Nevertheless, in several instances the uptake of nitrogen may be expected to occur as ammonium ions rather than as nitrate. This will be the case in acid soils or in soils covered with a dense vegetation where nitrification proceeds less readily and a

large part of ammonium salts is absorbed by the plant root before nitrification has taken place.

The ammonium ions derived from microbial decomposition of organic matter or from fertilization may be subjected to various types of immobilization, fixation by soil colloidal materials and volatilization reactions in addition to nitrification and uptake by the plant roots. Immobilization in the microbial cell material may be temporary but chemical fixation in humus compounds is much more stable and is highly resistant to microbial attack. Fixation of ammonium ions by clay minerals may render the nitrogen almost unavailable for plant growth. In alkaline soils part of the ammonium compounds may be lost by volatilization. On slightly acid soils volatilization losses of nitrogen may occur after the conversion of the ammonia to nitrite by bacteria of the genus Nitrosomonas.

Similarly to ammonia, nitrate may be subjected to a number of processes causing more or less serious losses of nitrogen. In contrast to ammonia which is readily utilized as the nitrogen source by the heterotrophic soil microflora, nitrate is only used as the nitrogen by the microorganisms after all the ammonia has been consumed. However, under anaerobic conditions, nitrate may be readily used by denitrifying bacteria, resulting in the formation of gaseous products like N_2 and N_2O which are lost into the atmosphere. Nitrate in contrast to ammonia is not adsorbed by the soil colloids and therefore, it is easily leached when precipitation exceeds evaporation.

The primary pathways by which nitrogen gas is converted to forms usable by higher plants are:-

(1). Fixation by Rhizobium and other microorganisms that live symbiotically on the roots of legumes and certain non-leguminous plants.

(2). Fixation by free living soil microorganisms and perhaps by organisms living on the leaves of tropical plants.

(3). Fixation as one of the oxides of nitrogen by atmospheric electrical discharges.

(4). Fixation by any of the various industrial processes for the manufacture of synthetic nitrogen fertilizers.

The supply of inorganic nitrogen gas is inexhaustible. This inert nitrogen is in dynamic equilibrium with the various fixed forms. Even as nitrogen is fixed by the different processes just indicated, so there is a release of elemental nitrogen to the atmosphere from the fixed forms by microbiological and chemical processes.

(1). Nitrogen fixation by Rhizobium or other symbiotic bacteria.

For centuries the use of legumes in crop rotations and the application of animal manures were the principal ways of supplying additional nitrogen to non-leguminous crops. Although they are still important sources of fixed nitrogen for agriculture, the importance of legumes and manures is dwindling with each passing year because of the rapid increase in the production of low cost synthetic nitrogen fertilizers.

The quantity of nitrogen fixed by properly nodulated legumes averages about 75 percent of the total nitrogen used in the growth of the plant. The legumes carry out the fixation in symbiosis with the soil bacteria, *Rhizobium*, which takes place in nodules located on the plant roots. Nodule formation follows infection with the appropriate strain of *Rhizobium*. In some situations inoculation to introduce the bacteria may be necessary if the legume is to be grown on a field for the first time. Amounts of nitrogen fixed by *Rhizobium* differ with *Rhizobium* strain, the host plant and environmental conditions under which the two develop. Fixation is inhibited below pH 6.0 and by high nitrogen fertilizer use. Well aerated conditions are needed for good nodulation.

(2). Nitrogen fixation by free living micro-organisms.

Three types of free living nitrogen fixers may be distinguished namely (a) aerobic bacteria, mainly those of the genus *Azotobacter* (b) anaerobic bacteria, particularly of the genus *Clostridium* and (c) Blue green algae. Nitrogen fixation by *Azotobacter* and presumably, also by other free living nitrogen fixers is carried out only by growing cells. The amount of nitrogen fixed is mainly used for the synthesis of the bacterial cells. Although the free living nitrogen fixing bacteria undoubtedly contribute to the nitrogen economy of natural vegetation and thus to soil fertility, their contribution to the nitrogen nutrition of agricultural plants in general is thought to be of little importance. There is evidence that contributions from the free living organisms may be higher

in temperate deciduous woodlands (20-30 kg N per ha per year), and exceptionally up to 100 kg per ha per year in some tropical forests (White, 1987).

(3). Addition from the atmosphere.

Nitrogen compounds are present in the atmosphere and are returned to the earth in rainfall. The nitrogen is in the form of ammonia, nitrate, nitrite and nitrous oxide. The ammonia comes largely from industrial sites where ammonia is used or manufactured and also from the animal manures. Some ammonia escapes from the soil surface because of the reactions taking place. The soil has a pronounced capacity for adsorbing ammonia gas from the atmosphere. This of course, is independent of that which may be added in rain fall. The presence of nitrate has been attributed to its formation during atmospheric electrical discharges. The total amount of nitrogen brought down has been estimated to range between 1 and 6 kg per hectare annually depending on location.

Nitrogen occurs in soils in organic as well as inorganic forms. The inorganic forms of soil nitrogen include ammonium, nitrate, nitrite and elemental nitrogen which can be directly utilized by plants. The organic form occurs as consolidated amino acids, amino sugars and other complexes. Practically all the nitrogen present in the surface soil is organically bound and cannot be utilized by plants unless it is mineralized by microbial processes during the growing season. Thus the soil organic matter supplies a considerable quantity of nitrogen for plant growth and acts as a natural store house for this

important nutrient. However, the amount of nitrogen released by the soil organic matter is not sufficient to meet the demand of the crop throughout the growing season and therefore, this has led to the use of nitrogen fertilizers.

1.2. Assessment of Nitrogen Fertilizer Requirement of Crops.

The nitrogen supply to a growing crop comes from 3 different sources. (i) residual inorganic nitrogen in the soil profile (ii) N mineralization during the growing season and (iii) fertilizer nitrogen.

Interest in developing improved methods for assessing soil nitrogen status becomes ever greater as the price of nitrogen fertilizer rises and concern increases over the environmental consequences of supplying more nitrogen than is required by the crop. A major concern of the present day farmer is the effective management of nitrogen fertilizers for maximum efficiency and minimal pollution of the environment.

The current system of nitrogen fertilizer recommendations in the U.K is based on a nitrogen index derived from the previous cropping. Soils are divided into 3 classes on the basis of the last crop grown. The nitrogen index system used by the Agricultural Development and Advisory Service (ADAS ,1983) and that used by the Scottish Colleges (Scottish Agricultural Colleges , 1985) are similar but differ in detail.

Recommended fertilizer levels can be modified to take

account of winter leaching by releasing information to the farmers via the press and television. To take better account of residual inorganic nitrogen in the soil, analysis of residual nitrogen in the soil profile in early spring is currently being evaluated by ADAS in parts of England. Samples are taken from the soil profile down to 90 cm, transfered to cold storage and analysed for ammonium and nitrate nitrogen. The method is expensive, requires rapid processing of samples and imposes a high seasonal workload on the analytical laboratories.

As manuring specification becomes more precise it is becoming increasingly important to allow for the amount of soil nitrogen released to crops during the growing season. Although in advisory work a rough idea of soil´s capacity to mineralize nitrogen can often be had from the results of past cropping, it is likely that the prediction could be usefully improved by a soil test for potentially available nitrogen that is quick and easy to do.

The potential value of a method providing an index of the availability of soil nitrogen has long been appreciated and it is now generally accepted that the most satisfactory methods available for assessment of the potential ability of soils to provide nitrogen for plant growth are those involving estimation of the mineral nitrogen formed when soil is incubated under conditions which promote mineralization of soil nitrogen. These methods have a rational basis because the agents responsible for release of mineral nitrogen during incubation of soils are those which make organic soil

nitrogen available for crop growth during the growing season. It is reasonable, therefore, to assume that these incubation techniques will provide a fairly accurate index of the availability of soil organic nitrogen to plants. The work involved in incubation experiments is so high that it cannot be used as a routine advisory method and no chemical extraction method for mineralizable nitrogen has been adopted.

Magdoff et al. (1984) have classified soil tests for nitrogen availability into 3 categories.

(1) Biological methods which include mineralization of soil organic nitrogen during various types of incubation (aerobic and anaerobic).

(2) Direct measurement of various nitrogen fraction such as nitrate nitrogen or organic nitrogen.

(3) Inorganic nitrogen released from organic matter by chemical treatment of the soil.

They recommended a soil test for nitrogen availability to corn in which nitrate nitrogen to 30 cm depth when plants are 15-30 cm tall is determined. They stated that the nitrate nitrogen at the time of sampling is a result of an integration of all the soil and weather factors that have influenced the availability of nitrogen prior to the time for fertilizer application. This does not account for nitrogen mineralization after sampling and may pose problems where organic manures have been used.

Computer models have been used to predict both the winter leaching of nitrate and to predict the transformations of soil nitrogen (mineralization,

immobilization, nitrification, denitrification, leaching and crop uptake). (Tanji, 1982).

Plant analysis has also been used as an indicator of plant nitrogen status. Nitrate reductase activity in leaves and nitrate levels in stems and leaves have been suggested. (Sylvester-Bradley et al., 1982 and Verstraeten and Vlassak, 1982).

Jenkinson (1982) has stated that the way forward is through improving the Nitrogen Index system, rather than through further development of soil testing. Computer based systems for keeping field records are now being developed and these records contain the very information needed for an improved Nitrogen Index system - soil type and depth, cropping history and yields on a year by year basis. Such an improved Nitrogen Index system could be extended to allow for winter leaching losses by regular input of meteorological data. The ultimate aim should be to predict the nitrogen supplying potential of the soil from our scientific knowledge of the behaviour of nitrogen in soil and from the history of the field, not from soil analysis (Jenkinson, 1982).

A better understanding of the process of nitrogen mineralization is important for the development and testing of computer models. Also if a rapid test for mineralizable nitrogen could be developed, it would be a useful tool for advisory work.

1.3. Measurement of Mineralizable Nitrogen.

Broadly speaking, tests for potentially mineralizable nitrogen can be separated into two groups, biological and chemical. Biological tests can be direct, where the quantity of organic nitrogen mineralized by the soil is measured after an incubation done under standard conditions - which can be aerobic or anaerobic. Indirect biological methods have also been proposed, for example the quantities of carbon dioxide produced when cellulose was incubated with a range of soils is correlated with the amounts of mineral nitrogen produced when the same soils were incubated without cellulose (Cornfield, 1961). None of the indirect methods have been widely used in the advisory work.

1.4. Biological Methods for Mineralizable Nitrogen.

All the biological methods suffer from two disadvantages. Firstly an incubation period is required which is inconvenient for laboratories engaged in predicting fertilizer requirements, particularly if large number of samples have to be processed. Secondly the results obtained are very dependent on when the soil is sampled and how it is handled before incubation. Bremner (1965a) concluded that the reliability and reproducibility of methods for measuring soil nitrogen mineralization depends on methods of sampling, drying, grinding and sieving, storing and incubating the soils. Moreover, total mineral nitrogen (ammonium, nitrate and nitrite) should be measured since most soils will not

nitrify all of the ammonium produced during incubation.

The earliest methods were those in which the soil sample was leached with water before incubation and only nitrate nitrogen produced on incubation was determined. In these methods the soil sample was mixed with vermiculite or sand and leached with water. The excess water was removed by suction and the samples incubated at 25 to 35 ^{o}C for 7 to 14 days and the nitrate produced during incubation was determined by leaching the incubated sample with water and analysing the extract colorimetrically.

Bremner (1965a) questioned the efficiency of preleaching to remove nitrate nitrogen, the use of vermiculite to improve leachability and aeration and the adjustment of water content by suction.

Bremner (1965a) proposed a method of aerobic incubation in which the air dried soil sample was mixed with acid washed sand in the ratio 1:3 and the mixture was moistened with water (0.6 cm^3/g soil) and incubated at 30 ^{o}C for 2 weeks in wide neck bottles fitted with a permeable membrane which permitted the diffusion of air but did not allow the passage of water vapour. The amount of ammonium, nitrate and nitrite nitrogen was then measured by extracting the sample with 2 M potassium chloride and determining the ammonium nitrogen liberated by steam distillation of an aliquot of the extract with magnesium oxide and Devarda alloy. This method has also been used with apparent success by Robinson (1968), Ryan et al. (1971) and Baerug et al. (1973). They obtained good correlations between nitrogen mineralized and

nitrogen uptake by plants grown in greenhouse or other indexes of soil nitrogen availability.

Some incubation methods involve preleaching with dilute salt solution, (0.01 M calcium chloride) before the initial and successive incubation periods to remove inorganic nitrogen from samples (Stanford and Smith, 1972; Stanford et al., 1974 and Griffin and Laine, 1983). Although no definite studies are available, it has been shown earlier by Bremner (1965a) that this procedure might be insufficient to remove all the ammonium from exchange sites, particularly in difficultly leached, high exchange capacity soils.

The importance of measuring both ammonium and nitrate following incubation is clear from the work by Vlassak (1970), Nommik (1976) and Geist (1977) who found that ammonium was the dominant product of mineralization in forest soils.

In flooded soils, the process of mineralization does not proceed past the ammonium stage because of lack of oxygen to carry the process through nitrification (Patrick and Mahapatra, 1968). The development of the anaerobic (waterlogged) nitrogen mineralization method (Waring and Bremner, 1964a) has attracted considerable attention because of its simplicity as compared to most aerobic incubation procedures. It has many advantages over the aerobic incubation in that (i) Only ammonium nitrogen need be measured (ii) Problems with establishment of optimal water content and loss of water during incubation are omitted (iii) More nitrogen is mineralized in a given

time than under aerobic conditions and (iv) Higher temperature can be used since optimum temperature for nitrification is of no concern. It still suffers from the disadvantages of requiring a considerable period of time and special incubation equipment.

Keeney and Bremner (1966a) showed that mineral nitrogen produced on incubation of soils under anaerobic conditions at 40 ^{o}C for 7 weeks provided a better index of nitrogen availability to ryegrass in Iowa soils than were amounts released anaerobically during 14 days incubation at 30 ^{o}C. They also noted that aerobic incubation correlated well with nitrogen uptake by ryegrass when soils were air dried and stored, but the anaerobic method was superior for field moist or air dried soils.

Hanway and Ozus (1966) found that ammonium released during anaerobic incubation was better correlated with nitrogen uptake by ryegrass than the nitrate produced during aerobic incubation using 24 moist soils. Similar results were reported by Kadirgamathoiyah and Mackenzie (1970).

Smith (1966) found no correlation between ammonium released by anaerobic incubation and nitrogen uptake by orchard grass but found a relatively good relation with aerobic incubation. Similar results were reported by Robinson (1967) who suggested modifying the method of Waring and Bremner (1964a) to provide for steam distillation of the filtered extract rather than the soil plus extract, in order to avoid release of ammonium by alkaline hydrolysis of soil organic matter.

Sims et al. (1967) incubated water submerged soils which were obtained from rice producing areas in Arkansas for 6 to 12 days at 35 °C. After incubation the samples were extracted with 1 M sodium chloride-0.1 M hydrochloric acid solution, then filtered and ammonium was recovered from the filtrate by distillation. With 19 soils, the amount of ammonium released during 6 days anaerobic incubation correlated well with rice grain yields in the greenhouse. They concluded that nitrogen availability indexes based on anaerobic incubation may be uniquely suited to estimating soil nitrogen supplying abilities of flooded rice soils. Similar results were reported by Lin et al. (1973) and Dolmat et al. (1980).

Cornforth (1968) found that aerobic incubation and anaerobic incubation gave values that correlated highly with nitrogen uptake by corn in the greenhouse.

Stanford and Smith (1972) modified the method of Waring and Bremner (1964a) by first extracting initial mineral nitrogen with 0.01 M calcium chloride followed by incubating the soil residue in "minus" nutrient solution at 35 °C for 14 days. The ammonium produced during incubation was recovered by distillation of the extract obtained by centrifuging and washing the soil residue with 0.01 M calcium chloride. Using 39 soils representing several important agricultural regions of the United States, Stanford and Smith (1972) compared amounts of ammonium released during anaerobic incubation with amounts of ammonium, nitrate and nitrite nitrogen produced during aerobic incubation. The amounts of ammonium nitrogen

produced anaerobically in five calcareous soils were much less than the amounts of mineral nitrogen (ammonium, nitrate and nitrite) released during aerobic incubation. For the remaining 34 soils, the amounts of mineral nitrogen released during 4 weeks of anaerobic and aerobic incubation were similar and relatively well correlated.

Baerug et al. (1973) found consistently higher correlation of nitrogen uptake by ryegrass in greenhouse culture with aerobic incubation than with anaerobic incubation (14 days, 30 ^{o}C).

Selmer-Olsen et al. (1974) modified the anaerobic procedure which involves incubation of air dried soils in 2 M potassium chloride solution instead of water. Interestingly, Selmer-Olsen et al. (1974) noted that 2 M potassium chloride inhibited denitrification but had little effect on mineralization during waterlogged condition.

Magdoff et al. (1983) incubated fresh soil samples aerobically for 17 weeks at 25 ^{o}C and the amount of nitrogen mineralized was found to be linearly related to the calcium chloride autoclaving index of nitrogen availability.

Hussain et al. (1984) performed aerobic incubation by incubating air dried soil samples at 30 ^{o}C for a period of 4 weeks. They found a good correlation between nitrogen mineralized during incubation test and nitrogen uptake by wheat plants.

Gianello and Bremner (1986b) incubated air dried soil samples under anaerobic condition at 40 ^{o}C for one

week, under aerobic condition at 30 ^{O}C for 2 weeks, at 35 ^{O}C for 12 weeks and at 35 ^{O}C for 2 weeks. They obtained a good correlation between the various biological indexes of nitrogen. The value of r ranging from 0.85 to 0.96.

Although Bremner (1965a) recommended an aerobic incubation method for the study of mineralizable soil nitrogen, in a more recent review Keeney (1982) proposed the anaerobic incubation of Waring and Bremner (1964a) but no aerobic procedure. Stanford (1982) has discussed the various aerobic and anaerobic techniques adopted by various research workers, particularly those recommended by Waring and Bremner (1964a) and Stanford and Smith (1972) but there is no recommendation on a specific incubation technique.

Many research workers have used the method of aerobic incubation recommended by Stanford and Smith (1972), sometimes with slight modifications e.g. Tabatabai and Alkhafaji (1980), Nuske and Richter (1981), Griffin and Laine (1983), Magdoff et al. (1983) and Darrah et al. (1985). Some have also used the method recommended by Bremner (1965a) with slight changes (Keeney and Bremner, 1966a; Gianello and Bremner, 1986b).

Some research workers have used different aerobic incubation methods. For example Reddy (1982) incubated intact soil columns which were obtained by slowly driving polyvinyl chloride pipes into soil profile to a depth of 70 cm. The columns were then leached with 0.01 M calcium chloride followed by 1 litre of distilled water to remove initial inorganic nitrogen and incubated for a period of

53 weeks. The columns were leached once every 25 days with a litre of water and the leachates measured for their inorganic nitrogen content.

Flowers and Arnold (1983) and Addiscott (1983) have used almost similar techniques for aerobic incubation of soil. They used fresh soil samples with a minimum of disturbance. Flowers and Arnold (1983) used polystyrene pots having a 2.5 cm dia hole to permit aeration which were placed in polyethylene boxes in which a moist atmosphere was maintained by pumping through ammonia free moist air. Soil samples were extracted with 0.5 M potassium sulphate and ammonium, nitrate and nitrite nitrogen was determined with a Technicon AutoanAlyzer.

Addiscott (1983) weighed fresh samples into unstoppered glass vials which were put in a metal box with a perforated lid. The air above the vials was kept moist by suspending a moist filter paper between the vials and the lid. Mineral nitrogen was determined at different intervals by extracting the soil with 2 M potassium chloride and analysing the extracts with a Technicon AutoanAlyzer.

Hussain et al. (1984) studied nitrogen mineralization by placing air dried samples in 125 cm^3 Erlenmeyer flasks. The moisture content was adjusted to field capacity by adding distilled water. The samples were thoroughly mixed and the flasks were closed with parafilm, which was pierced twice with a pin to allow for gas exchange. Incubation was done at constant temperature of 35 ^oC in a water bath for 5 weeks. Mineral nitrogen was

extracted by shaking samples for 30 minutes with 2 M potassium chloride solution.

1.5. Chemical Methods for Mineralizable Nitrogen.

Although biological studies of nitrogen availabilty indexes are quite reliable, they are time consuming and therefore, research efforts have been made to find a rapid chemical method that could provide reliable index of soil nitrogen availability. A chemical approach to the problem of obtaining a laboratory index of soil nitrogen availability is attractive because chemical methods of analysis are usually more rapid and convenient than biological methods. Also it seems reasonable to assume that the results of chemical analysis of soil samples will be less affected by preliminary handling and storage of samples than will the results of biological analysis.

Numerous chemical methods for assessing potentially mineralizable nitrogen have been proposed. Dahnke and Vasey (1973) listed 58 papers on chemical tests that have been used over the past 70 years of this century and more have been studied since then (Stanford, 1982). It is intrinsically unlikely that any single chemical method can measure the organic nitrogen about to be mineralized because no chemical reagent is likely to simulate the activities of soil microorganisms or to release selectively the fraction of soil nitrogen which is made available to plant growth by the soil microorganisms. The chemical methods involving total nitrogen, mineral nitrogen or organic matter appear to have very limited

value and it is difficult to see how such methods can be expected to give satisfactory results except under special circumstances. The most serious objection to chemical tests as distinct from biological incubation tests is that no single chemical measurement is likely to give due weight to both the processes leading to mineralization of nitrogen and those leading to immobilization. A soil with a large mineralization potential as indicated by a chemical test may in fact mineralize no nitrogen if immobilization is vigorous.

Soil scientists have long searched for a chemical method that would provide a reliable index of soil nitrogen mineralization (Bremner, 1965a; Stanford and Legg, 1968). Of the various chemical indexes that have been proposed, few have been shown to correlate well with nitrogen released by biological methods for a broad range of soils and none have been put to general practical use in assessing the nitrogen supplying capacities of soils. Most of the proposed chemical methods have involved measuring ammonium nitrogen or total organic nitrogen extracted by acid or alkaline reagents.

MacLean (1964) proposed a 15 minute room temperature extraction with 0.01 M sodium bicabonate solution. He found that the amount of nitrogen extracted by the method from 24 soils was clearly related to the nitrogen uptake by ryegrass in a greenhouse. Similar results were obtained by Smith (1966).

Keeney and Bremner (1966a) used a hot water extraction method and found that the amounts of nitrogen

extracted by 60 minutes boiling in water correlated well with nitrogen uptake by ryegrass or nitrogen mineralized by aerobic or anaerobic incubation methods. Good correlations of hot water extractable nitrogen with other nitrogen indexes or with greenhouse tests have been reported by a number of investigators (Jenkinson, 1968; Ryan et al., 1971; Lathwell et al., 1972; Gasser and Kalembasa, 1976; Osborne and Storrier, 1976).

Jenkinson (1968) found that sodium bicarbonate extractable organic nitrogen and glucose were more closely related to mineralized nitrogen using 14 different groups of soils than was the total nitrogen extracted by barium hydroxide or boiling water.

Stanford and DeMar (1969) modified the method by boiling the soil for 16 hours in 0.01 M calcium chloride to provide a solute concentration similar to that occurring naturally in non-saline soils. The nitrogen released was determined either by the Kjeldahl method or by distillation with sodium hydroxide. They found that the sodium hydroxide distillable fraction correlated well with the nitrogen mineralized during anaerobic incubation.

Smith and Stanford (1971) and Stanford and Smith (1976) proposed autoclaving in 0.01 M calcium chloride and determining the ammonium nitrogen released. They found that distillable ammonium nitrogen released during 16 hours autoclaving correlated well with nitrogen mineralized for a broad range of soils, but certain calcareous soils did not conform to the general relationship.

Oxidative release of ammonium nitrogen from soils by acid chromate or dichromate digestion has been reported as an index of soil nitrogen availability by NommiK. (1976). He found that the ammonium nitrogen extracted by various mixtures of phosphoric acid-chromic acid was significantly related to nitrogen mineralized during 9 weeks incubation at 20 ^{o}C.

Stanford (1978) studied the relation of potentially mineralizable soil nitrogen to hydrolytic and oxidative release of ammonium nitrogen from soil organic matter by extraction with alkaline permanganate using 62 different soil samples. The ammonium was recovered by steam distilling 1 g of soil sample for 4 minutes with several concentrations of sodium hydroxide and potassium permanganate in different combinations. The same concentration of sodium hydroxide without potassium permanganate was used to determine amounts of ammonium nitrogen released by hydrolysis during steam distillation. Oxidative release was estimated as the difference between the total ammonium nitrogen produced during alkaline permanganate extraction and that derived by sodium hydroxide distillation. He concluded that alkaline permanganate distillation is not as reliable or precise for predicting mineralizable soil nitrogen as acid permanganate extraction.

Fox and Piekielek (1978a) correlated results of 8 chemical nitrogen index methods. Soil organic matter, nitrate nitrogen, sulphuric acid extractable and potassium chloride extractable nitrogen were not significantly

correlated with nitrogen availability, whereas autoclave extractable ammonium and total soil nitrogen were significantly correlated.

Oien and Selmer-Olsen (1980) proposed a rapid laboratory method by heating 4 g of air dried soil or the equivalent amount of fresh soil for 20 hours at 80 °C in 40 cm^3 of 2 M potassium chloride. They found nearly the same amount of nitrogen in the extract as inorganic nitrogen in fresh soil after aerobic incubation for 14 days at 30 °C. The correlation between the two indexes for 43 soils with variable content of organic matter was very good ($r = 0.98$).

Sahrawat (1982) evaluated nitrogen supplying capacity of 39 different soils by two anaerobic incubation methods and six chemical methods. The results were compared with nitrogen uptake by rice crops grown on these soils and found a high correlation with the nitrogen supplying capacity determined by these methods. Among the chemical indexes used were, oxidative release of soil nitrogen by alkaline permanganate, acid permanganate, acid dichromate and hydrogen peroxide.

Sahrawat (1983) studied relationship between available nitrogen determined by seven chemical indexes (organic carbon, total nitrogen, ammonium released by acid dichromate, acid permanganate, alkaline permanganate, hydrogen peroxide and dilute sulphuric acid) and nitrogen percent in rice plant, nitrogen uptake and dry matter yield of rice. It was found that all the availability indexes were best correlated with nitrogen uptake of rice

and percent nitrogen in rice plants.

Magdoff et al. (1983) correlated mineral nitrogen released during autoclaving in a 0.01 M calcium chloride solution with that mineralized during 17 weeks of aerobic incubation at 25 °C. They found that the autoclaving test appears to be a reasonable basis for estimating soil nitrogen availability under field condition.

Hussain et al. (1984) used 0.05 M potassium permanganate in 0.5 M sulphuric acid for measurement of soil nitrogen availability index and they found a very good correlation with the nitrogen uptake by wheat plants.

Gianello and Bremner (1986a) studied a chemical method of assessing potentially available organic nitrogen which involves determination of ammonium nitrogen produced by treatment of the soil sample with 2 M potassium sulphate at 100 °C for four hours. They showed that the results of 33 Brazilian soils obtained by this method were highly correlated with those obtained by aerobic and anaerobic methods of assessing potentially available organic nitrogen in soil.

Gianello and Bremner (1986b) evaluated a rapid chemical method of assessing potentially available organic nitrogen in which the soil samples are steam distilled for 8 minutes with phosphate borate buffer solution of pH 11.2. They compared the results of this method with those of other chemical and incubation methods and reported that it is the best method for assessment of potentially available organic nitrogen in soil.

1.6. Aims of the Thesis.

The initial objective of the thesis was to investigate incubation experiments at temperatures below 15°C using fresh or air dried soil for the study of soil organic nitrogen mineralization. But during the initial experiments it was found that the method of extraction for the determination of inorganic nitrogen was prone to various sources of error while using soils with low levels of inorganic nitrogen and therefore, work was needed to refine the method of extraction.

The thesis is split up into two parts. The first part is devoted to purification of potassium salts and filter papers from inorganic nitrogen contamination and development of a suitable method of extraction of low levels of inorganic nitrogen from soil.

The second part of the thesis is devoted to measurements of nitrogen mineralization at low temperatures using biological incubation methods and to chemical extraction methods of measuring nitrogen availability in soil.

CHAPTER TWO

ANALYTICAL TECHNIQUES AND SOILS

2.1. TEXTURAL CLASS DETERMINATION

This was modified from the method of ADAS (1981) whereby following the treatment with hydrogen peroxide for the destruction of organic matter the soils were treated with 2 M hydrochloric acid to remove the cementing effects of carbonates and iron and aluminium oxides.

2.1.1. REAGENTS

(i). 30% Hydrogen peroxide .

(ii). Dispersing reagent (Calgon).

50.0 g sodium hexametaphosphate plus 7.0 g sodium carbonate (anhydrous) were dissolved in water and diluted to 1 litre.

(iii). Silicon antifoaming agent. 1 cm^3 of 30 % aqueous emulsion (BDH chemical Ltd.) was diluted to 100 cm^3 in water.

(iv). 2 M hydrochloric acid.

2.1.2. PROCEDURE

2.1.2.1. Dispersion

The soil was air dried and ground to pass a 2 mm sieve. 10.0 g was weighed accurately into a 600 cm^3 beaker for each soil. Approximately 10 cm^3 of 30 percent hydrogen peroxide and 2 drops of antifoaming agent were then added

to each beaker. The initial reaction was allowed to subside for 20 minutes. The beakers were then gently heated on a steam bath with occasional stirring with a glass rod. The heating was continued until the reaction ceased. The beakers were cooled, a further 10 cm^3 of 30 percent hydrogen peroxide was added washing down the sides of the beaker and heating continued until the reaction ceased completely. 20 cm^3 of hydrogen peroxide was sufficient for most of the soils except those high in organic matter where additions were continued until no reaction was observed.

For soils containing calcium carbonate, approximately 2 M hydrochloric acid was added dropwise and the contents of the beaker were stirred continuously until the effervescence ceased. For all soils a further 10 cm^3 of acid was added giving a dispersion of soil in approximately M/5 hydrochloric acid. The beakers were stirred at intervals during an hour and then the soil was allowed to settle. The soil suspension was filtered through a Whatman filter paper No. 50 under suction. The paper was washed with 3 successive portions of 50 cm^3 of hot water. The soil was scraped from the filter paper with a spatula and then the filter paper and spatula washed with a jet of hot water letting the washings into the beaker. The sides of the beakers were washed down with distilled water and sufficient water was added to give approximately 2 cm depth of suspension in the beaker. Then 10 cm^3 of Calgon solution was pipetted into each beaker. The solution was dispersed for 5 minutes using the

ultrasonic probe (MSE instruments limited). After the dispersion, any soil adhering to the probe was washed into the beaker using distilled water.

25 cm^3 of the Calgon solution was pipetted into a weighed dish, evaporated to dryness on a steam bath and then dried in a 110 ^oC oven overnight and reweighed. This was carried out to find out what weight of Calgon was added to the suspension as its weight must be subtracted from the weights obtained for silt plus clay and clay.

2.1.2.2. Fractionation of sand.

A one litre graduated cylinder was set up with a large filter funnel in the neck. A 180 micrometre and a 53 micrometre sieve were banked together and placed in the funnel with 180 micrometre sieve on the top. These sieve sizes allow the separation of the coarse plus medium and the fine sand fractions. The soil suspension was poured into the 180 micrometre sieve. The sides of the beakers were washed using a wash bottle and rubber coated glass rod to ensure that all the soil was removed from the beaker. The soil was washed through 180 micrometre sieve until the coarse and medium sand appeared clean. The contents of the 53 micrometre sieve were washed in the same way. The sieves and funnel were removed and the volume of the cylinder was made upto 1000 cm^3 with water. The contents of the sieves were washed into a weighed and labelled porcelain basins and evaporated to dryness on a steam bath. The basins were then transferred into a 110 ^oC oven and left overnight, cooled in a desiccator for 30

minutes and reweighed. The percent coarse sand plus medium sand and fine sand was then calculated on an oven dry basis in the soil mineral material.

2.1.2.3. Fractionation of silt plus clay.

The cylinders were kept at a constant room temperature of 20 ^{o}C. The temperature of the suspension was noted and appropriate time for the silt plus clay at a depth of 20 cm and a sampling depth of 10 cm for clay particles were selected from the table which was prepared by the Soil Survey of England and Wales (1976). Each cylinder was shaken thoroughly for one minute to ensure that all the soil was in suspension. The cylinder was then placed on the pipetting stand. Immediately a clean dry 25 cm^3 pipette was lowered down into the cylinder with the tap closed, until it just failed to touch the surface of the liquid and the height on the scale noted. About 20 seconds before the required time, the pipette was lowered down gently to exactly 20 cm depth. The tap was opened at the appropriate time and sample of slightly more than 25 cm^3 of the suspension was taken. The tap was closed and the pipette was then removed from the cylinder and the volume adjusted to 25 cm^3. The solution was run into a weighed basin. The shaking was then repeated to obtain a duplicate sample in the same way. The samples were evaporated to dryness on the steam bath and then dried at 110 ^{o}C in an oven overnight. The samples were then removed, cooled in a desiccator for 30 minutes and weighed.

The cylinder was left undisturbed on a flat table for the appropriate time to allow the silt particles to settle down. The temperature of the suspension was noted and a sample of clay was taken at 10 cm depth by similar procedure as described for silt plus clay except that the cylinder was not shaken between pipetting the samples. The solution was then run into a weighed and labelled porcelain dish, evaporated to dryness on a steam bath and then left in a 110 °C oven overnight.

Textural class of soil was determined with the help of a triangular chart prepared by the Soil Survey of England and Wales (Hodgson, 1976).

2.2. ORGANIC MATTER DETERMINATION

2.2.1. DRY COMBUSTION METHOD

Vitreosil basins were placed in a 110 °C oven for one hour, cooled in a desiccator for 30 minutes and weighed. Approximately 5 g of soil was weighed accurately into the basins. The basins were then placed in the oven overnight, after which they were removed, cooled in a desiccator and reweighed. The basins were then placed in the electric furnace. The furnace was ignited and the temperature increased to 450 °C. The furnace was switched off after 6 hours of ignition. The basins were cooled in a desiccator and reweighed. The organic matter content was obtained as a percentage loss on ignition.

2.2.2. DICHROMATE METHOD

This was the method recommended by ADAS (1981).

2.2.2.1. Reagents.

(i) Barium diphenylamine sulphonate.

0.5 g barium diphenylamine sulphonate was dissolved in approximately 250 cm^3 of warm water, 50.0 g of barium chloride was added, warming to dissolve, cooled and diluted to 500 cm^3.

(ii) Ferrous sulphate 0.4 M.

5 cm^3 of concentrated sulphuric acid was added into 1500 cm^3 of water. 320.0 g of ammonium ferrous sulphate was added to dissolve and diluted to 2 litres.

(iii) Potassium dichromate 66.7 mM.

Approximately 50.0 g of potassium dichromate powder was dried in a 110 ^oC oven overnight and then cooled in a desiccator. 39.23 g of the dried salt was dissolved in approximately 700 cm^3 of water. 800 cm^3 of concentrated sulphuric acid was added carefully and cooled. 400 cm^3 of orthophosphoric acid were added, stirred to dissolve the chromic acid, cooled and diluted to 2 litres.

(iv) Standardization of ferrous sulphate solution.

40 cm^3 of potassium dichromate solution was added into a 500 cm^3 round bottom flask then 100 cm^3 of water and 2 cm^3 of barium diphenylamine reagent was added. It was then titrated against 0.4 M ferrous sulphate solution. Ferrous sulphate solution was added dropwise from a 50 cm^3 graduated burette until the colour of the contents of flask changed from purple to bright green. The volume of ferrous sulphate used was noted and the standardization factor was calculated.

Standardization factor of 0.4 ferrous sulphate = 40/volume of ferrous sulphate used.

The standardization was carried out each day immediately before examination of the soils.

2.2.2.2. Determination in soils

Air dried soil samples previously ground to pass a 2mm sieve were ground to a powder form using mortar and pestle. 0.25 to 1.0 g of the sample was accurately weighed into a 500 cm^3 round bottom flask. Then 40 cm^3 of potassium dichromate solution was added. The flasks were then placed on individually controllable electric heaters and fitted with Quick fit Leibig condensers. They were then gently boiled at approximately 130 ^oC for 2 hours. After heating, the flasks were removed from the heaters, cooled and then 100 cm^3 of water and 2 cm^3 of barium diphenylamine sulphonate reagent were added. The samples were titrated with 0.4 M ferrous ammonium sulphate solution by adding it dropwise until the colour changed from purple to bright green. The volume of the ferrous sulphate used was noted. A blank titration was carried out exactly in the same way except that no soil sample was taken.

2.3. DETERMINATION OF SOIL BIOMASS

This was the method of Jenkinson and Powlson (1976) with slight changes. The sample weight and the size of the jar used for incubation were reduced to half of that recommended. Moreover, the chloroform was purified by a different method before use.

2.3.1. Purification of Analar chloroform.

Silica gel was first dried in oven at 80 °C overnight. It was then cooled in a desiccator and put into three clean and dry 100 cm^3 leaching columns. The leaching columns were fixed in three different stands. 100 cm^3 of Analar chloroform was filtered through each silica gel leaching column and in turn collected in an air tight conical flask after passing through the final column.

2.3.2. Soil preparation and sterilization

Soil was first passed through a 4 mm sieve in the fresh condition. 50 g of fresh soil sample was taken in eight 250 cm^3 glass beakers on oven dry basis for each soil. Half of the samples of each soil were fumigated with chloroform and half left unfumigated. The fumigation was done in a large desiccator lined with moist tissue papers. Approximately 50 cm^3 of the washed chloroform was transferred into a 250 cm^3 beaker containing few granules of silica gel. Beakers containing soil and chloroform were put into the desiccator. Air was evacuated from the desiccator until the chloroform started bubbling vigorously. The tap was closed and the desiccator was then left in a dark cupboard for 18 to 24 hours. The unfumigated soil samples were put into another desiccator lined with moist tissues and kept in the same cupboard with the fumigated samples for 18 to 24 hours. The beaker containing chloroform and the tissue were then removed, fresh air was allowed into the desiccator for 5 minutes to take off the fumes of chloroform. It was then evacuated

and this practice was repeated several times over a period of one and a half hours until the complete removal of chloroform vapour. After this the weight of each fumigated soil was adjusted to original weight by adding few cm^3 of deionized water. They were then inoculated with one g of fresh unfumigated soil which was mixed in with a spatula. The weight of unfumigated soil was also adjusted to original, but they were not inoculated.

2.3.3. Incubation

Each beaker of fumigated and unfumigated soil samples were placed in a 1.5 litres glass Kilner jar together with 25 cm^3 of 1 M sodium hydroxide in a 4 ounce glass screw cap bottle. The Kilner jars were kept moist by adding few cm^3 of deionized water to offset the drying effect of sodium hydroxide. Blank incubations in which the jars contained alkali (sodium hydroxide) but no soil were also included. The jars were large enough to ensure that there was enough oxygen for the period of incubation. The jars were then screwed down over a parafilm sealing ring to make them air tight and incubated for a period of 20 days at 25 ^{o}C. Carbon dioxide evolved was measured two times (0 to 10 and 10 t0 20 days) during the incubation period. At the end of first 10 days incubation the bottles containing sodium hydroxide were removed and closed tightly. Fresh air was allowed to enter the Kilner jars for 10 minutes. The weight of the soil samples was adjusted by adding deionized water and then incubated for another 10 days period with fresh sodium hydroxide bottles.

2.3.4. Titration

At the end of an incubation the sodium hydroxide was transferred from the bottle into a 250 cm^3 glass beaker. The bottles were rinsed 3 times with carbon dioxide free water into the beaker to ensure that no sodium hydroxide has been left in the bottle. Four drops of a solution of carbonic anhydrase which was prepared by dissolving 10 mg of pure enzyme in 10 cm^3 of deionized water was added into the beaker. Immediately after adding the enzyme, the pH of the solution was brought to about 10.0 by slow addition of 1 M hydrochloric acid and then to 8.3 by slow addition of 0.05 M hydrochloric acid, the solution being stirred with a magnetic stirrer. The solution was finally titrated with 0.05 M hydrochloric acid to bring the pH down to 3.7 and the amount of carbon dioxide evolved during incubation calculated from the volume of the acid required to bring the pH from 8.3 to 3.7 less that required by blanks.
1 cm^3 of 0.05 M hydrochloric acid is equivalent to 0.6 mg of carbon dioxide carbon in the sodium hydroxide solution.

2.4. TOTAL N DETERMINATION IN SOILS

Digestion of the soils was carried out using the Kjeldahl digestion method of Bremner and Mulvaney (1982). Ammonium-N in the digests was determined using the Technicon AutoAnalyzer II system.

Air dried soil sieved through a 2 mm sieve was thoroughly ground using mortar and pestle. 1.0 g of soil was carefully weighed into a digestion flask. For organic

soils the weight was reduced to 0.50 g. The soil was soaked by adding 2 to 3 cm^3 of deionized water. Then 2.5 g Kjeltab tablets (IB/61 5 g each containing 100 parts potassium sulphate, 6 parts copper sulphate pentahydrate and 1 part selenium, manufactured by Thompson and Capper Ltd. Runcorn, Cheshire England) was added and 5 cm^3 of concentrated sulphuric acid (AR) was also added. The flasks were first heated gently on gas heaters and then more strongly until the solution became clear. The flasks were then left at slow heating for approximately 2 hours. They were then cooled and very carefully 20 to 30 cm^3 of deionized water was added to dissolve the digest. The solution was filtered through a Whatman filter paper No 40 into a 100 cm^3 volumetric flask. The digestion flask was rinsed several times with deionized water and the aliquots added to the volumetric flask through the filter funnel into the volumetric flask. The digest was then allowed to cool before making up to volume. The ammonium nitrogen content of the digest was determined by colorimetric method using the Technicon AutoAnalyzer.(see section 2.9.2)

2.5. DETERMINATION OF SOIL pH

Soil pH was determined in a 5:1 water/soil mixture by a combined glass/reference electrode. It was first standardized with buffer solutions of pH 7.0 and 4.0. The buffer solutions were prepared by dissolving a tablet in a 100 cm^3 of deionized water. After this 2 g of air dried soil ground to pass a 2 mm sieve was taken in a glass

vial. 10 cm^3 of deionized water was added to the vial which was then shaken for 15 minutes. The electrode was then immersed in the vial and the soil suspension stirred by swirling the electrode slightly. The pH was read immediately. The soil pH was also determined exactly the same way by substituting 0.01 M calcium chloride solution for deionized water.

2.6. % MOISTURE DETERMINATIONS

Porcelain basins were washed and cleaned and then left in the oven at 110 ^{o}C for an hour to dry. They were cooled in a desiccator and weighed. 100 to 150 g soil was weighed into each basin and were placed for 24 hours in a oven at 110 ^{o}C, cooled in a desiccator and reweighed. The % moisture content was determined on an oven dry basis.

2.7. AUTOCLAVING OF SOIL IN 0.01 M CALCIUM CHLORIDE

This was the method proposed by Stanford and Smith (1976)

10 g of air dried soil ground to pass a 2 mm sieve was weighed into 2 ounce glass bottles. 25 cm^3 of 0.01 M calcium chloride solution was added into each bottle and the total weight (weight of bottle + soil + solution) was noted. The bottles were closed with plastic tops but not tightly and were put in a 5 litres glass beaker. The top of the beaker was then covered with aluminium foil. The samples were then heated in the autoclave (manufactured by George and Gurr limited) at 120 ^{o}C for 16 hours. After heating the samples were allowed to cool,

adjusted to its original weight with deionized water and then filtered through Whatman No. 40 filter paper. The ammonium, nitrate and nitrite nitrogen content of the samples were determined with the Technicon AutoAnalyzer.

2.8. DETERMINATION OF MOISTURE CONTENT AT -0.5 BAR

Determination of moisture content at a soil water potential of -0.5 bar was carried out with the pressure plate apparatus (Moisture Equipment Company Santa Barbara California). The soil samples were placed on the plate and flooded with water. They were allowed to soak overnight. Excess water was removed from the plate which was then placed in the pressure plate apparatus and the pressure adjusted to 0.5 bar using nitrogen gas from a cylinder. Samples were then allowed to equilibrate for three days, by which time water loss had ceased. The % moisture content was determined on an oven dry basis.

2.9. AUTOMATED DETERMINATION OF SOIL INORGANIC NITROGEN.

2.9.1. INTRODUCTION

Potentiometric, colorimetric and distillation methods for the determination of inorganic forms of nitrogen (ammonium, nitrate and nitrite nitrogen) are all used in soil and water analytical laboratories and the limitations imposed by the expense, rapidity, versatility and precision of the individual methods are the deciding factors in selecting which methods are to be used in a particular laboratory. A large number of soil inorganic

nitrogen extractants like potassium chloride, potassium sulphate, sodium chloride, calcium chloride and potassium acetate are all available and the adoption of a single solution for the extraction of soil is also important for routine inorganic nitrogen determinations.

The distillation procedures are time consuming and the potentiometric methods particularly the nitrate electrodes are prone to chloride ion interference. The use of automated colorimetric analysis for determination of inorganic nitrogen in various extracting solutions is an attractive alternative because large numbers of samples can be analysed quickly and with a high degree of reproducibility.

In a recent development any AutoAnalyzer system can be upgraded by adding microprocessor based controller and data handler. The sampling rate and sample to wash ratio may be set to any desired rate and ratio. As a data handler, the microprocessor can provide drift, gain and carry over corrections through peak height adjustments. Also the microprocessor produces a printed record of the calculated and corrected final analytical results.

The Technicon AutoAnalyzer II was used in this study for analysis of ammonium, nitrate and nitrite nitrogen in various soil extracting solutions and Kjeldahl digests. The system comprised a sampler, a pump, a constant temperature water bath and a spectrophotometer. Results of the samples were recorded with a single pen chart recorder. The system was connected to a BBC microcomputer which was used for the measurement of peak heights and

calculation of results.

2.9.2. DETERMINATION OF AMMONIUM.

Ammonium was determined by a modification of the indophenol green method using a complexing reagent to prevent interferences due to the precipitation of hydroxides in the reagent system. With the inclusion of a sodium nitroprusside catalyst the sensitivity of the method was such that ammonium nitrogen could be determined in the range 0 to 1 ppm and with care 0 to 0.1 ppm (Brown, 1973).

The schematic diagram of the flow system for ammonium is shown in fig. 2.1.

2.9.2.1. Reagents

Analar grade reagents and nitrogen free deionized water were used throughout.

(i). Alkaline phenol.

50.0 g of phenol was dissolved in approximately 600 cm^3 deionized water. 22.5 g sodium hydroxide was dissolved separately in approximately 200 cm^3 deionized water. The solutions were mixed carefully together and the volume made to 1 litre.

(ii). Complexing reagent.

50.0 g potassium sodium tartrate, 50.0 g sodium citrate and 1.2 g sodium nitroprusside were weighed carefully and dissolved in 800 cm^3 deionized water and the volume was made to one litre. 0.5 cm^3 of 30 % Brij-35 solution was also added.

(iii). Sodium hypochlorite solution (approximately 0.5 %).

50 cm^3 of sodium hypochlorite solution (12 % w/v available chlorine) was diluted to one litre with deionized water.

(iv) Ammonium nitrogen standard stock solution (1000 mg/l).

4.717 g of dried ammonium sulphate was dissolved in deionized water and the volume made to 1 litre. The solution was stored at 2°C. Working standard were prepared by dilution in the appropriate extracting solutions.

In addition to the above reagents required for measurement of ammonium, the following were also used for the analysis of ammonium nitrogen in the Kjeldahl digest of soils.

(a). Wash chamber solution. 50 cm^3 of concentrated sulphuric acid was diluted to one litre with deionized water.

(b). Neutralizing solution. 3.6 g of sodium hydroxide was dissolved in a litre of deionized water.

2.9.2.2. Procedure.

The manifold (fig. 2.1) was used for determination of ammonium in water, 2 M and 1 M potassium chloride, 0.5 M potassium sulphate, 1 M sodium chloride, 0.5 M calcium chloride, 1 M potassium acetate, acidified potassium chloride and potassium sulphate and Kjeldahl digest

solutions. The filtered solutions were analysed directly on a Technicon AutoAnalyzer No II. The samples were run at the rate of 50 per hour. Where dilution was required, the sampling rate was then reduced to 40 per hour. Colour development was carried out at a constant temperature of 37 ^{o}C in the heating bath. The colour intensity was measured at 650 nm. The air was cleaned from atmospheric ammonium by bubbling through 5 % sulphuric acid solution. The calibration curve for the system was linear over the full range. Dilution was necessary above 5 ppm standard solutions.

For the analysis of Kjeldahl digest, the following modifications were made in the standard ammonium-N manifold:

(i) Sampler wash solution-5% v/v H_2SO_4

(ii) Dilution ratio 20:1

Sample 0.1 cm^3 per minute

Diluent 2.0 cm^3 per minute

Air 0.8 cm^3 per minute

The composition of the diluent neutralizing solution was checked by sampling some of the wash chamber solution containing a few drops of methyl red indicator. Sodium hydroxide (1 M) was added to the neutralizing solution until the indicator just changed colour from red to yellow in the diluter mixing coil.

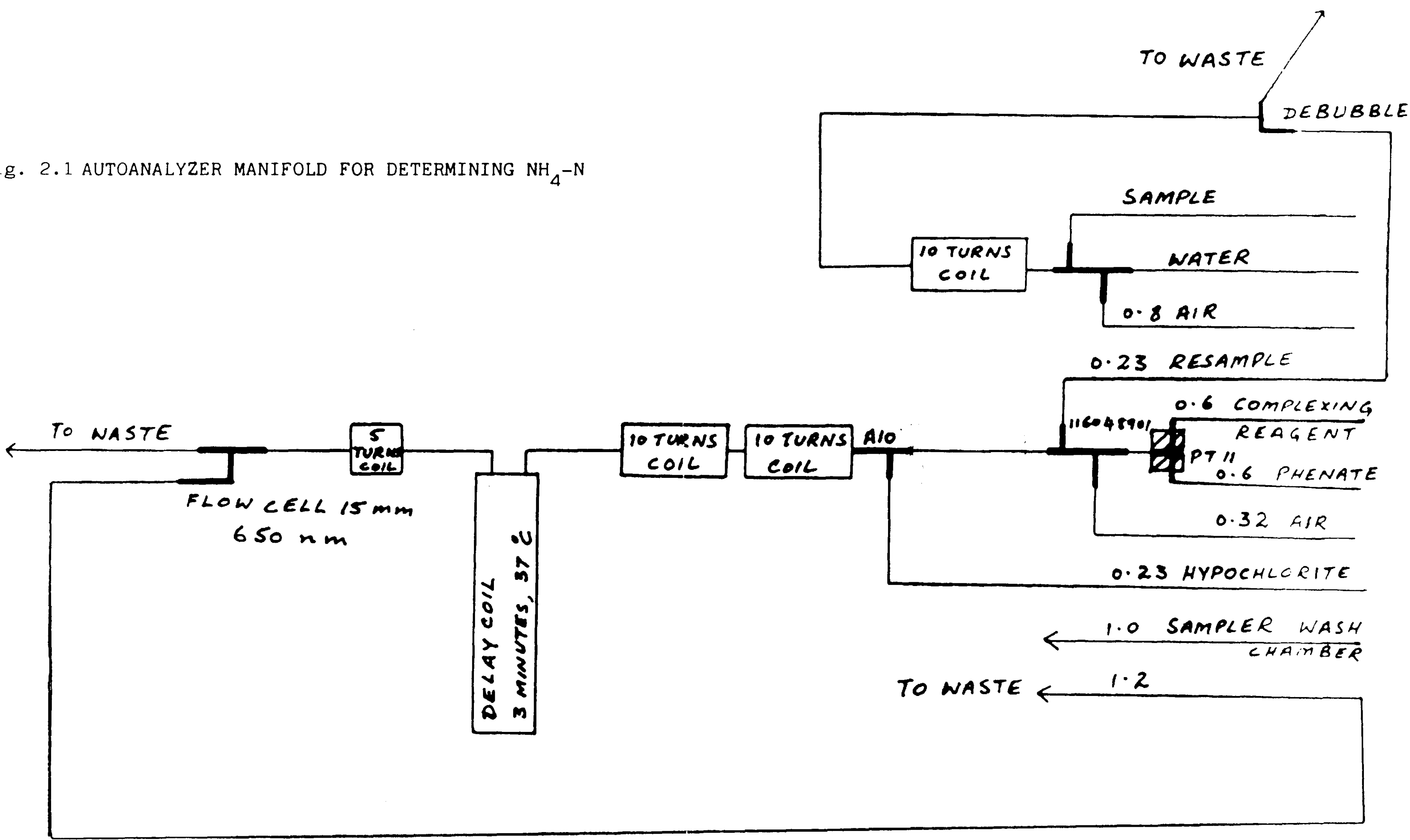


Fig. 2.1 AUTOANALYZER MANIFOLD FOR DETERMINING NH_4-N

2.9.3. NITRATE AND NITRITE NITROGEN DETERMINATION.

In the automated system, nitrate nitrogen was quantitatively reduced to nitrite nitrogen followed by determination of the nitrite using the Greiss reagent. The method, therefore, measured nitrate plus nitrite. The nitrite nitrogen was measured separately on the same manifold by omitting the reduction step.

The Schematic diagram of the flow system for nitrate and nitrite is shown in fig. 2.2. (Best, 1976)

2.9.3.1. Reagents.

(i). Buffer solution.

22.5 g sodium tetraborate and 2.5 g sodium hydroxide were dissolved in deionized water and the volume made to one litre.

(ii). Greiss reagent.

100 cm^3 concentrated hydrochloric acid was carefully added into approximately 800 cm^3 deionized water. 10.0 g sulphanilamide and 0.5 g N-1-naphthylene diamine dihydrochloride was dissolved in the acid solution and the volume was made to 1 litre.

(iii). Reducing reagent.

The reducing reagent was divided into two components.

(a). Hydrazine sulphate. 0.30 g of hydrazine sulphate was dissolved in a litre of deionized water which was taken in a one litre volumetric flask. The flask was made up to the mark without shaking and the hydrazine sulphate was dissolved with a magnetic stirrer keeping the top of the flask closed in order to prevent access of oxygen. 0.5 cm^3

of Brij-35 30 % solution was then mixed with the solution. If the hydrazine sulphate is shaken in a flask which is half filled with deionized water, it will react with the oxygen content of the flask and would, therefore, decrease its reducing power.

(b). Catalyst solution. 1 cm^3 of 2.47 % copper sulphate pentahydrate solution was added into approximately 800 cm^3 deionized water and the volume made to 1 litre. The solution was stored in a plastic bottle. 0.5 cm^3 of Brij-35 30 % solution was also added and mixed with the solution. For the determination of nitrite nitrogen, the reducing reagents were replaced with nitrogen free deionized water containing 0.5 cm^3 per litre of a 30 % Brij-35 solution.

(iv) Nitrate nitrogen standard stock solution (1000 mg/l).

6.068 g of dried sodium nitrate or 7.218 g of dried potassium nitrate was dissolved in deionized water and the volume made to 1 litre. The solution was stored at $2^{o}C$. Working standard were prepared by dilution in the appropriate extracting solutions.

(v) Nitrite nitrogen standard stock solution (1000 mg/l).

4.926 g of dried sodium nitrite was dissolved in deionized water and the volume made to 1 litre. The solution was stored at $2^{o}C$. Working standard were prepared by dilution in the appropriate extracting solutions.

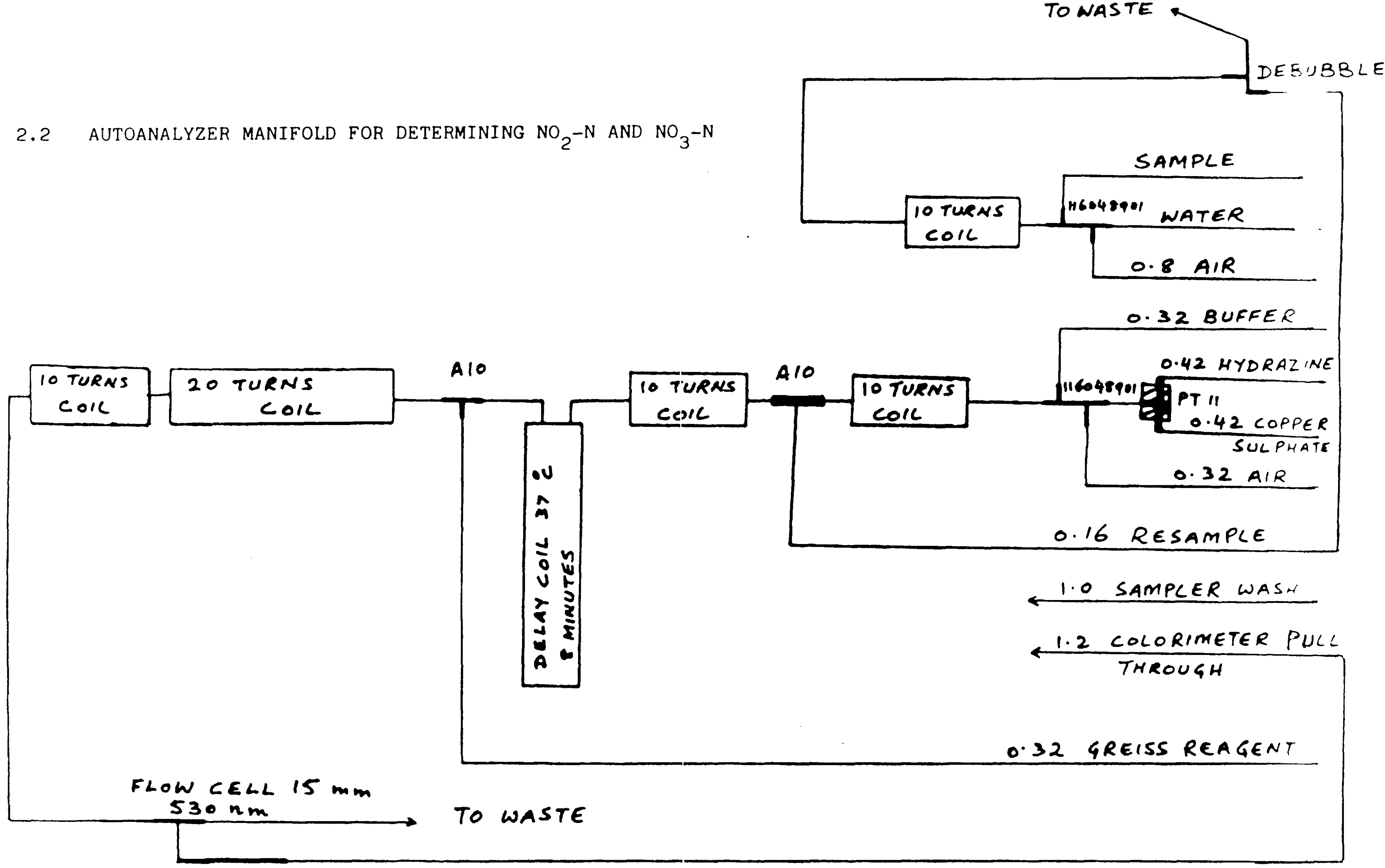
2.9.3.2. Procedure.

The manifold (fig. 2.2) was used for determination of nitrate and nitrite nitrogen in different extracting solutions. The samples were run at the rate of 50 per hour. The sampling rate was reduced to 40 per hour where dilution step was necessary. The nitrate nitrogen was reduced to nitrite by adding copper sulphate and hydrazine sulphate solutions to the sample stream and conducting it through constant heating bath at 37 ^{o}C. Then, all the nitrite nitrogen initially present and that reduced from nitrate nitrogen were determined in the sample by a diazotization coupling reaction. In the diazotization reaction a soluble pink colour was formed, the colour intensity was measured at 530 nm. The calibration curve was linear at 0 to 1 ppm range and it was curved at 0 to 5 ppm. Dilution was necessary above 5 ppm standard solution.

2.10. EXTRACTION OF INORGANIC NITROGEN FROM SOIL

The method is discussed in chapters 3, 4 and 5 and details of the method finally adopted are given in section 5.5.

Fig. 2.2 AUTOANALYZER MANIFOLD FOR DETERMINING NO_2-N AND NO_3-N



2.11. COLLECTION AND PREPARATION OF SOIL SAMPLES.

Soil samples were taken in the fresh condition from the upper 0-15 cm depth of soil profile and were brought to the laboratory in labelled plastic bags as soon as possible. The samples were spread on clean plastic sheets in the laboratory and air dried just sufficiently to pass through a sieve with 4 mm opening. The samples were then stored at 2 ^{o}C if not used at once. Half of each of the sieved samples was spread on plastic sheets and left at room temperature until completely air dried.

Some of the samples were collected from various locations in England (Rivington, Midelney 1, Midelney 2, Downholland and Alluvium) but most of the samples were collected from different locations in Scotland. The system of classification of the Soil Survey of England and Wales is different from that of Scotland. The Soil Survey of England and Wales classify soils into Series. A Series is a uniform soil forming on a particular parent material. The Soil Survey of Scotland classify them into Soil Associations. The Soil Associations are subdivided into Series on the basis of point in a catena and drainage status. The Soil Association is the catenary sequence of soils on a particular parent material. Soil Series within the Association are distinguished on the basis of drainage status.

2.12. SOIL SAMPLING SITES

A brief description of each of the soil sampling sites is given below. Soils were attributed to Soil Series using the Soil Memoirs and Soil Maps for each area. Where the area had not been Mapped, Soil Memoirs for an adjacent area were used. (Mitchell and Jarvis, 1956; Grant et al., 1962; Seale, 1975; Ragg et al. 1976).

Soil No.1. Midelney 1 (Fen grass)

The site is located at Bank Farm, Norfolk, England. Grid reference No. is TF 588022. The soil was ploughed out for permanent grass in 1979. Now it is used for intensive arable crop cultivation. It belongs to Midelney Series which is formed from calcareous alluvial clay parent material. The Series has been classed as a ground water gley.

Soil No.2. Midelney 2 (Fen arable)

The site is located at Bank Farm, Norfolk, England. Grid reference No. is TF 588022. The soil is used for intensive arable crop production. e.g. wheat, potatoes and sugar beet It belongs to the Midelney Series which is developed from calcareous alluvial clay parent material. The Series has been classed as a ground water gley.

Soil No.3. Downholland

The site is located at Bank Farm, Norfolk, England. Grid reference No. TF 586021. The soil is used for intensive cultivation of wheat, potatoes and sugar beet.It

belongs to Downholland Series which originated from peat remnant cultivated into the Fen clay to produce a organic matter rich soil. The Series has been classed as a humic gley.

Soil No.4. Alluvium

The site is located at Bank Farm, Norfolk, England. Grid reference No. is TF 588022. The soil is cultivated as a garden. It is originated from calcareous river alluvium produced by dredging the mud from the river Great Ouse in 1961.

Soil No.5. Rivington

The site is located at University of Newcastle Experimental Farm, Cockle Park, England. Grid reference No. is NZ 203916. The soil is used for grass-arable rotation. The site was under 5 years of grass adjacent to experimental plots. The soil belongs to Rivington Series which developed on coarse grained Carboniferous sandstone. The Series is classed as a brown earth.

Soil No.6. Darvel

The site is located at Westerton Farm, Lennoxtown, Scotland. Grid reference No. is NS 635773. The soil is devoted to permanent grass and rough grazing. It belongs to the Darvel Association which is formed from fluvioglacial sands and gravels derived from Carboniferous igneous and sedimentary rocks. The soil comes under the Darvel Series which has been classed as a freely drained

brown forest soils of low base status.

Soil No.7. Caprington

The site is situated at West of Scotland College of Agriculture, Auchincruive, Ayr, Scotland. Grid reference No. is NS 376232. It is devoted to permanent grass and managed for grazing by dairy cows. The soil belongs to the Rowanhill Association which is developed from glacial till derived from sandstone and shale of the productive coal measures. The soil comes under the Caprington Series which is classed as an imperfectly drained brown forest soil.

Soil No.8. Dreghorn 1 (Ayr)

The site is situated at West of Scotland College of Agriculture, Auchincruive, Ayr, Scotland. Grid reference No. is NS 373232. The soil is under permanent grass adjacent to greenhouses. It belongs to the Dreghorn Association which is developed from raised beach deposits. The Series is Dreghorn which has been classed as freely drained brown forest soil.

Soil No.9. Bargour

The site is situated at West of Scotland College of Agriculture Auchincruive, Ayr, Scotland. Grid reference No. is NS 379234. The soil is under permanent grass just near the meteorological station. It belongs to Bargour Association which is developed from till derived from Carboniferous sandstone. The Series is Bargour which has been classed as imperfectly drained brown forest soil.

Soil No.10. Dreghorn 2 (Arkleston)

The site is located at Arkleston Farm, Paisley, Scotland. Grid reference No. is NS 508655. The soil is used for intensive cultivation of potatoes, barley and oil seed rape. It belongs to Dreghorn Association which is formed from raised beach deposits. The Series is Dreghorn which has been classed as freely drained brown forest soil.

Soil No.11. Darleith 1 (Drumboy)

The site is located at South Drumboy Farm, Fenwick, Ayrshire, Scotland. Grid reference No. is NS 498485. The soil is under poor pasture and used for sheep grazing and beef cattle. The soil belongs to Darleith Association which is developed on till derived from Carboniferous age igneous rocks (Basalt). The soil Series is Darleith which has been classed as freely drained brown forest soil.

Soil No.12. Darleith 2 (Carbeth)

The site is located at Carbeth, Strathblane Dumbartonshire, Scotland. The Grid reference No. is NS 521785. The soil is under permanent grass and used for sheep grazing with some beef cattle. The soil belongs to Darleith Association which is developed on till derived from Carboniferous age igneous rocks (Basalt). The soil Series is Darleith which has been classed as a freely drained brown forest soil characteristic of the steeper

slopes.

Soil No.13. Dunlop

The site is located at South Drumboy Farm, Fenwick, Ayrshire, Scotland. The Grid reference No. is NS 500484. The soil is under permanent grass and used for sheep grazing and beef cattle. The site receives occasional dressing of lime. The soil belongs to Darleith Association which is developed on till derived from Carboniferous age igneous rocks (Basalt). The Soil Series is Dunlop which is classed as an imperfectly drained brown forest soil.

2.13. SOIL PROPERTIES

Routine soil analytical properties are given in tables 2.1 and 2.2.

SOILS	%Coarse sand	%Fine sand	%Silt	%Clay	Textural class
Midelney 1	2.3	15.5	40.3	42.3	Clay
Midelney 2	1.5	7.4	50.8	40.4	Silty Clay
Alluvium	0.6	65.0	18.2	16.1	Sandy Loam
Darvel	33.5	20.0	22.0	24.4	Sandy Clay Loam
Darleith 1	39.3*		19.2	41.5	Sandy Silt Loam
Darleith 2	18.8	27.9	30.2	22.9	Clay Loam
Dreghorn 1	46.9	25.9	19.0	8.1	Sandy Loam
Dreghorn 2	32.7	35.3	16.3	15.7	Sandy Loam
Caprington	29.2	22.0	25.7	23.1	Sandy Clay Loam
Dunlop	15.7	12.2	39.7	32.5	Clay Loam
Rivington	69.8*		17.5	12.7	Sandy Loam
Bargour	39.4	27.5	11.3	21.8	Sandy Loam
Downholland	8.0*		2.1	47.5	Clay

Table 2.1 Textural Properties of Soils

(*) = Total sand as coarse and fine sand content of these samples were not determined individually.

Coarse sand > 0.18 mm, Fine sand= 0.18-0.05 mm, Silt= 0.05-0.002 mm and Clay < 0.002 mm

SOILS	Total C %	LOI %	pH water	pH $CaCl_2$	Total N %
Midelney 1	5.9	16.2	7.6	7.0	0.55
Midelney 2	4.4	14.7	7.4	7.0	0.40
Alluvium	1.9	7.1	8.1	7.3	0.16
Darvel	3.5	9.1	5.6	4.7	0.25
Darleith 1	8.5	23.3	5.8	4.4	0.70
Darleith 2	6.8	15.3	5.5	4.4	0.45
Dreghorn 1	2.3	6.7	5.8	4.8	0.20
Dreghorn 2	2.9	6.5	5.6	4.8	0.19
Caprington	3.8	11.7	6.6	5.8	0.28
Dunlop	8.3	18.5	5.8	4.8	0.64
Rivington	1.9	4.0	6.0	5.3	0.14
Bargour	1.8	6.8	5.4	4.8	0.18
Downholland	12.5	31.2	5.1	4.6	0.92

Table 2.2 Soil Properties

CHAPTER THREE

PURIFICATION OF POTASSIUM SALT SOLUTIONS FROM INORGANIC NITROGEN CONTAMINATION

3.1. INTRODUCTION

Extraction is a most important step in the colorimetric determination of ammonium and other inorganic forms of nitrogen in the soil. The use of potassium salts, particularly potassium chloride, is the standard method for the extraction of mineral nitrogen from the soil (Bremner, 1965b).

In the course of mineral nitrogen determination it becomes necessary to use an extractant that contains very low levels of inorganic nitrogen as impurities. The levels of many inorganic impurities in the different potassium salts like chloride, sulphate and acetate are stated by the manufacturers. They describe the levels of inorganic nitrogen impurities in these salts in terms of total nitrogen or simply nitrogen compounds, but there is not always information on the levels of impurities of specified forms as ammonium, nitrate and nitrite (see table 3.1). Although the levels of inorganic nitrogen impurities in the Aristar grade of potassium salts are too small to interfere during inorganic soil nitrogen analysis the cost is so high that it cannot be used for routine analysis.

Salt	Grade	Specification	Molarity of extractant	Concentration in extractant (ppm)
Chloride				
	AR	0.001 % N	1 M	0.75
	ARISTAR	1 ppm NO3-N	1 M	0.08
		0.1 ppm NH4-N		0.008
Sulphate				
	AR	0.0005 % N	0.5 M	0.43
	ARISTAR	1 ppm NO_3-N	0.5 M	0.087
		2 ppm NH_4-N		0.174
Acetate				
	AR	0.005 % NH_4-N	1 M	0.49

Table 3.1. Manufacturers specifications for nitrogen impurities in potassium salts used for extraction.

There appears to be no information in the literature on the effect of inorganic nitrogen impurities in potassium salts on soil nitrogen determination nor is there any standard method for their purification. If the actual levels of inorganic nitrogen in the potassium salts are as high as the maximum permissible levels stated by the manufacturers, then there is a possibility of their interference in the analysis of a soil that contains very

low inorganic nitrogen levels. It, therefore, becomes imperative to test and purify the solution before it is used for extraction. The aim of the present work was to select a wide range of different makes and batch numbers of potassium chloride, sulphate and acetate for determination of their initial values of ammonium, nitrate and nitrite nitrogen and if necessary to find a suitable method of purification.

The method of removal of ammonium and nitrite contamination from the potassium salt is simple. Ammonium nitrogen can easily be converted into ammonia by making the pH of the solution alkaline with a potassium hydroxide solution. Ammonia is then expelled by boiling and stirring the solution for a certain period of time. Nitrite nitrogen is also very easy to remove under acid conditions as it decomposes to a mixture of nitric and nitrous oxides. However, nitrous oxide may dissolve to produce nitric and nitrous acids. Removal of nitrate nitrogen seems to be very difficult as it is unaffected by both acidic and alkaline pH values.

3.2. MATERIALS AND METHODS

Different batches of potassium sulphate, potassium chloride and potassium acetate supplied by different manufacturers (BDH, Hopkin and Williams, Koch-Light Limited, May and Baker Limited and Riedel deHaen) were collected in sealed bottles and tested as follows:-

3.2.1. Nitrogen levels in potassium salts.

Thirteen different batches of potassium sulphate (11 manufactured by BDH, and one each by Hopkin and Williams and Riedel deHaen), five different batches of potassium chloride (2 manufactured by Koch-light Ltd. and one each by BDH, May and Baker Ltd. and Riedel deHaen) and three different batches of potassium acetate (2 manufactured by BDH and one by Riedel deHaen) were selected for determination of their ammonium, nitrate and nitrite nitrogen levels. 0.5 M potassium sulphate, 1 M potassium chloride and 1 M potassium acetate solutions were prepared from their respective salts using deionized water. The solutions were then analysed for their mineral nitrogen content (ammonium, nitrate and nitrite) with a Technicon AutoAnalyzer.

3.2.2. Purification of potassium sulphate solution.

(i). Effect of various pH values and boiling treatments on the ammonium-N content.

Four litres of potassium sulphate solution were prepared. This was divided into four 1 litre volumes which were adjusted to pH values of 6.0 , 9.0 , 10.0 or 11.0 with 1 M potassium hydroxide solution. Three 250 cm^3 aliquots of each solution were transferred to 500 cm^3

beakers and boiled for a period of 0, 5 or 15 minutes. After boiling the solutions were cooled to room temperature, the pH was adjusted to pH 6.0 with 0.5 M sulphuric acid and volume was made to 250 cm^3. Solutions from each beaker were stored in plastic bottles and analysed for their ammonium nitrogen content.

(ii). Effect of different boiling treatments.

0.5 M potassium sulphate solution was prepared in a 4 litre beaker. The pH of the solution was adjusted to 11.0 with 1 M potassium hydroxide solution. 250 cm^3 aliquots of the solution were boiled for 15 minutes, boiled for 15 minutes with beads, stirred for 3 hours at 90 ^oC or boiled dry. After the different boiling treatments the solutions were allowed to cool, the pH adjusted to 6.0 with 0.5 M sulphuric acid and the volume made to 250 cm^3 and then each of the solution was analysed for its ammonium nitrogen content.

(iii). Testing of the boiling treatment.

In this experiment a batch of potassium sulphate (batch No. 9611822E) that contained high ammonium levels was selected to test the validity of the method for purification of potassium sulphate solution on a large scale. Three litres of solution were prepared from the above batch number. The pH was made alkaline with potassium hydroxide solution to pH 11.0. 250 cm^3 of the solution was taken in a separate beaker and was boiled and stirred for 15 minutes. The rest of 3 litres solution was boiled and stirred for 15 minutes separately. Both the solutions were allowed to cool, made up to volume and the

pH adjusted to (pH 6.0) with 0.5 M sulphuric acid. They were then analysed for their ammonium nitrogen content.

3.3. RESULTS

Ammonium, nitrate and nitrite nitrogen levels in different salt solutions of potassium sulphate, potassium chloride and potassium acetate are given in table 3.2.

Manufacturer	Batch No	Ammonium (ppm N)	Nitrate (ppm N)	Nitrite (ppm N)
Sulphate				
BDH	9611822E	2.74	0.49	0.02
BDH	9611822E	3.04	nd	nd
BDH	9296180E	0.36	0.46	0.06
BDH	9437962E	0.70	0.40	0.03
BDH	9456080E	0.34	nd	nd
BDH	9558982E	3.88	nd	nd
BDH	9437960E	0.61	nd	nd
BDH	32862	0.62	0.03	0.0
BDH	9549520E	2.23	0.02	0.0
BDH	9611820E	3.22	0.07	0.0
BDH	9611820E	3.78	nd	nd
H.W	715600	0.25	0.68	0.13
RDL	oE60390	0.27	nd	nd
Chloride				
BDH	5750573B	0.85	0.58	0.06
K.L	91111	0.46	0.55	0.09
K.L	91111	0.54	0.60	nd
M&B	L402	0.40	0.39	0.05
RDL	4214	1.00	0.35	0.05
Acetate				
BDH	9524802E	0.10	4.18	nd
BDH	965544OE	0.20	3.69	0.19
RDL	2327	0.18	1.45	0.14

Table 3.2. Inorganic nitrogen content of different potassium salts.

nd = Not determined.
BDH = British Drug Houses
H.W = Hopkin and Williams.
RDL = Riedel - deHaen.
K.L = Koch - Light Limited.
M&B = May and Baker Limited.
ppm = micrograms per g of salt.

The results show that there was a variable amount of ammonium and nitrate nitrogen in the different potassium salt solutions. The values of ammonium were different for different batches of the same salt solution. For example

some of the batches of potassium sulphate (batch number 9296180E and 9456080E) gave low ammonium values (0.34 and 0.36 ppm respectively) while some batch numbers (No. 9611822E and 9558982E) gave comparatively high values (3.04 and 3.88 ppm respectively). On the whole the levels were higher in potassium sulphate compared with potassium chloride and potassium acetate. The lowest ammonium levels were obtained in potassium acetate solution (0.10 ppm). The nitrate levels were reasonably low in most of the potassium sulphate and potassium chloride salt solutions but were much higher in all the different batches of potassium acetate. The levels of nitrite nitrogen were negligibly low in all the different batches of potassium sulphate and potassium chloride salts (Table 3.2). It was slightly higher in batches of potassium acetate (ranging from 0.14 to 0.19 ppm).

The effects of different boiling and pH treatments on the purification of ammonium nitrogen content of potassium sulphate solution are presented in table 3.3.

pH	Boiling Time	Ammonium (ppm N)
6.0	0	0.98
6.0	5	0.91
6.0	15	0.86
9.0	0	0.98
9.0	5	0.89
9.0	15	0.61
10.0	0	0.92
10.0	5	0.77
10.0	15	0.59
11.0	0	0.88
11.0	5	0.53
11.0	15	0.41

Table 3.3. Effect of various purification methods on nitrogen contamination of potassium sulphate salt.

The results show that although there was not a high initial level of ammonium in the test solution of potassium sulphate (0.98 ppm), there was a gradual decline in the levels of ammonium when the pH of the solution was increased from 6.0 to 10.0 and boiling period from 0 to 15 minutes. Minimum levels of ammonium were obtained (0.41 ppm) when pH raised to 11.0 and the solution boiled for 15 minutes.

Data in table 3.4 shows the effect of different boiling treatments at pH 11.0 on the ammonium-N content of potassium sulphate solution. It reveals that the different

boiling treatments had similar effect on the ammonium nitrogen content of potassium sulphate solution. The level of ammonium nitrogen was effectively reduced after all the boiling treatments. The ammonium levels of the solution ranged from 0.20 to 0.24 ppm after these treatments.

Treatment	Ammonium (ppm N)
Unboiled Control pH 6	2.74
Boiled 15 minutes	0.24
Boiled 15 minutes with beads	0.25
Unboiled stirred for 3 hours	0.24
Boiled dry	0.20

Table 3.4. Effect of different boiling treatments at pH 11.0 on the purification of potassium sulphate from ammonium impurities.

Treatment	Ammonium (ppm N)
Untreated	2.74
$250cm^3$ batch preparaton	0.19
3 litres batch preparation	0.33

Table 3.5. Testing of method of purification for large scale potassium sulphate solution preparation (boiled and stirred for 15 minutes at pH 11.0)

Table 3.5 shows the effect of boiling and stirring on the ammonium nitrogen content of both large and small scale potassium sulphate solution preparation. The results reveal that although there was a high initial ammonium level in the potassium sulphate solution (2.74 ppm), after boiling and stirring for 15 minutes at pH 11.0 it dropped to a reasonably low level (0.33 ppm for large scale and 0.19 ppm for small scale preparation).

3.4. DISCUSSION

Results of this study indicated that there were variable levels of ammonium, nitrate and nitrite nitrogen in the different potassium salt solutions (potassium sulphate, potassium chloride and potassium acetate). The levels of ammonium were higher and more variable in the potassium sulphate solutions compared with potassium chloride and potassium acetate. Nitrate was highest in potassium acetate and was reasonably low and uniform in most of the batches of potassium sulphate and potassium chloride (table 3.2). Nitrite levels were very low in all various batches of potassium salts (table 3.2). It ranged from 0.02 ppm to 0.06 ppm in various batches of potassium sulphate and potassium chloride salts. It was slightly higher in batches of potassium acetate (0.14 to 0.19 ppm). Due to the presence of only trace amounts of nitrite, it was not considered to be a source of error during inorganic nitrogen analysis of the soil extract. Although the levels of ammonium and nitrate were low in different potassium salts compared with the levels described by the manufacturers (table 3.1), even this could introduce an error to the analysis of a soil that contains low mineral nitrogen levels.

There is no reference in the literature to the problem of inorganic nitrogen contamination of extraction solution nor is there any recommendations on the method of purification. Due to the lack of availability of a simple and reliable method of removal of nitrate from the solution, it is recommended that a batch number of

potassium sulphate that contains low levels of nitrate should be used for extraction.

The presence of high and variable levels of ammonium in the potassium salts definitely needs purification before use for the extraction of a soil. The method of purification is simple. It can be easily removed by raising the pH of the solution and then boiling and stirring for a certain period of time. The main difficulty is the accurate analysis of levels of ammonium after purification of solution because of the non availability of ammonium free potassium salts to be used as a blank. Deionised water is the other alternative which could be used as a blank against the test solution, but there is a problem of refractive index which has been explained by Froelich and Pilson (1978) as follows:- "The cells in the Technicon AutoAnalyzer colorimeter are designed with curved ends. Light passing through these cells is refracted at the non-perpendicular ends. Curved ends, therefore, cause differences in apparent absorbance measured at the phototube which are related to differences in the refractive indices of the solutions in the cells. This can cause significant error in the colorimetric analysis of solutions with different refractive indices (caused for example by variation in salinity). This effect is responsible for a systematic error which, for typical AutoAnalyzer determination of phosphate is 0.2 micromolar in sea water. If the size of the error is known then deionized water blanks can readily be used and a correction made for the refractive index error". Since the

size of the error is not known in the present case and the boiling treatments at pH 11.0 all produced similar apparent ammonium levels it is assumed that the lowest values of ammonium obtained (0.19 and 0.20 ppm) may be the effect of refractive index of the potassium salt and not the level of ammonium in the solution.

A comparison of the effects of different pH and boiling treatments on the purification of potassium sulphate from ammonium-N shows that boiling for a period of 15 minutes at pH 6.0 (the original pH of the potassium sulphate) reduced the ammonium content only to a small extent. There was a measurable effect on the ammonium-N content when the solution was boiled at pH 9.0 and 10.0. Maximum amount of ammonium was expelled when the pH of the solution was kept at 11.0 and boiled for a period of 15 minutes. The explanation to this could be that nearly all of the ammonium is converted to ammonia at a pH value 11.0 and that boiling and stirring for a period of 15 minutes would expel all the ammonia from the solution.

From table 3.4 it is seen that different boiling treatments at pH 11.0 have almost similar effects on the ammonium-N content of potassium sulphate. It also appears that boiling and stirring for a period of 15 minutes at pH 11.0 provides a simple method of potassium sulphate purification. It seems from table 3.5 that the method is suitable for both small and large scale solution preparation. The results reveal that although the initial levels of ammonium were very high in the potassium sulphate, after boiling and stirring they were lowered to

levels of 0.19 and 0.33 ppm for a small and large scale solution preparation respectively.

The present study recommends a method of purification of ammonium from potassium salts which is both simple and reliable. Ammonium can easily be converted into ammonia by increasing the pH of the solution to 11.0 with the addition of potassium hydroxide. Boiling and stirring for a period of 15 minutes will expel all the ammonia in the solution. The pH of the solution can be readjusted to 6.0 with 0.5 M sulphuric acid, M hydrochloric acid or acetic acid depending on which salt solution has been purified. As there is no simple method for the removal of nitrate it is recommended that the potassium salts are tested and a batch number with low levels of nitrate impurities selected for use where soil nitrate levels are expected to be low.

CHAPTER FOUR

PURIFICATION OF FILTER PAPERS FROM INORGANIC NITROGEN CONTAMINATION

4.1. INTRODUCTION

Prior to the colorimetric determination of mineral nitrogen in soil extract, filtration is necessary to remove all suspended materials both organic and inorganic. This can only be done without a serious error if the filter papers being used are of negligibly low impurities and this is what the chemist wishes, to perform the filtration stage of the analysis without interfering with the consistency or magnitude of the final results.

In initial study of the mineral nitrogen content of soil extracts, it was found that the ammonium content of the extracts varied irregularly. The nitrate and nitrite content of the soil extracts were quite uniform and the results obtained were consistent. The degree of variability in ammonium contents of the soil extracts was so great that it was not possible to carry out further work before finding some explanation or correction for these variations. Various tests were made to find out the possible source of contamination. Deionized water used for washing of various equipment was tested for its ammonium content, plastic bottles used for shaking of soil samples and sample cups were tested but no obvious sign of

ammonium contamination was noticed. It seemed, therefore, logical to conclude that the variation in ammonium content was due in some manner to the filtration procedure and probably to the mineral nitrogen content of the filter papers.

There is not a considerable amount of information in the recent literature on the inorganic nitrogen impurities in filter papers which might interfere in the mineral nitrogen determination of soil extracts. A search of the literature revealed very few references. Whatman in its laboratory product catalogue has shown typical levels of nitrogen in different grades of filter papers. (No.1 23 ug per g of paper , No.42 12 ug per g , No. 542 260 ug per g) but has made no recommendations on methods of purification. Leitch and Wells (1946) and Lucas (1955) first reported the presence of ammonium in filter papers. Leitch and Wells (1946) stated that ammonium contaminated filter papers can cause a serious error in total and non-protein nitrogen determination in aqueous extracts of various organs. They have recommended the use of small sintered glass filters for complete elimination of the error. In the more recent literature O´Halmhain and O´Danachair (1974) tested a variety of filter papers for their ammonium content and found significant levels of ammonium in all the filter papers. They recommended carrying out blank determinations with all reagents including filter papers and of taking the blank determination through all the stages of analysis. Hattori et al. (1983) evaluated the effect of ammonium-N in

filter papers on the determination of ammonium-N in soils. They recommended the use of filter papers with the lowest ammonium content and subtraction of amount of ammonium-N eluted from blank filter papers of the same package from the measured value.

In the present study it was decided to make a survey of different grades and batches of filter papers for their initial nitrogen content and to find a suitable method of washing and drying of filter papers so that they can be used for almost all levels of nitrogen determinations in the soil without causing any serious problem in the results.

After this work had been completed, Sparrow and Masiak (1987) tested filter papers for ammonium and nitrate nitrogen contamination by leaching them with 2 M potassium chloride and analysing the leachate. They found significant amounts of ammonium or nitrate in various grades and kinds of filter papers. They recommended washing of filter papers with distilled water or 2 M potassium chloride solution before use.

4.2. MATERIALS AND METHODS

Different grades and batches of Whatman filter papers, except two which were manufactured by W. and R. Balston Limited, were collected in sealed boxes and were tested as follows.

4.2.1. Nitrogen levels in the filter papers

Thirteen different batches of filter papers (11 manufactured by Whatman and 2 manufactured by W. and R. Balston limited) were selected for measurement of their initial nitrogen levels. Filter papers were taken from each box randomly, ignoring the first few at the top and bottom of the box. They were folded and placed in the plastic funnels in the usual mode for filtration. 50 cm^3 of 0.5 M potassium sulphate solution was passed through each filter paper in two successive 25 cm^3 portions. The filtrate of each 50 cm^3 of potassium sulphate was collected separately in plastic bottles. The ammonium, nitrate and nitrite nitrogen content of the filtrate was measured with the Technicon AutoAnalyzer .

4.2.2. Potassium sulphate versus deionized water washing.

In this experiment a batch of filter paper which contained high and variable levels of ammonium-N (Whatman No. 40, 11 cm batch No. 3594) was selected. Ten filter papers were collected from a sealed box, five of these were used for washing with 75 cm^3 of 0.5 M potassium sulphate solution in three successive 25 cm^3 portions. The filtrate of each 25 cm^3 portion was collected separately for the measurement of their ammonium-N content. The other

five filter papers were used for washing with 100 cm^3 of deionized water in four successive 25 cm^3 portions. The filtrates of each 25 cm^3 portions of deionized water was collected in separate plastic bottles and analysed for their ammonium-N content. Filter papers washed with deionized water were finally leached with 25 cm^3 of 0.5 M potassium sulphate solution and ammonium content of the filtrate determined.

4.2.3. Method of drying of filter papers

Thirty filter papers from a box of Whatman No. 40, 11 cm batch No. 3594 were taken. They were first washed with 50 cm^3 of 0.5 M potassium sulphate solution in two successive 25 cm^3 portions and the filtrate ignored. Then they were washed twice with deionized water to wash away traces of potassium sulphate salt and were dried as follows.

1. five filter papers left undried.
2. five filter papers air dried at room temperature (approximately 18 to 20 ^oC)
3. five oven dried at 40 ^oC for four hours.
4. five oven dried at 40 ^oC overnight.
5. five oven dried at 70 ^oC for 40 minutes.
6. five oven dried at 70 ^oC for four hours.

After the various methods of drying, the filter papers were leached with 25 cm^3 of 0.5 M potassium sulphate. The filtrates were collected and analysed for the ammonium-N. Results of the different methods of drying

were compared.

4.2.4. Washing and drying of various batches of filter papers.

To check the suitability of method of washing and drying, nine different batches of Whatman filter papers were collected. Five filter papers from each batch were folded into the plastic funnels. They were washed with 50 cm^3 of 0.5 M potassium sulphate solution in two successive portions and the filtrate ignored. Then they were washed two times with deionized water and were then transferred into the oven along with funnels and dried at 70 ^oC for four hours. After drying they were filtered with 25 cm^3 of 0.5 M potassium sulphate. The first 2.5 cm^3 of the filtrate and the rest of 25 cm^3 were collected in separate plastic bottles for determination of their ammonium-N content.

4.3. RESULTS

4.3.1. Initial Nitrogen levels of the filter papers.

Data on the initial ammonium nitrogen content of various grades and batches of filter papers is presented in table 4.1. The results reveal that there was a considerable amount of ammonium-N in almost all the boxes of filter papers tested. Some of the boxes contained very high ammonium levels, mean values ranged from 0.74 to 39.27 micrograms of N per paper. Different values were obtained for different batches of the same grade of filter papers. There was considerable variation in ammonium levels of the filter papers obtained from different boxes of the same batch number. The values were not uniform even within a given box of filter papers. Table 4.2 contains data on the nitrate nitrogen content of filter papers. It reveals that the levels of nitrate in different grades and batches of filter papers were very low and the results obtained were more uniform.

Filter paper	Batch number	Papers tested	Ammonium-N Content (µg N per paper) Mean	SD
No 40 12.5 cm	5512*	5	3.98	0.55
No 40 11.0 cm	59045	5	4.79	0.65
No 40 15.0 cm	2581	5	1.03	0.18
No 40 15.0 cm	2438	5	0.74	0.03
No 40 11.0 cm	3594	10	12.90	1.51
No 40 11.0 cm	4575	5	11.25	4.94
No 40 11.0 cm	4??6	5	0.54	0.14
No 40 12.5 cm	0519	5	1.09	0.34
No 40 9.0 cm	****	5	39.27	2.97
No 42 9.0 cm	****	5	5.88	0.28
No 42 11.0 cm	9500	5	3.46	0.40
No 44 11.0 cm	????	10	7.70	1.30
N0 42 11.0 cm	0508	5	1.65	0.40
No 40 15.0 cm	2581	5	5.20	2.96
No 40 15.0 cm	2438	5	5.14	4.83
No 40 11.0 cm	4575	5	26.52	2.72
No 40 11.0 cm	4??6	5	1.55	0.18
No 40 12.5 cm	0519	5	3.92	2.53
No 42 11.0 cm	9500	5	19.77	1.74
No 40 11.0 cm	3594	40	16.37	2.92

Table 4.1. Ammonium-N content of various batches of filter papers.

* All filter papers were manufactured by Whatman Ltd. except those marked **** which were manufactured by W. and R. Balston Ltd.

Filter paper	Batch number	Papers tested	Nitrate-N content (μg N per paper) Mean	SD
No 40 12.5 cm	5512 *	5	0.75	0.15
No 40 11.0 cm	59045	5	0.72	0.09
No 40 15.0 cm	2581	5	0.53	0.02
No 40 11.0 cm	3594	5	0.13	0.02
No 40 11.0 cm	4575	5	0.24	0.03
No 40 12.5 cm	0519	5	0.38	0.12
No 42 9.0 cm	****	5	0.07	0.00
No 42 11.0 cm	9500	5	0.47	0.07
No 44 11.0 cm	????	5	0.08	0.08

Table 4.2. Nitrate-N content of various batches of filter papers.

* All filter papers were manufactured by Whatman Ltd. except those marked **** which were manufactured by W. and R. Balston Ltd.

4.3.2. Potassium sulphate versus deionized water washing.

Results of washing of filter papers with 0.5 M potassium sulphate and deionized water are presented in table 4.3. The results show that after initial washing of filter papers with 25 cm^3 of 0.5 M potassium sulphate or deionized water, the level of ammonium in the subsequent washing declined to a satisfactory uniform level. However, after washing of filter papers with deionized water, the ammonium content of the final wash with 25 cm^3 of potassium sulphate increased again to a higher level (from 0.09 to 5.86 microgram of N per paper). It has, therefore, been confirmed that washing with potassium sulphate can remove more ammonium from the filter papers than deionized water.

	K_2SO_4 washing (µg N per paper)		Water washing (µg N per paper)	
	Mean	SD	Mean	SD
1ST 25 cm^3	12.70	0.74	9.00	0.99
2ND 25 cm^3	2.60	0.31	2.20	0.35
3RD 25 cm^3	1.20	0.03	1.20	0.13
4TH 25 cm^3	---	---	0.09	0.08
Total	16.50	0.65	13.25	0.84
Final K2SO4	---	---	5.86	0.40

Table 4.3. Washing of filter papers with 0.5 M potassium sulphate and deionized water.

Each reading is the mean of 5 replicates.

4.3.3. Drying of filter papers.

Results of various methods of drying of washed filter papers are shown in table 4.4. The results indicate that the method of drying does not significantly change the ammonium content of the filter papers with the exception of air drying at room temperature which slightly increased the ammonium levels of the filter papers.

Drying treatment	Ammonium-N (µg N per paper)
Undried	0.79
Air dried overnight	1.00
Oven dried 40 °C 4 hours	0.74
Oven dried 40 °C overnight	0.68
Oven dried 70 °C 40 minutes	0.60
Oven dried 70 °C 4 hours	0.59

Table 4.4. Effect of drying treatment on the Ammonium-N content of washed filter papers.

Each figure is the mean of 5 replicates.

4.3.4. Washing and drying of various batches of filter papers.

Data in table 4.5 show the effect of washing and drying treatment on the ammonium levels of different kinds of filter papers. It reveals that although there was initially a wide range of ammonium in the filter papers (ranging from 1.55 to 26.52 micrograms), after washing and drying treatment it dropped to a uniform level. The Whatman No. 42 and 44 had higher ammonium levels compared to Whatman No. 40 which indicates that the method is suitable only for Whatman No. 40 filter papers. The data also reveal that after washing and drying the first 2.5 cm^3 of the filtrate contained much higher ammonium levels than the rest of the filtrate.

Filter paper	Batch number	Total N in washings (μg N)	Weight N in first $2.5cm^3$ (μg N)	Total N per paper (μg N)
No 40 15.0 cm	2581	5.20	0.21	0.37
No 40 15.0 cm	2438	5.14	0.15	0.31
No 40 11.0 cm	3594	13.94	0.18	0.50
No 40 11.0 cm	4575	26.52	0.17	0.42
No 40 11.0 cm	4??6	1.55	0.08	0.45
No 40 12.5 cm	0519	3.92	0.34	0.74
No 42 11.0 cm	9500	19.77	0.72	1.25
No 44 11.0 cm	????	8.87	0.47	0.80
No 42 11.0 cm	0508	1.65	0.31	0.57

Table 4.5. The ammonium contents of filter papers after washing and drying at 70 °C.

Each figure is the mean of 5 replicates

4.4. DISCUSSION

The present study indicates that different grades and batches of filter paper vary widely in their ammonium-N content. The nitrate-N content of the filter papers was lower and more uniform and, therefore, it was not a matter for concern in the present study. The variability in ammonium content existed between different boxes of the same batch number, even within filter papers of the same package. These fluctuating values obtained for ammonium in the filter papers agrees with the results that have been reported previously by O´Halmhain and O´Danachair (1974) and Hattori et al. (1983). It should be emphasized, however, that they found uniform levels of ammonium in the filter papers taken from the same package, while results in table 4.1 show that there was an obvious variability in the levels of ammonium of the filter papers taken from the same box.

The evidence available on the presence of high and variable levels of ammonium in the filter papers indicates that a definite error is introduced into ammonium-N determination in the soil extract through the filter papers. It may not affect results of a soil extract that has high ammonium levels but will definitely interfere in the low level determination.

A general survey of the pertinent literature reveals very few references to a correction for nitrogen in the filter papers. Hattori et al. (1983) while working on microammonium determinations of the soil extracts have recommended the use of the filter papers that contain the

lowest ammonium content and subtraction of the amount of ammonium eluted from a blank filter paper of the same package from the measured value. In the present study the variability of ammonium content in filter papers of the same package is so great that there is no guarantee to get uniform results even after subtraction of blank values from the measured values of the soil extract. Also it seems impossible to single out a particular batch number that will contain low and uniform levels of ammonium. Data in table 4.1 shows that there is no uniformity at all in the levels of ammonium of the filter papers taken from different boxes of the same batch number. For example batch number 2438 has a mean value in one box as low as 0.74 micrograms of N per paper while in the other it is 5.20.

Leitch and Wells (1946) recommends that washing of filter papers with distilled water prior to use would be one procedure for eliminating the error produced by ammonium-N of the filter papers or the use of small sintered glass filters would completely eliminate this error. However, results in table 4.3 show that washing with deionized water is not an effective method of removal of ammonium compared with potassium sulphate solution. There seems to be some exchangeable ammonium in the filter papers which cannot be washed away unless they are rinsed with a salt solution such as potassium sulphate. Other salt solutions like potassium chloride or dilute hydrochloric acid may also do the same job as potassium sulphate for the removal of both exchangeable and water

soluble ammonium content of the filter papers.

The present study recommends a method of washing and drying of filter papers which is more convenient and reliable. Washing of filter papers with 50 cm^3 of potassium sulphate solution (2 successive washings each of 25 cm^3) can remove most of the ammonium from the filter papers (see table 4.5). Rinsing twice with deionized water will wash away any potassium sulphate salt left in the filter papers. Drying of washed filter papers in the oven at 70 ^oC for 4 hours does not affect the ammonium levels in the filter papers. However, air drying at room temperature does affect ammonium levels of the washed filter papers. It increases the values of ammonium (see table 4.4). The increase may be attributed to the adsorption of atmospheric ammonia when the filter papers are left open in the room for a period of 24 hours. After the washing and drying treatment the ammonium levels of the filter papers will be very low and uniform. The data in table 4.5 shows that much of the ammonium is flushed out in the first 2.5 cm^3 of the filtrate. Therefore, results for blanks and samples could be improved by discarding the first few cm^3 of the filtrate. However, the uniformity in the results has been achieved for Whatman No. 40 filter papers only. There were slightly higher ammonium levels in Whatman No.42 and 44 filter papers even after washing and drying treatment which suggests that the method is less suitable for these grades of filter papers.

After this work had been completed, Sparrow and Mosiak (1987) reported significant levels of ammonium and

nitrate nitrogen in different grades and batches of filter papers. They have used similar techniques for testing of filter papers for ammonium and nitrate nitrogen contamination and arrived at similar conclusions. But results of the present work disagree with their final recommendations on the method of purification of filter papers. This could be due to the fact that their potassium chloride was not purified of ammonium and would possibly be less effective than in the present study where potassium sulphate was purified of ammonium nitrogen and low in nitrate nitrogen. They recommended washing of filter papers with distilled water or 2 M potassium chloride prior to use for work that requires a high degree of precision. The current work shows clearly that water alone is ineffective in removing ammonium nitrogen from filter papers especially the ammonium which is adsorbed on the filter paper. Therefore, it is essential to use a suitable salt solution which can remove both soluble and exchangeable ammonium from the filter paper.

CHAPTER FIVE

EXTRACTION OF INORGANIC NITROGEN FROM SOIL

5.1. INTRODUCTION

The inorganic nitrogen in soil (ammonium, nitrate and nitrite) can be readily determined once it has been brought into solution, but it is necessary that the ions are brought quantitatively into the extracting solution and that subsequent changes in the concentration with time are prevented.

The nitrate and nitrite nitrogen ions are not held by the soil colloids but are readily and completely extractable when the soil is shaken with water or aqueous solution. When ammonium, nitrate and nitrite all are to be determined on the sample, the extraction procedure needs to be modified because of the presence of adsorbed ammonium on the colloidal complex of soil. Most methods use a salt solution such as calcium sulphate, sodium sulphate, potassium sulphate or potassium chloride with an intent to include exchangeable ammonium. To be satisfactory, the method of extraction should possess the following properties.

(i). It should be rapid and simple.

(ii). The ammonium, nitrate and nitrite nitrogen should be liberated quantitatively and none should be produced from organic matter compounds during the work even after the

addition of such compounds.

(iii). In the case of soil to which ammonium has been added, the method must be able to recover this quantitatively together with soil nitrogen. Moreover, the extract must be susceptible to analysis by the methods available for the determination of the forms of nitrogen under investigation.

Bremner and Keeney (1966) developed a method for the extraction of inorganic soil nitrogen and determined this by the steam distillation method. Their study of the method of extraction revealed that the amount of ammonium extracted from soil by potassium chloride solution at room temperature is essentially maximal if 2 M potassium chloride is used and the soil sample is shaken with this reagent for one hour using 10 cm^3 of 2 M potassium chloride per g of soil. They further showed that the amount of ammonium extracted by shaking the soil with potassium chloride at room temperature for one hour is the same whether 1 M , 2 M or 4 M potassium chloride is used, provided that the amount of potassium chloride solution employed contains the equivalent of 20 meq of K per g of soil and that it is not increased if the meq of K per g of soil exceeds this value or if the time of shaking is increased.

Comparison of the most recent reviews shows (Keeney and Nelson, 1982 and Bremner and Hauck, 1982) that there have been no major advances in the method of extraction for inorganic soil nitrogen analysis and the one recommended by Bremner and Keeney (1966) remains the

method of choice for most research on nitrogen transformations in soil. It is desirable to apply colorimetric procedures for the determination of ammonium, nitrate and nitrite in a single soil extract because steam distillation is somewhat time consuming. Potassium chloride has been one of the most popular salts for extraction of ammonium-N. However, the chloride ion interferes with many of the methods generally favoured for colorimetric determination of nitrate nitrogen.

The Soil Science Society of America (1979) has defined exchangeable ammonium as that ammonium which is extractable by neutral potassium salt solution (e.g. 0.5 M potassium sulphate, 2 M potassium chloride) at room temperature. Potassium sulphate salt solution was selected as an extractant in the present study because the extract was satisfactory for use in the automated analysis of nitrate and nitrite as well as ammonium in this department while potassium chloride solution interfered in the automated analysis of nitrate. Flowers and Arnold (1983) also used 0.5 M potassium sulphate during inorganic nitrogen analysis of soil and they did not mention any significant interference of the sulphate ion. The soil solution ratio was maintained by using 2.5 g of soil per 50 cm^3 of 0.5 M potassium sulphate solution which was compatible with the Bremner and Keeney (1966) recommendations.

When soil extracts cannot be analysed immediately after filtration, they should be treated or stored so that further changes are minimised. In the past several

attempts have been made to preserve soil extracts for inorganic nitrogen analysis but detailed information on the method of preservation is scanty. In recent years many workers have used storage at low temperature as a means of preserving soil extracts for inorganic nitrogen analysis. Bremner and Keeney (1966) recommend that storage of 2 M potassium chloride soil extract in a refrigerator after filtration will be stable for several months but they do not mention a specific temperature.

For the automated method of inorganic nitrogen determinations, quite a large number of samples are needed and most of the time it is not convenient to prepare such a large number of extracts in a single day. It normally takes several days and during this period any chemical, biological or enzymatic reaction may occur which can cause error to the inorganic nitrogen content of the extract.

The purpose of this work was to determine whether the use of potassium sulphate salt solution as an extractant is applicable to a range of soils particularly those containing very low inorganic nitrogen levels and also to find out how far certain factors like period of shaking, extracting solutions and temperature influence the mineral nitrogen contents of the extract.

An experiment was conducted to examine how long the soil extracts can be stored in a 2 ^{o}C refrigerator without altering their inorganic nitrogen status.

5.2. MATERIALS AND METHODS

5.2.1. Extraction of mineral nitrogen from soil.

The soil used in this experiment was Rivington Soil Series . The sample was air dried and ground to pass a 2 mm sieve. 2.5 g of soil (oven dry basis) was weighed into a wide mouth 100 cm^3 plastic bottle and then 50 cm^3 of 0.5 M potassium sulphate solution was added to maintain the soil : solution ratio at 20 meq of K per g of soil as recommended by Bremner and Keeney (1966). The plastic bottles were stoppered and then shaken on two different shaking machines (orbital and end over end shaking machines) for periods of 0.5, 1, 2, 4, 6, 12 and 24 hours at room temperature (20 ^{o}C). The speed of the orbital shaker was maintained at 200 rpm while the end over end shaker was run at 41 rpm. The samples were shaken in triplicate and the suspensions obtained were then filtered through Whatman No. 40 filter papers. The extracts were collected in plastic bottles. Analyses for ammonium, nitrate and nitrite nitrogen contents of the extracts were carried out with a Technicon AutoAnalyzer. (see section 2.9). Blanks were prepared by shaking 50 cm^3 of 0.5 M potassium sulphate solution in plastic bottles, filtering through Whatman No. 40 filter papers and then analysing the solution for ammonium, nitrate and nitrite nitrogen.

5.2.2. Extraction of ammonium with different extractants.

2.5 g of air dried samples from Rivington Soil Series were weighed into 100 cm^3 plastic bottles and were shaken with 50 cm^3 of 0.5 M potassium sulphate and 1 M potassium chloride solutions for periods of 1 and 24 hours at room temperature. The shaking was done on an orbital shaker at a speed of 200 rpm. The suspensions were filtered through Whatman No. 40 filter papers. Blanks were prepared by shaking and then filtering 50 cm^3 of the extracting solutions through Whatman No. 40 filter papers. Ammonium nitrogen in the extract was measured with a Technicon AutoAnalyzer (see section 2.9). All determinations were made in triplicate.

5.2.3. Effect of periods of shaking on the mineral nitrogen content of different soils.

The effect of period of shaking at room temperature was further tested on five soil samples (air dried and fresh condition) which were taken from Darvel, Rivington, Midelney 1 (Grass), Downholland and Midelney 2 (Arable) Series. The samples were shaken in 0.5 M potassium sulphate solution for a period of 1 or 24 hours at room temperature with an orbital shaker. The speed of the shaking machine was maintained at 200 rpm. The samples were replicated 5 times for each soil and soil : solution ratio was maintained as 20 meq of K per g of soil. After shaking, the suspensions were filtered through Whatman filter paper No. 40 and the filtrates were collected in plastic bottles. Appropriate blanks were also prepared in a similar way. Analyses of the extract for ammonium,

nitrate and nitrite nitrogen were performed by the Technicon AutoAnalyzer.(see section 2.9)

5.2.4. Effect of different temperatures on the extractable mineral nitrogen content of soils.

This experiment was set up to find out the effect of different temperatures on the extraction of mineral nitrogen content of soils after 1 and 24 hours shaking. Three samples were taken in a fresh condition from Rivington, Midelney 1 (Grass) and Downholland Series. 2.5 g of each soil was weighed into plastic bottles to which was added 50 cm^3 of 0.5 M potassium sulphate solution. The samples were then shaken with an orbital shaker for periods of 1 and 24 hours at temperatures of 0, 2, 5, 10, 20 and 30 ^{o}C. The speed of the shaking machine was maintained at 200 rpm. The suspensions were filtered through Whatman filter paper No. 40. The samples were replicated 5 times for each soil. Blanks were prepared by shaking 0.5 M potassium sulphate solution for 1 and 24 hours at different temperatures and filtering through Whatman No. 40 filter papers. The extracts obtained were analysed for ammonium, nitrate and nitrite nitrogen content with a Technicon AutoAnalyzer. (see section 2.9).

5.2.5. Study of method of extraction for soil inorganic nitrogen analysis.

Seven different soil samples in the fresh state were shaken in 0.5 M potassium sulphate solution at 2 ^{o}C temperature for 0.5, 1, 2, 3, 4, 6, and 8 hours duration

with an orbital shaker. The shaking machine was run at a speed of 200 rpm and the soil : solution ratio was maintained at 20 meq of K per g of soil. Blank solutions were also shaken on the same shaker. The suspensions were filtered through Whatman filter paper No. 40. Each soil sample was replicated 5 times. Ammonium, nitrate and nitrite nitrogen content of the filtered extracts were determined colorimetrically with a Technicon AutoAnalyzer. (see section 2.9).

5.2.6. Mineral nitrogen preservation in the soil extract.

Filtered soil extracts were collected in 50 cm^3 plastic bottles and were stored at two different temperatures to check their stability. Half of the extracts were stored at room temperature (20 ^oC) and half in a 2 ^oC refrigerator. Extracts stored at room temperature were analysed for ammonium, nitrate and nitrite nitrogen after 0, 7, 14 and 21 days duration while those stored in the refrigerator were analysed after storage periods of 0, 9, 17, 29, and 71 days. The analysis were carried out with a Technicon AutoAnalyzer. (see section 2.9).

5.3. RESULTS

5.3.1. Extraction of mineral N from Rivington soil.

Data presented in fig. 5.1 show the effect of different shaking periods and two different shaking machines on the extraction of mineral nitrogen from Rivington soil. There was a considerable effect of period of shaking on the ammonium nitrogen extracted from the soil. There was a steady increase in the levels of ammonium when the period of shaking was increased. The increase was approximately fourfold after 24 hours shaking (12.68 ppm) compared with 1 hour (2.85 ppm). The orbital shaking machine extracted more ammonium nitrogen from the soil after 24 hours shaking (12.68 ppm) than the end over end shaker (8.88 ppm). In the case of nitrate nitrogen, the influence of time of shaking was negligible. The levels after 24 hours shaking with end over end shaker and orbital shaker were 36.6 and 36.3 ppm respectively. There were no traces of nitrite nitrogen in the soil after the different shaking periods.

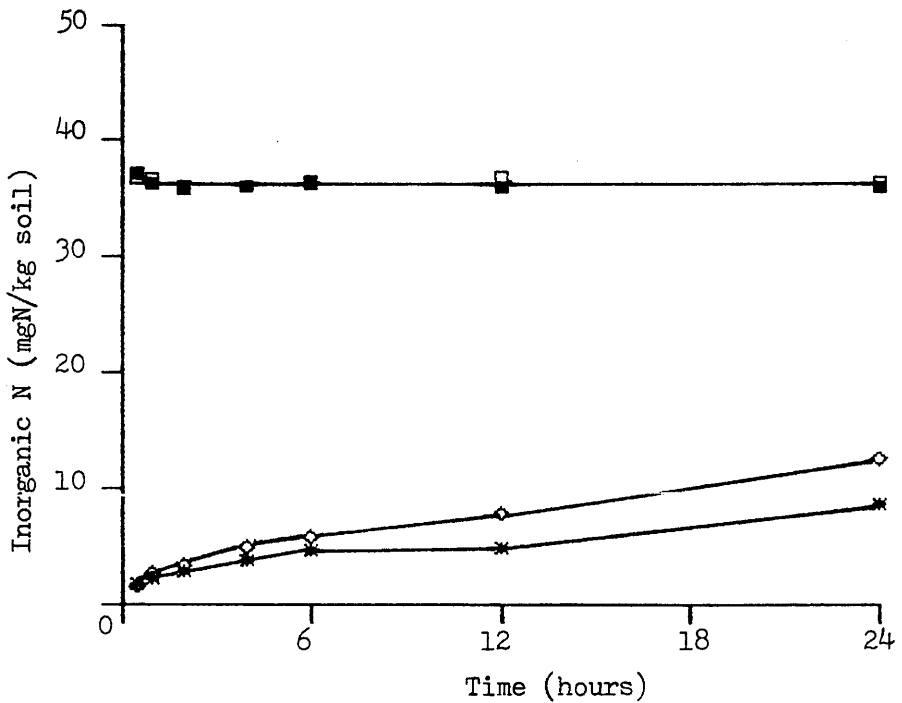


Fig. 5.1 Effect of different shaking periods and shaking machines on the extraction of inorganic N from Rivington soil at room temperature

NH_4-N with end over end shaker (*)

NO_3-N with end over end shaker (□)

NH_4-N with orbital shaker (◇)

NO_3-N with orbital shaker (■)

5.3.2. Effect of different extractants on the ammonium nitrogen content of soil.

The amount of ammonium nitrogen extracted by different extracting solutions (0.5 M potassium sulphate and 1 M potassium chloride solutions) are given in table 5.1. The data indicates that the ammonium content of the extracts were significantly affected ($p < 0.001$) by the period of shaking at room temperature. The values increased from 1.21 to 5.08 ppm after 24 hours shaking in the 0.5 M potassium sulphate extract and from 1.04 to 3.28 ppm when the extraction was done with 1 M potassium chloride solution.

Extractant	Ammonium-N(mg/kg soil)		
	1 hr	24 hrs	
0.5 M Potassium sulphate	1.2	5.1	***
1.0 M Potassium chloride	1.0	3.3	***

Table 5.1. Extraction of ammonium nitrogen from Rivington soil (air dried) with different potassium salt solutions at room temperature.

Each value is the mean of 3 replicates.

Differences between 1 and 24 hours for a particular extractant are significant at the following levels. * $p < 0.05$, ** $p< 0.01$ and *** $p< 0.001$

5.3.3. Effect of period of shaking on the mineral N levels of different soils.

The data in table 5.2 show the effect of time of shaking on the ammonium content of five different soils extracted with 0.5 M potassium sulphate solution at room temperature. For all soils in both air dried and fresh state, significant increases in the measured ammonium content occurred when the period of shaking was increased from 1 to 24 hours. The amount of ammonium extracted was greater for the air dried soils than for the fresh soils after 1 hour shaking but not in all cases after 24 hours.

In table 5.3 are shown the contents of nitrate nitrogen extracted from soils at room temperature after different periods of shaking. The data shows that the content of nitrate nitrogen in extracts of some of the soils was not significantly changed by the period of shaking. However, there were considerable changes in the nitrate nitrogen contents of other soils. There was a significant increase in the nitrate nitrogen level in the case of Midelney 1 (Grass) soil in air dried and fresh state after 24 hour shaking. In the case of Downholland soil a significant decrease in nitrate nitrogen occurred (air dried and fresh state) after 24 hours shaking period. The nitrate nitrogen extracted was higher in the fresh soils compared with air dried soils at both periods of shaking.

There was no detectable nitrite nitrogen in the

Darvel and Rivington soils but measurable amounts in Midelney 1 (Grass), Downholland and Midelney 2 (Arable) soils (table 5.4). The amount of nitrite nitrogen in Midelney 1 (Grass), Downholland and Midelney 2 (Arable) soils was significantly higher after 24 hours shaking compared with 1 hour in both air dried and fresh samples.

Soil	Air dried 1 hr	Air dried 24 hrs		Fresh 1 hr	Fresh 24 hrs	
Darvel	5.6	12.6	***	3.5	12.0	***
Rivington	4.5	13.4	***	3.7	12.6	***
Midelney 1	6.4	24.2	***	2.0	3.2	**
Downholland	4.3	17.1	***	2.2	19.0	***
Midelney 2	4.3	12.2	***	0.8	7.0	***

Table 5.2. Extraction of ammonium-N with 0.5 M potassium sulphate solution from air dried and fresh soils after 1 and 24 hours shaking at room temperature.

Each value is the mean of 5 replicates.

Differences between 1 and 24 hours for a particular soil are significant at the following levels. * $p<0.05$, ** $p<0.01$ and *** $p<0.001$

Soil	Air dried 1 hr	Air dried 24 hrs		Fresh 1 hr	Fresh 24 hrs	
Darvel	4.6	4.7		6.6	7.0	*
Rivington	39.6	38.7		41.7	40.9	**
Midelney 1	42.9	45.9	*	49.7	64.3	***
Downholland	41.5	34.9	***	51.0	41.9	***
Midelney 2	24.3	22.2	***	31.5	31.0	

Table 5.3. Extraction of nitrate-N with 0.5 M potassium sulphate solution from air dried and fresh soils after 1 and 24 hours shaking at room temperature.

Each value is the mean of 5 replicates.

Differences between 1 and 24 hours for a particular soil are significant at the following levels. * $p<0.05$, ** $p<0.01$ and *** $p<0.001$

Soil	Air dried 1 hr	Air dried 24 hrs		Fresh 1 hr	Fresh 24 hrs	
Darvel	N.D	N.D		N.D	N.D	
Rivington	N.D	N.D		N.D	N.D	
Midelney 1	0.7	3.4	***	0.5	5.2	***
Downholland	0.1	0.3	***	0.0	0.3	***
Midelney 2	0.5	1.2	***	0.2	1.0	***

Table 5.4. Extraction of nitrite-N with 0.5 M potassium sulphate solution from air dried and fresh soils after 1 and 24 hours shaking at room temperature.

Each value is the mean of 5 replicates.

Differences between 1 and 24 hours for a particular soil are significant at the following levels. * $p<0.05$, ** $p<0.01$ and *** $p<0.001$

N.D means not detectable

5.3.4. Effect of different temperatures on the mineral nitrogen extracted from soils by shaking for 1 and 24 hours.

The effect of different temperatures on the ammonium and nitrate nitrogen extracted from the soils after 1 and 24 hours shaking is shown in tables 5.5, 5.6 and 5.7. The data reveal that for all soils a significant increase in the ammonium nitrogen content occurred at all temperatures. There were small increases in the ammonium nitrogen content of soils between 0 and 5 ^{o}C. The increases were found to be appreciable when the temperature was raised to 10 ^{o}C and increased further at 20-30 ^{o}C.

The nitrate nitrogen extracted from Rivington soil

was not significantly affected by different shaking periods over the ranges of temperatures (table 5.5) but in the cases of Midelney 1 (Grass) and Downholland soils, there was a significant decrease in the levels of nitrate nitrogen at all temperatures when period of shaking was increased from 1 to 24 hours. The decrease was small at temperatures between 0 and 5 ^{o}C and increased when the temperature was increased from 10 to 30 ^{o}C. There were no traces of nitrite nitrogen in all the soils at different temperatures.

Temperature ^{o}C	Ammonium-N (mg/kg soil)			Nitrate-N (mg/kg soil)		
	1 hr	24 hrs		1 hr	24 hrs	
0	12.7	14.5	***	35.3	30.2	
2	6.0	8.2	***	35.0	34.9	
5	8.7	10.1	***	35.0	35.1	
10	9.0	12.8	***	34.6	34.7	
20	13.8	20.2	***	43.5	44.7	*
30	17.2	25.1	***	41.6	42.9	

Table 5.5. Extraction of mineral nitrogen from Rivington Soil Series with 0.5 potassium sulphate solution after 1 and 24 hours shaking at different temperatures.

Each value is the mean of 5 replicates.

Differences between 1 and 24 hours for a particular temperature are significant at the following levels. * $p<0.05$, ** $p<0.01$ and *** $p<0.001$.

Temperature °C	Ammonium-N (mg/kg soil) 1 hr	24 hrs		Nitrate-N (mg/kg soil) 1 hr	24 hrs	
0	2.5	5.1	***	65.6	60.2	***
2	0.7	4.3	***	55.1	50.4	***
5	1.8	4.1	***	61.9	57.7	***
10	1.4	5.6	***	62.9	58.3	***
20	2.6	10.7	***	73.0	68.5	***
30	5.0	22.4	***	69.0	62.0	***

Table 5.6. Extraction of mineral nitrogen from Midelney 1 (Grass) soil with 0.5 M potassium sulphate solution after 1 and 24 hours shaking at different temperatures.

Each value is the mean of 5 replicates.

Differences between 1 and 24 hours for a particular temperature are significant at the following levels. * $p<0.05$, ** $p<0.01$ and *** $p<0.001$.

Temperature °C	Ammonium-N (mg/kg soil) 1 hr	24 hrs		Nitrate-N (mg/kg soil) 1 hr	24 hrs	
0	5.6	7.3	***	59.0	53.1	*
2	1.0	5.4	***	50.7	44.1	***
5	3.6	5.7	***	54.2	50.5	***
10	3.0	6.4	***	57.8	51.3	***
20	4.5	12.6	***	68.1	58.5	***
30	5.3	23.7	***	66.0	51.5	***

Table 5.7. Extraction of mineral nitrogen from Downholland soil with 0.5 M potassium sulphate solution after 1 and 24 hours shaking at different temperatures.

Each value is the mean of 5 replicates.

Differences between 1 and 24 hours for a particular temperature are significant at the following levels. * $p<0.05$, ** $p<0.01$ and *** $p<0.001$.

5.3.5. Study of method of extraction for soil inorganic nitrogen analysis.

The preliminary experiments (tables 5.5, 5.6 and 5.7) showed that shaking of soil at room temperature is not a successful method for estimating mineral nitrogen content of all kinds of soils and particularly those containing very low initial ammonium nitrogen levels.

The changes in the mineral nitrogen content of the extracts after 1 and 24 hours shaking were smaller at temperatures of 0-5 ^{o}C compared to 10, 20, and 30 ^{o}C. A study of the effect of 2 ^{o}C was, therefore, undertaken. Seven different soils in fresh state were compared in this experiment. The soils were shaken for 0.5, 1, 1.5, 2, 3, 4, 6 and 8 hours. Data for the ammonium nitrogen content of all the soils are given in Figures 5.2, 5.3, 5.4, 5.5, 5.6, 5.7 and 5.8. The data show that there were significant differences ($p < 0.001$) in the ammonium levels of all soils over shaking periods up to 8 hours. There was an upward trend in the ammonium nitrogen levels, however, the changes were not significant (Scheffe´s Least Significant Difference $p < 0.05$) at shaking periods between 1 and 3 hours. Increased ammonium content could occur when the period of shaking is extended to 4-8 hours.

Data for nitrate nitrogen are given in figures 5.9, 5.10, 5.11, 5.12, 5.13 and 5.14 and 5.15. It shows that significant changes in the nitrate nitrogen content of soils also occurred ($p < 0.001$) in all soils over the

different shaking periods. In most of the soils the changes between 1 and 3 hours shaking periods were not significant (Scheffe´s LSD $p < 0.05$). In Darleith (Drumboy) soil and some other soils there were irregularities in the levels of nitrate nitrogen, not a part of the general trend which seems to be due to a systematic error in the analysis. There was a measurable amount of nitrite nitrogen in some of the soils (figures 5.16 and 5.17). The changes in the levels of nitrite nitrogen were not significant at shaking periods of 2-3 hours in the Midelney 2 (Arable) soil but were significant in case of Midelney 1 (Grass) soil over all shaking periods from 0.5 to 8 hours (Scheffe´s LSD $p <$ 0.05).

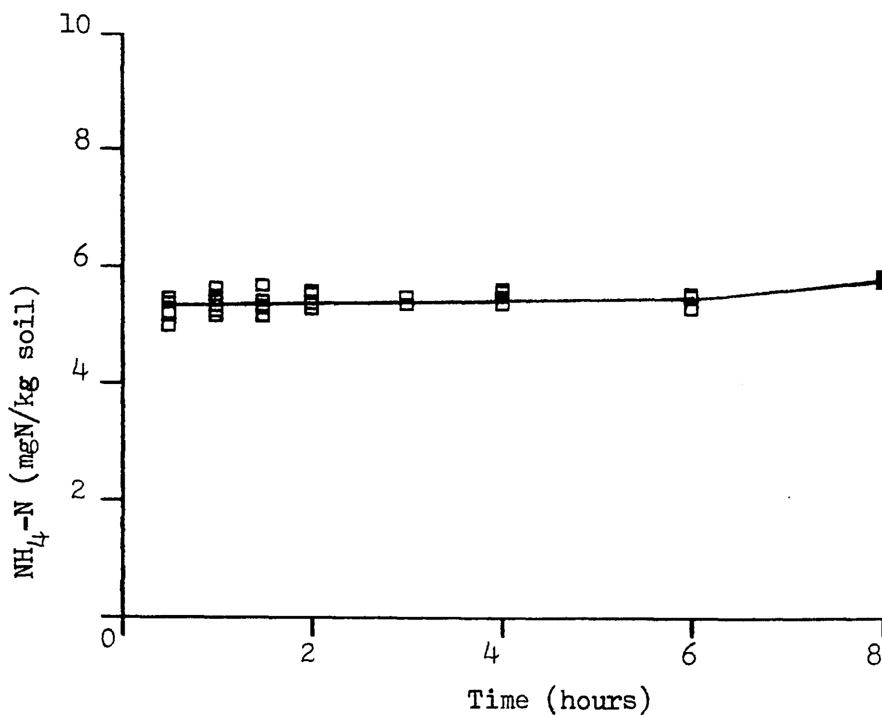


Fig. 5.2 Effect of period of shaking on the extraction of NH_4-N from Dreghorn 1 (Ayr) soil at 2°C

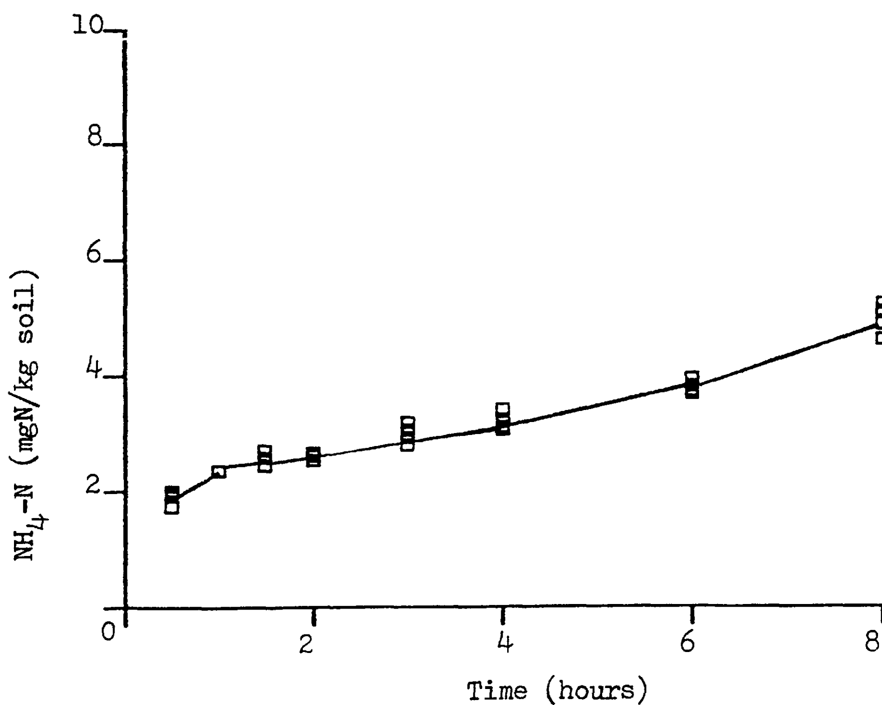


Fig. 5.3 Effect of period of shaking on the extraction of NH_4-N from Dunlop soil at 2°C

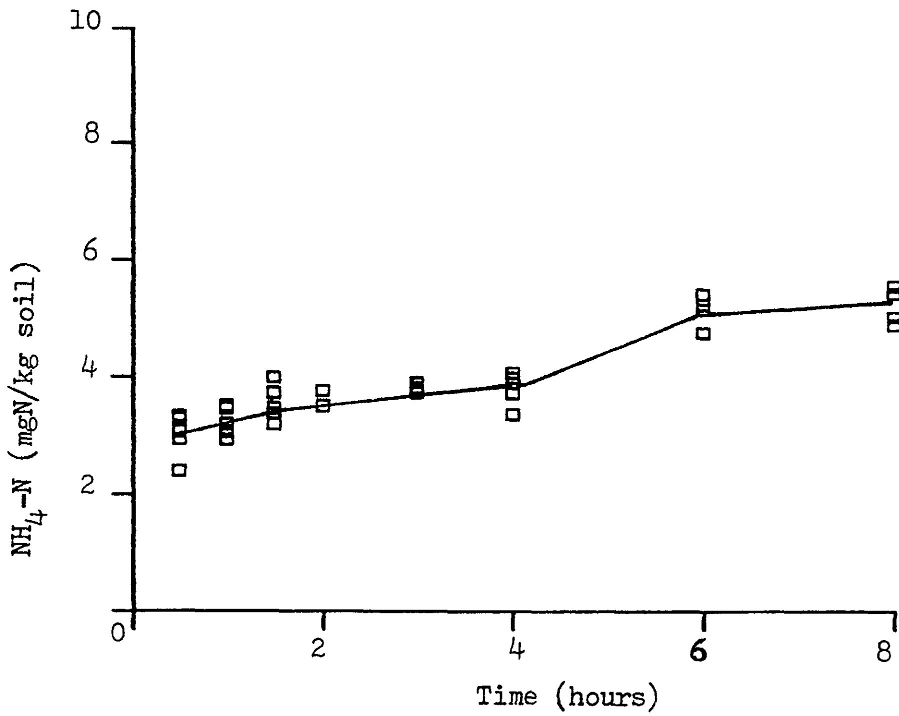


Fig. 5.4 Effect of period of shaking on the extraction of NH_4-N from Darleith 1 (Drumboy) soil at 2°C

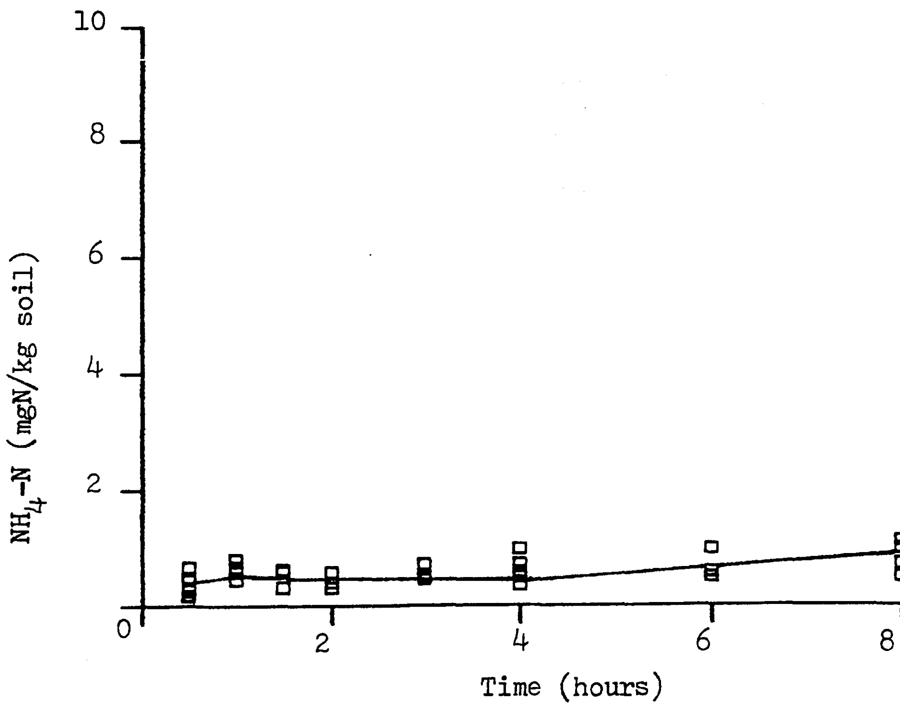


Fig. 5.5 Effect of period of shaking on the extraction of NH_4-N from Midelney 1 (Grass) soil at 2°C

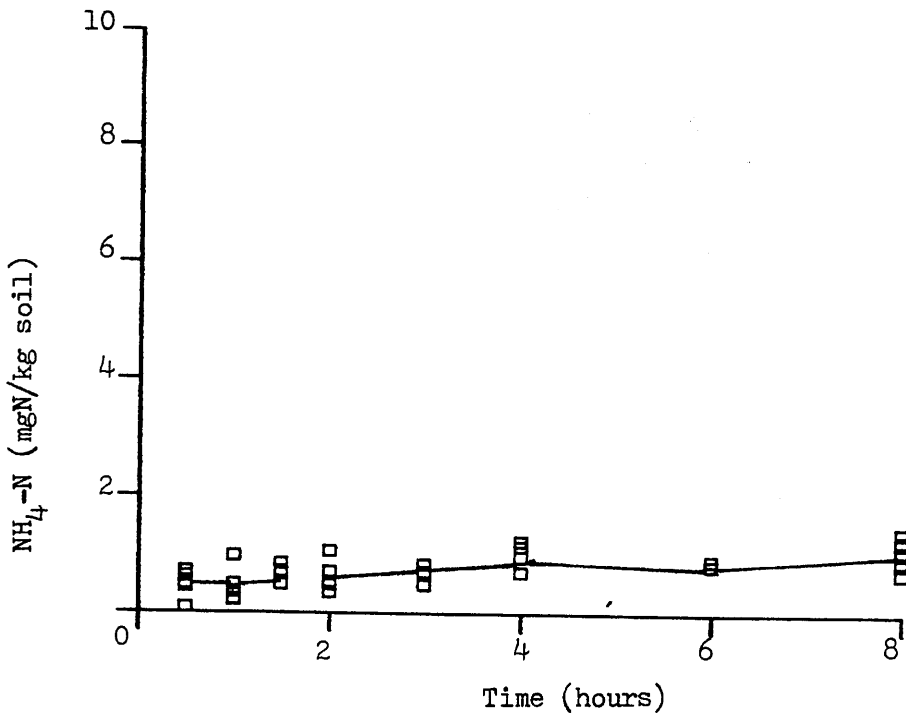


Fig. 5.6 Effect of period of shaking on the extraction of NH_4-N from Downholland soil at 2°C

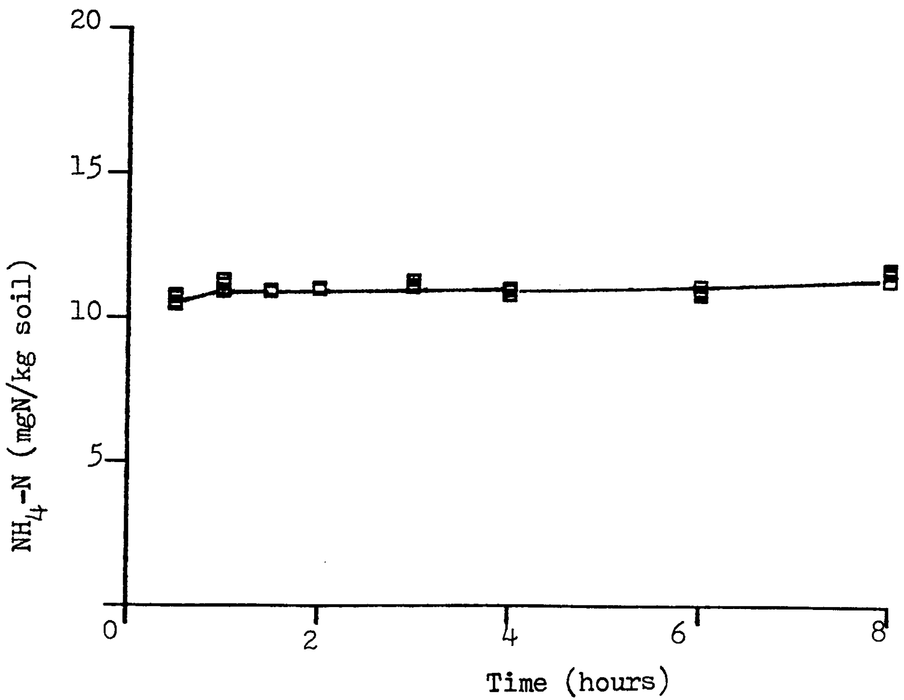


Fig. 5.7 Effect of period of shaking on the extraction of NH_4-N from Bargour soil at 2°C

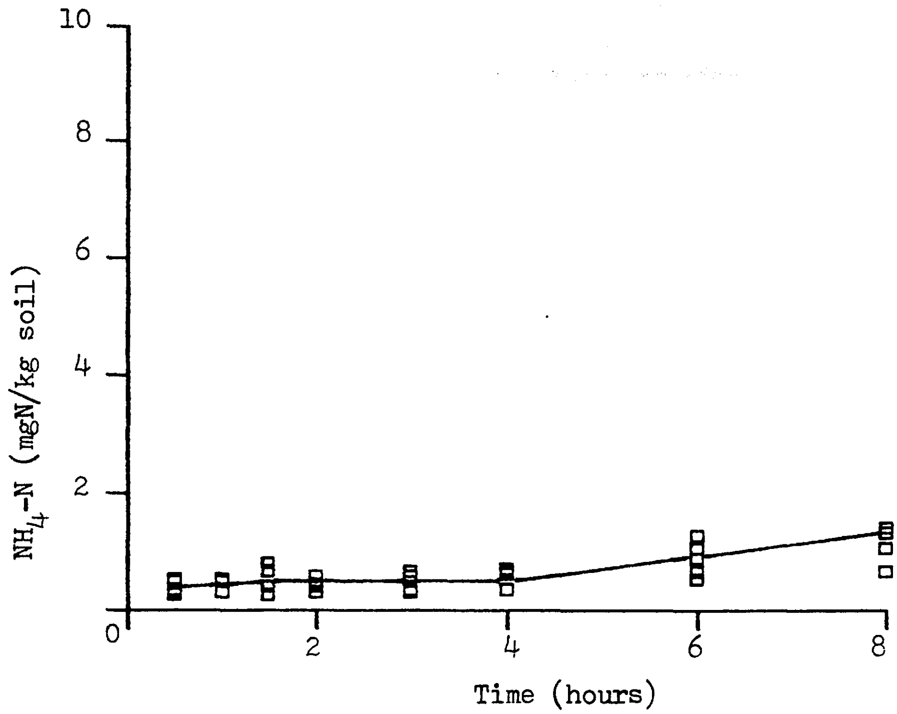


Fig. 5.8 Effect of period of shaking on the extraction of NH_4-N from Midelney 2 (Arable) soil at 2°C

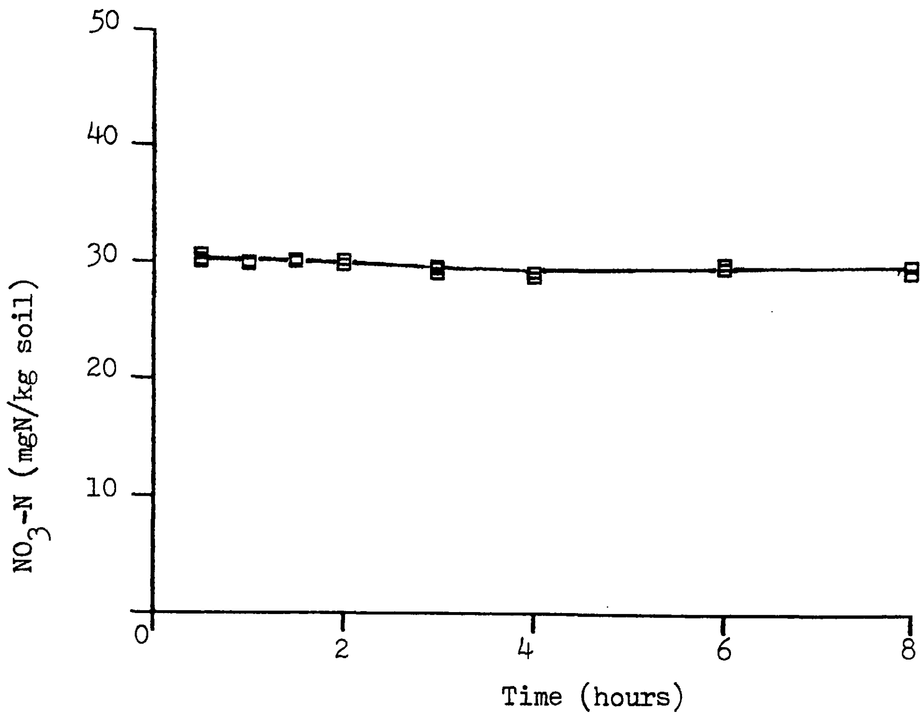


Fig. 5.9 Effect of period of shaking on the extraction of NO_3-N from Dreghorn 1 (Ayr) soil at 2°C

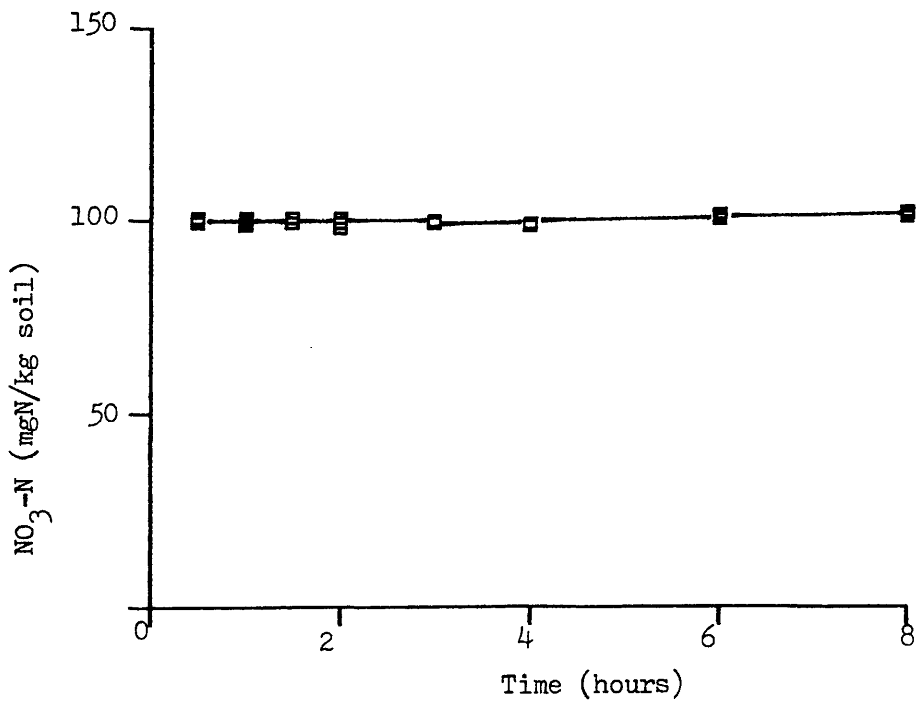


Fig. 5.10 Effect of period of shaking on the extraction of NO_3-N from Dunlop soil at 2°C

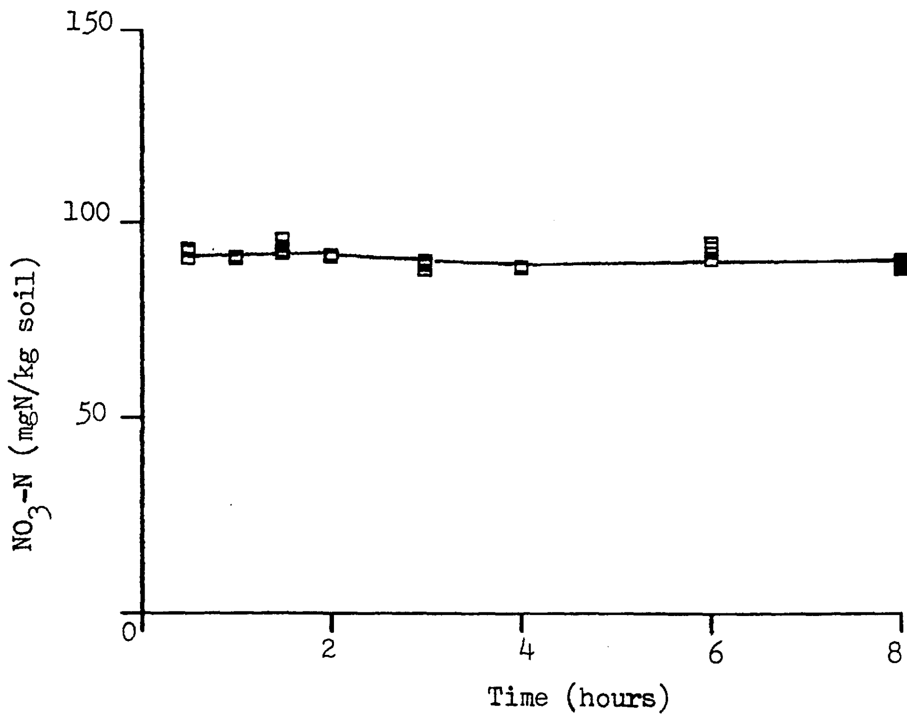


Fig. 5.11 Effect of period of shaking on the extraction of NO_3-N from Darleith 1 (Drumboy) soil at 2°C

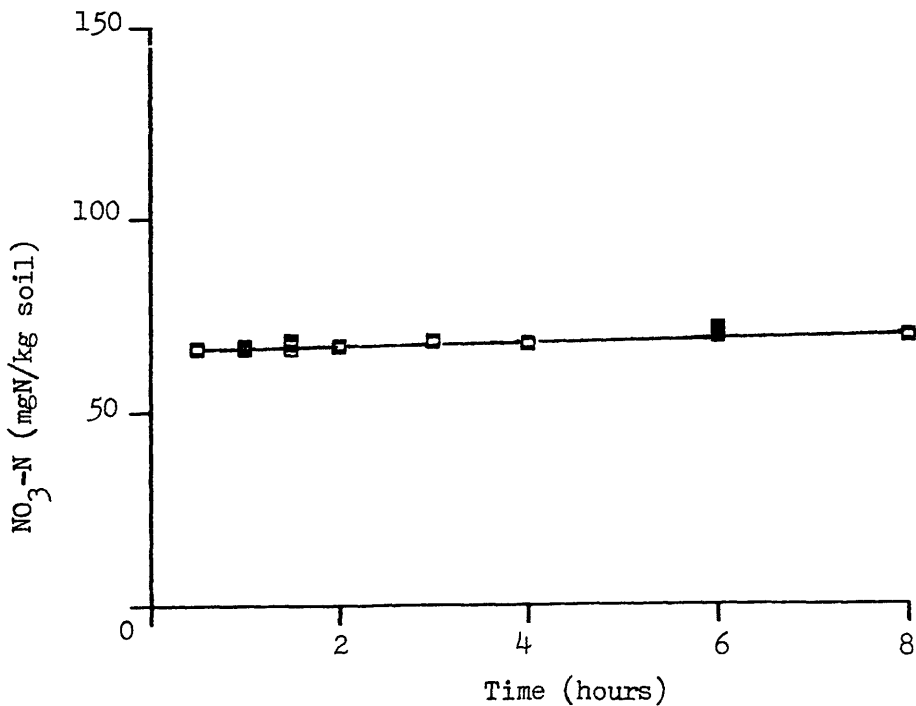


Fig. 5.12 Effect of period of shaking on the extraction of NO_3-N from Midelney 1 (Grass) soil at 2°C

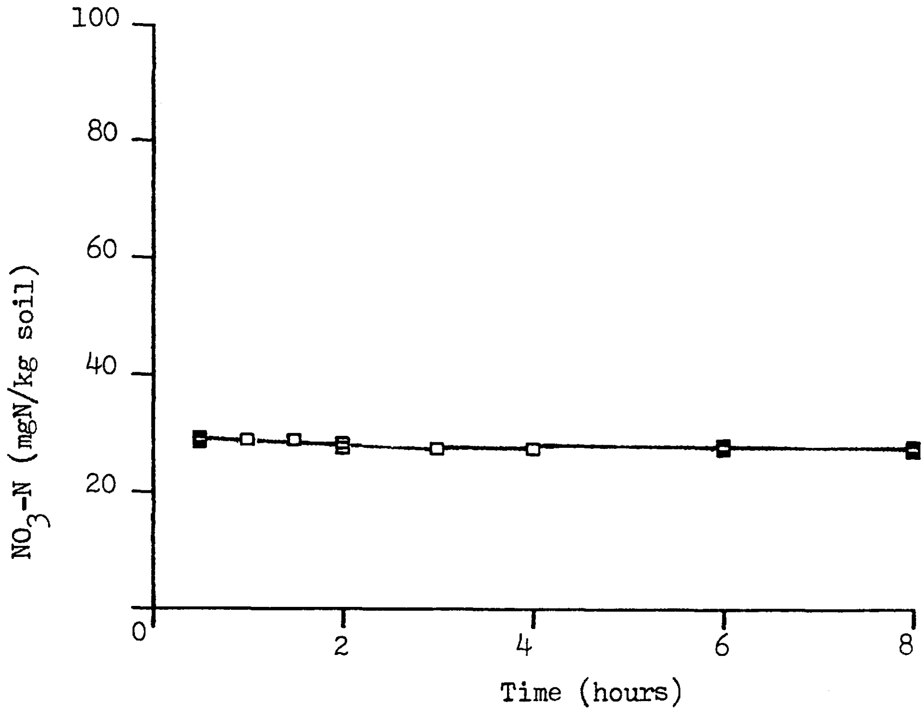


Fig. 5.13 Effect of period of shaking on the extraction of NO_3-N from Downholland soil at 2°C

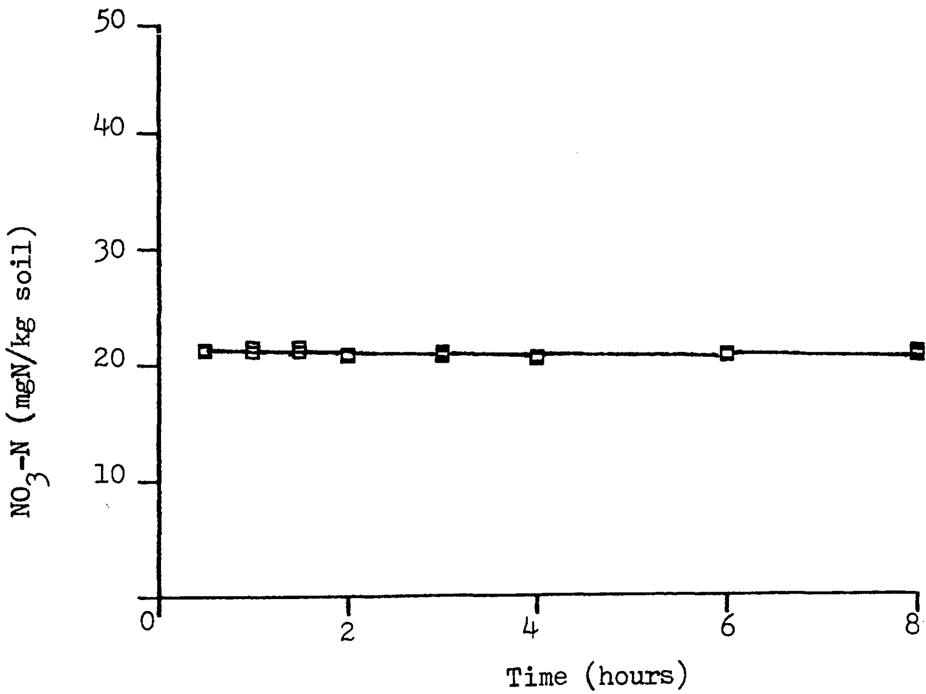


Fig. 5.14 Effect of period of shaking on the extraction of NO_3-N from Bargour soil at 2°C

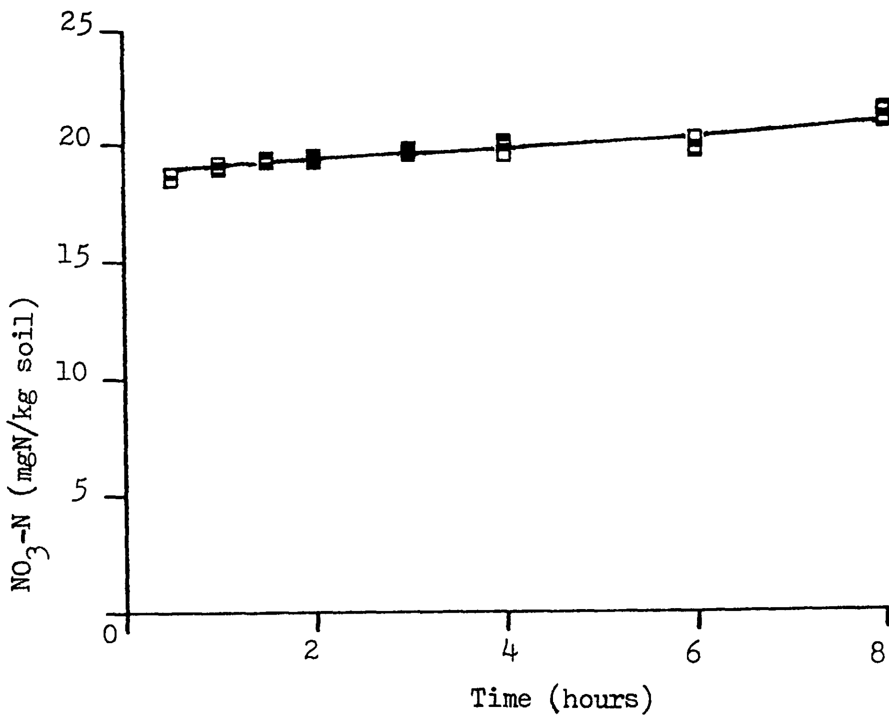


Fig. 5.15 Effect of period of shaking on the extraction of NO_3-N from Midelney 2 (Arable) soil at 2°C

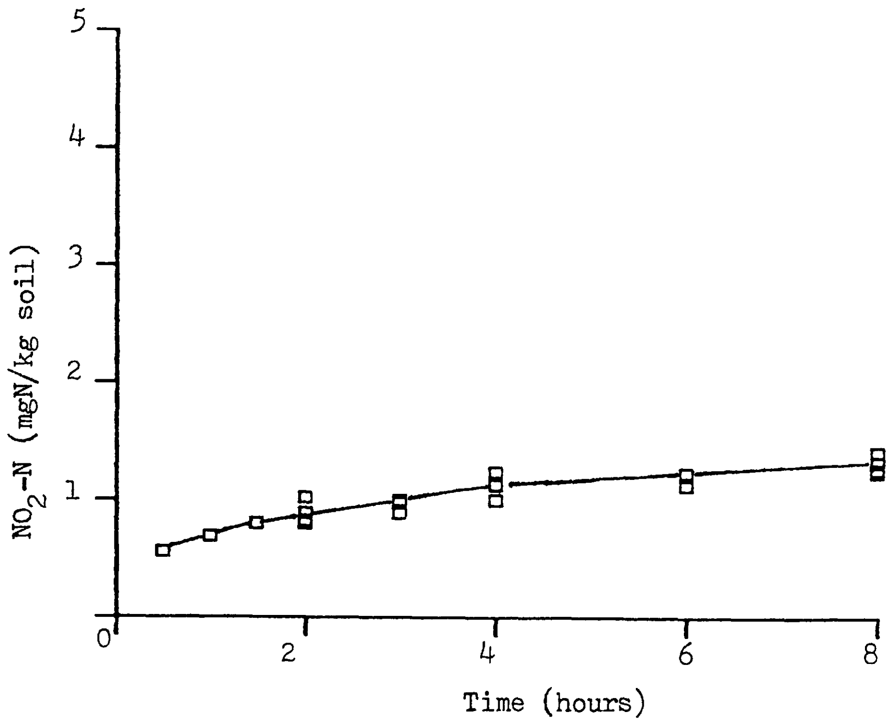


Fig. 5.16 Effect of period of shaking on the extraction of NO_2-N from Midelney 1 (Grass) soil at 2°C

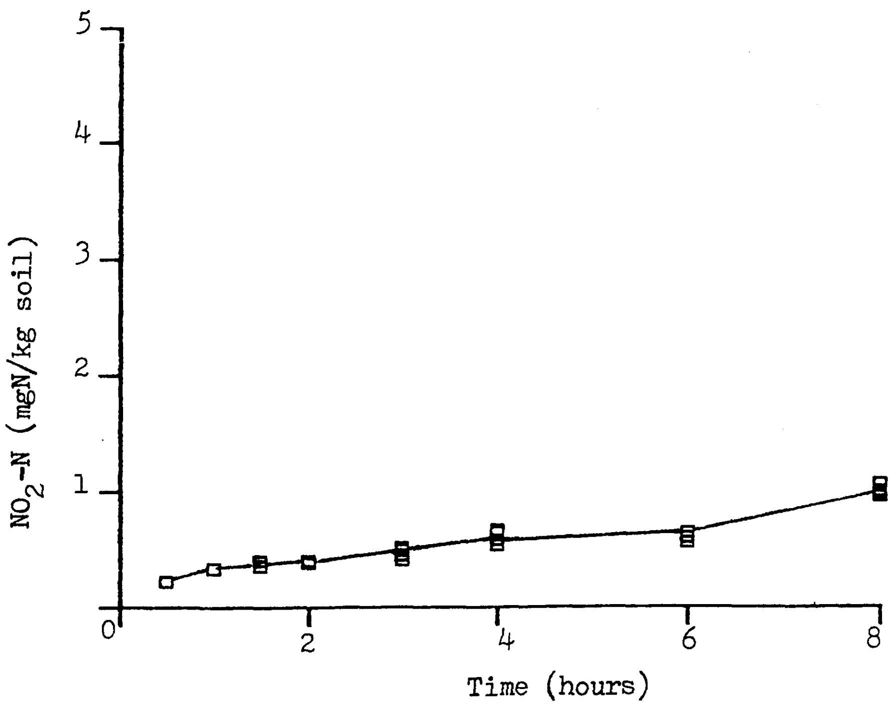


Fig. 5.17 Effect of period of shaking on the extraction of NO_2-N from Midelney 2 (Arable) soil at 2°C

5.3.6. Mineral nitrogen preservation in the soil extract.

Data presented in figures 5.18, 5.19 and 5.20 show the mineral nitrogen content of 3 different soil extracts (Rivington, Midelney 1 (Grass) and Downholland soils) in 0.5 M potassium sulphate solution over a storage period of up to 3 weeks at room temperature. There was a significant difference ($p < 0.001$) in the ammonium nitrogen content of all the soil extracts over the 3 weeks storage period. The levels increased after 1 and 2 weeks storage and then suddenly declined after 3 weeks storage period. There was a significant difference in ammonium nitrogen level of 5 of the 6 treatments between 0 and 7 days storage (Scheffe´s LSD $p < 0.05$). The nitrate nitrogen content of all the soil extracts also showed significant differences ($p < 0.001$) over the 3 weeks storage period (figure. 5.18). There was a gradual increase in the nitrate nitrogen level over the storage period. There was a significant difference in nitrate nitrogen level of all of the 6 treatments between 0 and 7 days storage (Scheffe´s LSD $p < 0.05$).

Figures 5.21, 5.22 and 5.23 show the changes in the mineral nitrogen content of the extracts during cold storage in the refrigerator at 2 ^{o}C over a period of 71 days. It shows that there were no significant changes ($p < 0.001$) in the ammonium nitrogen content of the extracts obtained from Rivington soil during the storage period of 71 days. There were, however, significant changes in the ammonium nitrogen contents of the extracts taken from

Midelney 1 (Grass) and Downholland soils ($p < 0.01$). The levels of nitrate nitrogen in the extracts of all the soils showed significant changes ($p < 0.01$) during storage for 71 days at 2 ^{o}C in the refrigerator. In all the extracts there was no change in both ammonium and nitrate nitrogen between 0 and at least 9 days storage. There is evidence that some of the differences may have been due to systematic errors in the analysis rather than changes during the storage as there are some points which do not follow the general trend of changes.

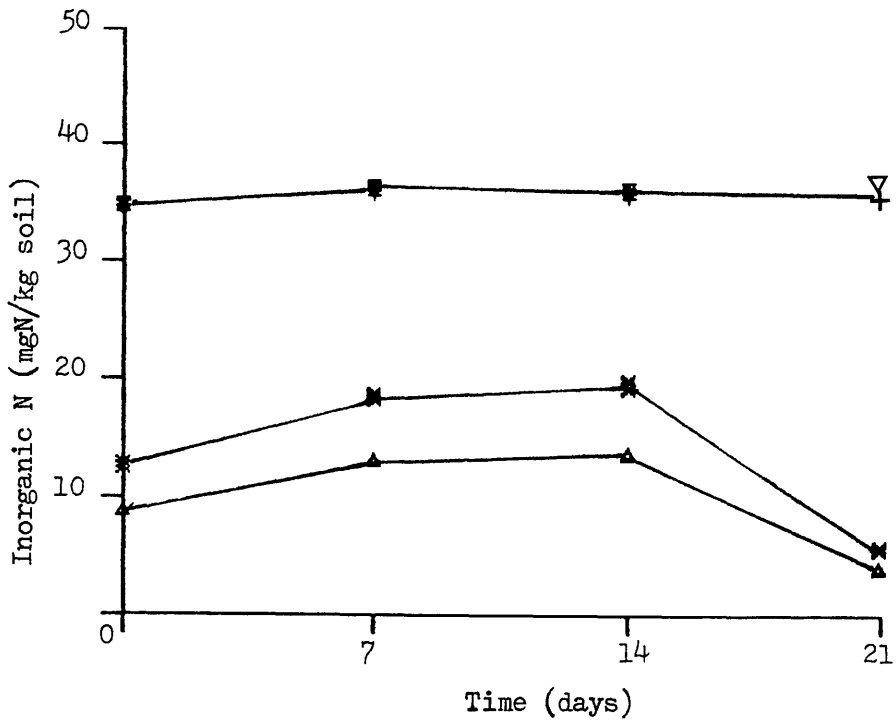


Fig. 5.18 Effect of storage at room temperature on the inorganic N content of Rivington soil extracted after 1 and 24 hours shaking
NH_4-N, 1h shaking (△), NH_4-N, 24h shaking (×)
NO_3-N, 1h shaking (▽), NO_3-N, 24h shaking (+)

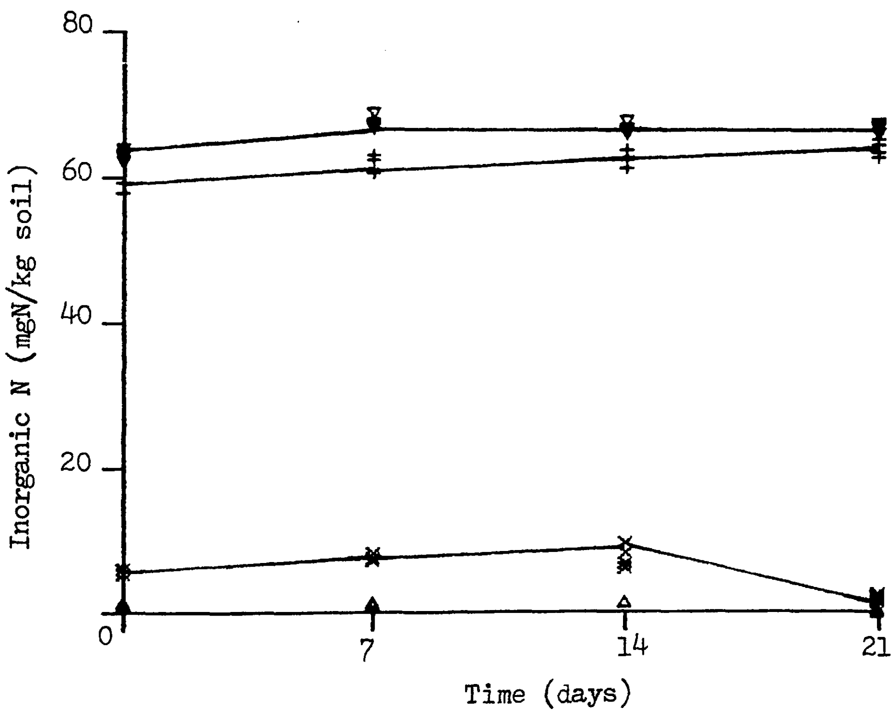


Fig. 5.19 Effect of storage at room temperature on the inorganic N content of Midelney 2 (Arable) soil extracted after 1 and 24 hours shaking
NH_4-N, 1h shaking (△), NH_4-N, 24h shaking (×)
NO_3-N, 1h shaking (▽), NO_3-N, 24h shaking (+)

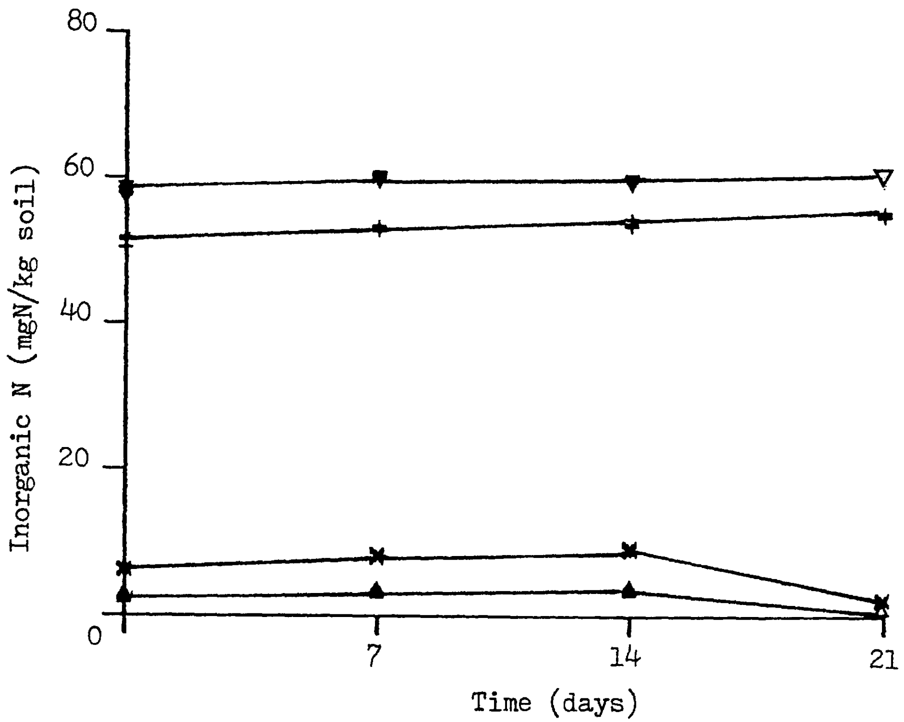


Fig. 5.20 Effect of storage at room temperature on the inorganic N content of Downholland soil extracted after 1 and 24 hours shaking
NH_4-N, 1h shaking (△), NH_4-N, 24h shaking (×)
NO_3-N, 1h shaking (▽), NO_3-N, 24h shaking (+)

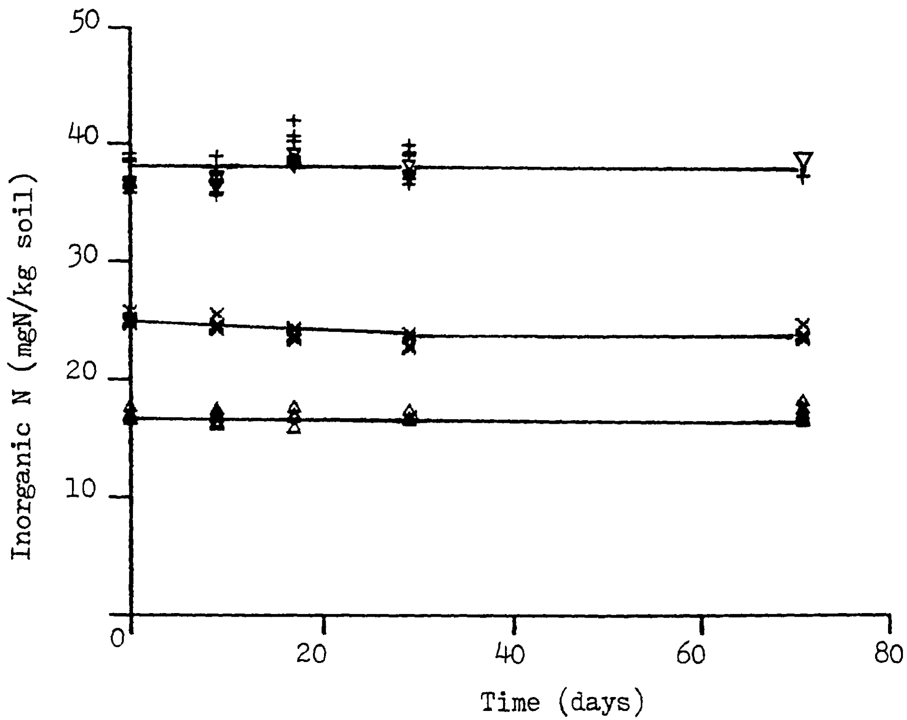


Fig. 5.21 Effect of storage at 2°C on the inorganic N content of Rivington soil extracted after 1 and 24 hours shaking
NH_4-N, 1h shaking (△), NH_4-N, 24h shaking (×)
NO_3-N, 1h shaking (▽), NO_3-N, 24h shaking (+)

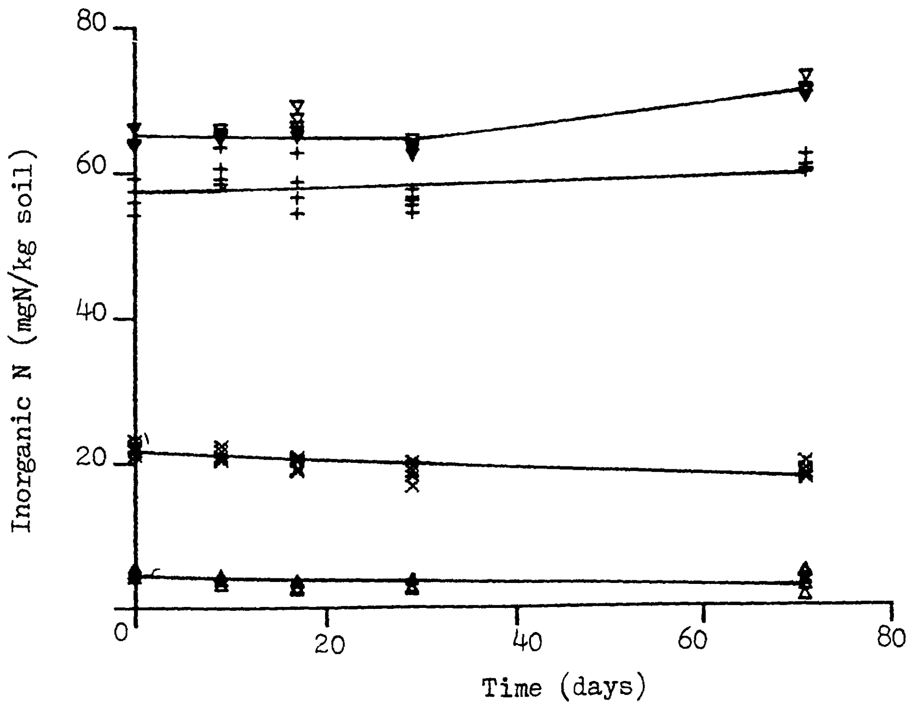


Fig. 5.22 Effect of storage at 2°C on the inorganic N content of Midelney 2 (Arable) soil extracted after 1 and 24 hours shaking
NH_4-N, 1h shaking (△), NH_4-N, 24h shaking (×)
NO_3-N, 1h shaking (▽), NO_3-N, 24h shaking (+)

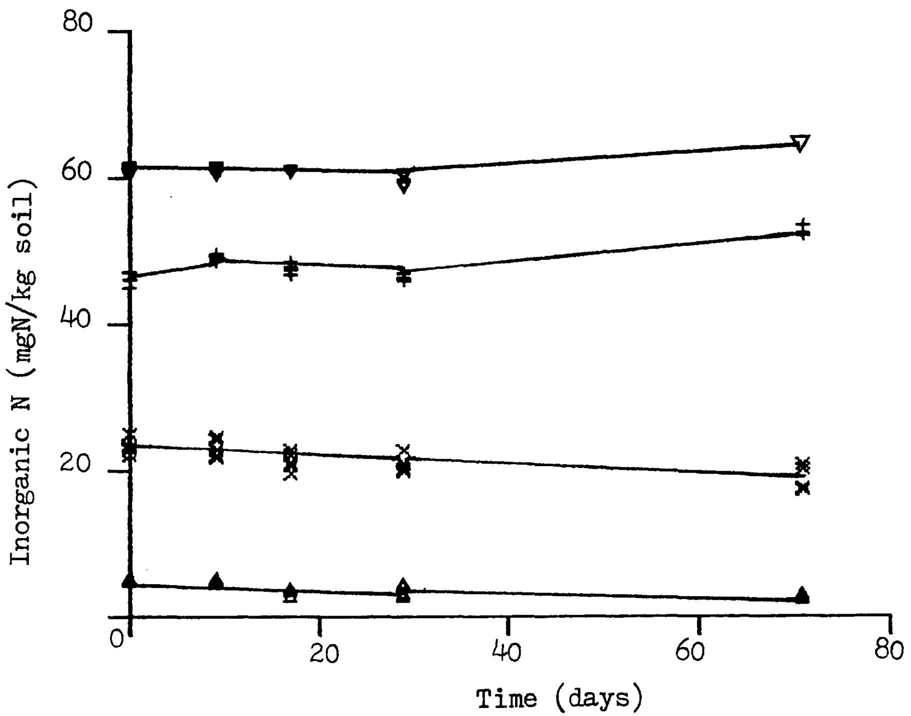


Fig. 5.23 Effect of storage at 2°C on the inorganic N content of Downholland soil extracted after 1 and 24 hours shaking
NH_4-N, 1h shaking (△), NH_4-N, 24h shaking (×)
NO_3-N, 1h shaking (▽), NO_3-N, 24h shaking (+)

5.4. DISCUSSION

Tests were carried out to check that the proposed modification to the method of extraction of Bremner and Keeney (1966) is suitable for the extraction of low levels of inorganic nitrogen from soils which were used in this study. The soil to potassium ratio was maintained at 20 meq of K/g of soil. The use of 0.5 M potassium sulphate instead of 2 M potassium chloride was proposed in order to avoid chloride ion interference with the colorimetric determinations of nitrate nitrogen. 0.5 M Potassium sulphate solution has previously been used successfully by Flowers and Arnold (1983) for the extraction of inorganic nitrogen while studying mineralization and immobilization in soils.

In the initial test an air dried sample from Rivington Soil Series was shaken with two different shaking machines for different periods of time at room temperature. There was no major change in the nitrate nitrogen levels but a drastic change in the ammonium nitrogen levels of soil occurred after different shaking periods and with different shaking machines. The exact cause of the increase in ammonium nitrogen content was not known, it was suspected that it might have occurred due to the microbial breakdown of organic nitrogen in the soil.

A more comprehensive study was similarly performed by taking samples from five different Soil Series (Rivington, Darvel, Midelney 1 (Grass), Downholland and

Midelney 2 (Arable)) in both air dried and fresh conditions. In all of the soils the results still followed the same pattern in the case of ammonium nitrogen. In most of the soils some significant changes in the nitrate nitrogen occurred. Its levels increased in most of the soils with increase in the period of shaking, with exception of Downholland soil where the levels of nitrate nitrogen decreased. The nitrite nitrogen also increased significantly in Midelney 1 (Grass), Downholland and Midelney 2 (Arable) soils when the shaking period was increased from 1 to 24 hours. These results, therefore, confirmed that variability in the inorganic nitrogen levels after different shaking periods may be attributed to biological changes including mineralization, immobilization, nitrification and denitrification. Extraction of soil at room temperature although simple and quick would seem to suffer from variable biological interference effects and hence doubtful accuracy would be obtained if the biological interference was not removed. Bremner and Keeney (1966) carrying out extraction with 2 M potassium chloride solution at room temperature did not find any differences in the amount of ammonium nitrogen extracted by shaking for periods between 0.5 and 8 hours. In the present study it was found that 1 M potassium chloride showed a smaller increase in ammonium nitrogen extracted after 24 hours shaking compared with 1 hour shaking than did 0.5 M potassium sulphate. It, therefore, seems reasonable to assume that biological processes may be reduced by the use of high concentration of the

extracting solution. Selmer-Olson et al. (1974) studied the effect of different concentrations of potassium chloride solution on the ammonium nitrogen levels of soils. They incubated the soils for 14 days in 0, 0.25, 0.5, 1.0, 2.0 and 2.5 M potassium chloride and found that smaller concentrations of potassium chloride (0.25 M) had a stimulating effect on the formation of ammonium nitrogen whereas higher concentrations (2 or 2.5 M) were, in general, found to be inhibitory. In the present study 0.5 M potassium sulphate was not strong enough to arrest biological transformations and because of its solubility, it was not practically feasible to use a higher concentration of potassium sulphate salt.

There were three options to check biological activities during the extraction process. The first option was the use of biocides which would completely inhibit biological activities in the soil. But according to the findings of Bremner (1965b), Storrier (1966), Robinson (1973) and Keeney and Nelson (1982) the use of biocides generally interferes with the commonly used methods of determining inorganic nitrogen in soils. The second option was to use an acidified extractant which has been tried in the past by Olsen (1929). He used potassium chloride hydrochloric acid mixture to give the soil suspension a pH value of 1.0. Potassium sulphate sulphuric acid mixture has also been used by many workers to prepare extracts for determination of both ammonium and nitrate in soils. However, the use of acidified mixtures has been criticised by Bremner (1965b) for the reasons

that it cannot be used for determination of nitrite or for determination of nitrate in soils containing significant amounts of nitrite and that storage of acidic extracts involves the risk of ammonium formation through hydrolysis of organic nitrogen. The last option was to perform the whole process of extraction at a low temperature which appears to be the most promising method of minimising microbial activities during the extraction procedure.

An experiment was designed to see whether low temperature would be effective in minimising biological activities during the extraction process. Fresh samples from Midelney 1 (Grass), Downholland and Rivington Soil Series were shaken for 1 and 24 hours at ranges of temperatures 0, 2, 5, 10, 20 and 30 ^{o}C. Release or loss of ammonium and nitrate nitrogen due to biological processes (mineralization, nitrification, denitrification or immobilization) seemed to occur at a higher rate in most of the soils at the temperatures 10 to 30 ^{o}C. There appeared to be biological immobilization or denitrification in some of the soils (Midelney 1 (Grass) and Downholland) because of the losses of nitrate nitrogen after 24 hours shaking compared with one hour. At temperatures 0 to 5 ^{o}C, microbial activities were reasonably slow because of the small variability in the inorganic nitrogen levels of soils between 1 and 24 hours shaking at these temperatures.

Although in this Department it was not possible to carry out the whole process of extraction at sub zero temperature, there was a facility of using a 2 ^{o}C room and

therefore, to confirm the conclusion that biological activities are minimised to a sufficient degree at low temperature (2 ^{o}C), 8 different soils were studied that exhibited low initial inorganic nitrogen levels. Fresh soil samples were shaken in a 2 ^{o}C room for periods of 0.5, 1, 1.5, 2, 3, 4, 6 and 8 hours. Results of the ammonium and nitrate nitrogen of the extracts were stable between 1 to 3 hours shaking periods except for Darleith 1 (Drumboy) soil where there was a variable amount of ammonium nitrogen over all periods of shaking but that seemed to be a systematic error in the analysis. There were variable amounts of nitrite nitrogen in Midelney 1 (Grass) and Midelney 2 (Arable) soils after different shaking periods. The variability was small between 1 and 3 hours in the case of Midelney 2 (Arable) soil but was significant in Midelney 1 (Grass) soil.

The present study recommends that shaking of soil in 0.5 M potassium sulphate solution at a soil solution ratio 2.5 : 50 cm^3 at 2 ^{o}C temperature for a period of 2 hours is the most suitable method of extraction for soil inorganic nitrogen analysis which is applicable to a broad range of soils. Soils which contain low levels of extractable inorganic nitrogen are not uncommon in natural soils and therefore, this method seems to be suitable for determination of such low levels of inorganic nitrogen. The study has shown that the ammonium, nitrate or nitrite nitrogen levels of the soil may not always be stable unless extraction is done at 2 ^{o}C. The method seems to be adaptable for routine use in soil

analysis laboratories where it is necessary to avoid chloride ion interference in the analysis.

An experiment was also conducted to check the stability of the filtered soil extracts at room temperature and in a 2 ^{o}C refrigerator. It was recommended that if immediate analysis of the filtered soil extract is not possible, it can be stored in a 2 ^{o}C refrigerator safely for approximately one week. Although significant changes do occur even in the refrigerator, the magnitude is relatively small and some soil extracts can be stored safely for several months. Bremner and Keeney (1966) have stated that 2 M potassium chloride salt solution extracts when filtered may be stored safely at sub zero temperature in the refrigerator for several months. Selmer-Olsen et al. (1971) also observed that the ammonium and nitrate contents of soil extracts remained the same when stored in a 4 ^{o}C refrigerator for 17 days.

5.5. CONCLUSION

The various aspects of the method of inorganic nitrogen extraction from soil are described in chapters 3, 4 and 5. Details of the final recommended method are given below.

5.5.1. Materials

(i) Washing of glassware.

The shaking bottles, bottle tops, filter funnels, beakers, stirring rods, volumetric flasks were first cleaned with hot water and soaked overnight in a 2 % solution of Decon 90 (Decon Laboratories Limited). They were then thoroughly washed with hot water, rinsed twice with deionized water and finally dried in a 70 ^{o}C oven.

(ii) Preparation of extracting solution

Analar grade potassium sulphate reagent was used throughout the experiment. 0.5 M potassium sulphate solution was prepared by dissolving 87.12 g per litre of deionized water. The solution was purified of ammonium nitrogen contamination by first raising its pH to pH 11.0 with 1.0 M potassium hydroxide. It was then boiled and stirred for a period of 15 minutes to give off the ammonia gas. The solution was allowed to cool and the pH was readjusted to pH 6.0 with 0.5 M sulphuric acid.

As there was no simple method of removal of nitrate nitrogen from the extracting solution, the potassium sulphate was first tested and a batch number with a low level of nitrate impurities was selected for use.

(iii) Washing of filter papers

The filter papers were purified of inorganic nitrogen contamination by washing with 50 cm^3 of 0.5 M potassium sulphate (2 successive washings each of 25 cm^3) and then rinsing twice with deionized water to wash away any potassium sulphate salt left in the filter papers. The washed filter papers were then dried in the oven at 70 ^oC for 4 hours.

5.5.2. Procedure.

2.5 g of soil (oven dry basis) was weighed into a wide mouth 100 cm^3 plastic bottle. 50 cm^3 of 0.5 M potassium sulphate solution was added to maintain the soil : solution ratio at 20 meq of K per g of soil as recommended by Bremner and Keeney (1966).

The plastic bottles were stoppered and then shaken on an orbital shaking machine for 2 hours at 2 ^oC. The speed of the orbital shaker was maintained at 200 rpm. The suspensions obtained were then filtered through previously washed Whatman No. 40 filter papers. The first 2.5 cm^3 of the filtrate were ignored as most of the residual ammonium from the filter papers flushes out in the first 2.5 cm^3.

Blank extraction solutions were prepared in exactly the same way except that no soil was added.

If immediate analysis of the filtered soil extract was not possible, it was then stored at 2 ^oC. The extracts were safe for at least one week at 2 ^oC. The extracts were analysed with a Technicon AutoAnalyzer II for their ammonium, nitrate and nitrite nitrogen content.

CHAPTER SIX

AEROBIC INCUBATION

6.1. INTRODUCTION

The need for a satisfactory laboratory method of obtaining an estimate of the amount of nitrogen likely to be made available for crop growth by mineralization of soil organic matter during the growing season has long been evident and numerous chemical and biological methods have been proposed by different workers. However, it is generally accepted that the most satisfactory methods currently available are those involving determination of the inorganic nitrogen produced by incubation of the soil sample under aerobic or anaerobic conditions for various times. These methods have a rational basis because the agents responsible for release of mineral nitrogen during incubation of soils are those which make soil organic nitrogen available for crop growth during the growing season. It is reasonable, therefore, to assume that these incubation techniques will provide a fairly accurate measure of the availability of soil nitrogen to plants. Bremner (1965a) stated that the method of incubation should possess the following qualities:-

(i). It should determine the total mineral nitrogen formed (ammonium, nitrate and nitrite), should be simple, accurate and should not be subject to interference by

organic or inorganic soil constituents.

(ii). It should be applicable to a wide variety of soils and should give reproducible results with both field moist and air dried soils. It should also be simple enough to be suitable for routine soil testing.

All the biological methods suffer from 2 major disadvantages. Firstly a long incubation period is required which is inconvenient for laboratories engaged in predicting fertilizer requirements, particularly if large numbers of samples have to be processed. Secondly the results obtained are very dependent on when the soil is sampled and how it is handled before incubation (Bremner, 1965a).

In the earliest methods of incubations, the soil samples were first mixed with vermiculite or sand and then leached with water before incubation. The excess water was removed by suction and the samples incubated at 25 to 35 ^{o}C for 7 to 14 days. The nitrate nitrogen produced during incubation was determined by leaching the incubated sample with water and analysing the extract colorimetrically. These nitrification methods were open to many criticisms and more recently it has been emphasised that both ammonium and nitrate nitrogen production should be estimated and preferably also nitrite nitrogen (Bremner, 1965a).

Stanford and Smith (1972) used method of aerobic incubation which involve preleaching with dilute salt solution (0.01 M calcium chloride) before the initial and successive incubation periods to remove inorganic

nitrogen from samples (Stanford et al., 1974 and Griffin and Laine, 1983). Although no definite studies are available, it has been shown by Bremner (1965a) that this procedure might be insufficient to remove all the ammonium from exchange sites, particularly in difficultly leached, high exchange capacity soils. The importance of measuring both ammonium and nitrate following incubation is clear from the work by Nommik (1976) and Geist (1977) who found that ammonium was the dominant product of mineralization in forest soils.

Extensive work has been done on the incubation methods under aerobic conditions for the estimation of mineralizable nitrogen. Keeney and Bremner (1967) used a method of incubation which involved the determination of ammonium, nitrate and nitrite nitrogen produced when 10 g of soil was mixed with 30 g of quartz sand, moistened with 6 cm^3 of distilled water and the incubation carried out under aerobic conditions at 30 ^{o}C for 14 days.

Stanford and Smith (1972) used a method of incubation whereby 15 g of soil was mixed with 15 g of quartz sand and the mixture was transferred to 50 cm^3 leaching tubes. The soil was retained in the tubes by means of a glass wool pad. After leaching with 100 cm^3 of 0.01 M calcium chloride and 25 cm^3 of nitrogen free nutrient solution, the samples were then incubated under aerobic conditions at 35 ^{o}C for 30 weeks. Leaching with 0.01 M calcium chloride was done at different intervals and the extracts obtained were analysed for their inorganic nitrogen content.

Tabatabai and Alkhafaji (1980) mixed fresh soil samples with glass beads instead of sand and packed the mixture into glass columns. They were then leached with 100 cm^3 of 0.01 M potassium chloride and incubated at 20 or 30 ^{o}C for 26 weeks. The leaching procedure was repeated every 2 weeks and the leachates were analysed for mineral nitrogen.

Flowers and Arnold (1983) incubated fresh soil samples in polystyrene pots, closed with lids having a 2.5 cm diameter hole to permit aeration. The pots were placed in polyethylene boxes in which a moist atmosphere was maintained by pumping through a stream of ammonia free moist air. The soils were incubated at a range of temperatures for 178 days. Ammonium, nitrate and nitrite nitrogen were extracted by shaking samples for 1 hour with 0.5 M potassium sulphate at a soil solution ratio 1:20.

Farooqi et al. (1983) studied nitrogen mineralization by placing air dried samples in 125 cm^3 Erlenmeyer flasks. The moisture content was adjusted to field capacity by adding distilled water. The samples were thoroughly mixed and the flasks were closed with Parafilm sealing film, which was pierced twice with a pin to allow for gas exchange. Incubation was done at a constant temperature of 35 ^{o}C in a water bath for 5 weeks. Mineral nitrogen was extracted by shaking samples for 30 minutes with 2 M potassium chloride solution.

Addiscott (1983) weighed fresh samples into small unstoppered glass vials which were put in a metal box with a perforated lid. The air above the vials was kept moist

by suspending a moist filter paper between the vials and the lid. The tins were stored in constant temperature rooms for 20 weeks. Mineral nitrogen was determined at different intervals by extracting the soil in the vials for 2 hours with 2 M potassium chloride and analysing on the Technicon AutoAnalyzer.

Magdoff et al. (1983) used samples in the fresh condition which were transported to the laboratory in coolers containing ice. The soil was mixed with perlite at a ratio of 1:1 and placed inside a glass tube with a small cotton wad in the bottom. The initial inorganic nitrogen was leached with 0.01 M calcium chloride. The tubes were then incubated under aerobic conditions at 25 ^{O}C for 17 weeks and were leached with calcium chloride solution at 2 week intervals and the inorganic nitrogen in the leachate determined by steam distillation.

Nordmeyer and Richter (1985) incubated undisturbed soil columns obtained from the upper 30 cm soil layer in PVC tubes at 35 ^{O}C. The PVC tubes were closed air tight at the bottom and were covered on the top with foil to prevent evaporation. After each period of 1, 2, 4 and 8 weeks, 5 columns were analysed for mineral nitrogen (ammonium and nitrate nitrogen).

In the present study the method of aerobic incubation was chosen to use as the measure of soil nitrogen mineralization because of its operational ease and the fact that it can be used for measuring both inorganic nitrogen and carbon dioxide evolved in the soil simultaneously.

6.2. METHODS AND MATERIALS

Nine soil samples were collected from different Soil Series (Midelney 1 (Grass), Alluvium, Darvel, Darleith 2 (Carbeth), Midelney 2 (Arable), Dreghorn 1 (Ayr), Dreghorn 2 (Arkleston), Caprington and Dunlop Series) in a field moist state (fresh condition). They were partially air dried to allow handling and then sieved through a 4 mm sieve. A portion of each sieved sample was air dried at room temperature and then stored in a plastic bag. The fresh soil samples were stored in a refrigerator at 2 ^{o}C in plastic bags (see section 2.11).

6.2.1. Aerobic incubation

50 g (oven dry equivalent) of fresh and air dried samples was weighed into glass beakers. The moisture contents of the samples were adjusted to the water content equivalent to a soil moisture potential of -0.5 bar by adding deionized water. The beakers were then placed in 1.5 litres Kilner jars together with a 2 ounce glass bottle containing 25 cm^3 of 1 M sodium hydroxide. Blank incubations in which the jars contained only sodium hydroxide but no soil were also included. The samples were replicated 4 times. The jars were screwed down and incubated for a period of 12 weeks at 10 ^{o}C. The samples were analysed for inorganic nitrogen content (ammonium, nitrate and nitrite nitrogen) and carbon dioxide evolved after periods of 1, 2, 3, 4, 6, 8, 10 and 12 weeks. Carbon dioxide evolved was determined by titrating the sodium

hydroxide solution with 0.05 M hydrochloric acid. The amount of carbon dioxide evolved was calculated from the volume of the acid required to bring the pH from 8.3 to 3.7 less that required by blanks (see section 2.3.4). The inorganic nitrogen content of the samples was determined in potassium sulphate extracts with the Technicon AutoAnalyzer (see section 2.9).

6.3. RESULTS

6.3.1. Carbon dioxide evolution.

The carbon dioxide evolved in both air dried and fresh soil samples during 12 weeks of aerobic incubation are shown in figs. 6.1 to 6.9. Air dried samples evolved more carbon dioxide than fresh samples with the exception of Caprington soil where the total carbon dioxide evolved was almost the same in both air dried and fresh soil samples after the 12 weeks incubation period. In some of the samples (Darleith 2 (Carbeth) and Dreghorn 1 (Ayr) soils) the increase in carbon dioxide was 2 to 3 times more in air dried than in fresh samples.

There was an initial flush of carbon dioxide evolution in all the fresh soils during the first week of incubation. In some of the soils the rate of carbon dioxide evolution was linear over the period 1 to 12 weeks. In the other soils the carbon dioxide evolution became linear by 4 weeks at the latest.

In the air dried soil samples, there was a large flush of carbon dioxide in the first week and the graphs of carbon dioxide were strongly curved during the whole incubation period. The rate was, however, approximately linear from 6 or 8 weeks until the end of the incubation period.

Carbon dioxide evolution from fresh samples was measured as a linear rate which was expressed in mg/kg/week. Regression lines were, therefore, fitted to the linear regions in order to calculate mineralization

rate constants (table 6.1). The carbon dioxide evolved from air dried soils was tabulated as total carbon mineralized after 12 weeks expressed in mg/kg of soil (table 6.2) as a rate measurement was not applicable.

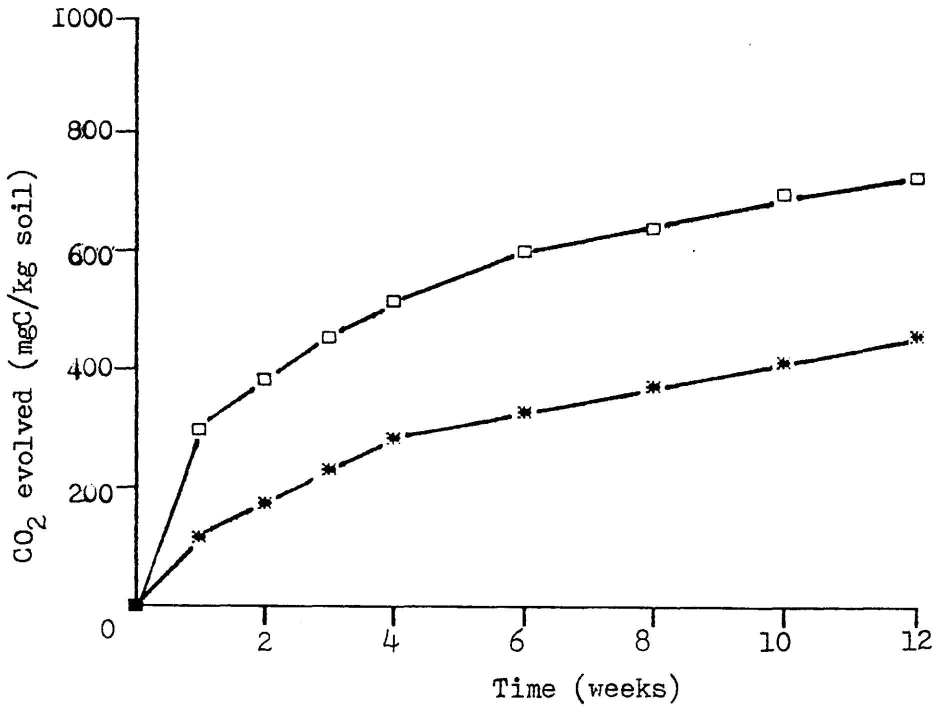


Fig. 6.1 CO_2 evolution by Midelney 1 (Grass) soil
Fresh sample (*), Air dried sample (□)

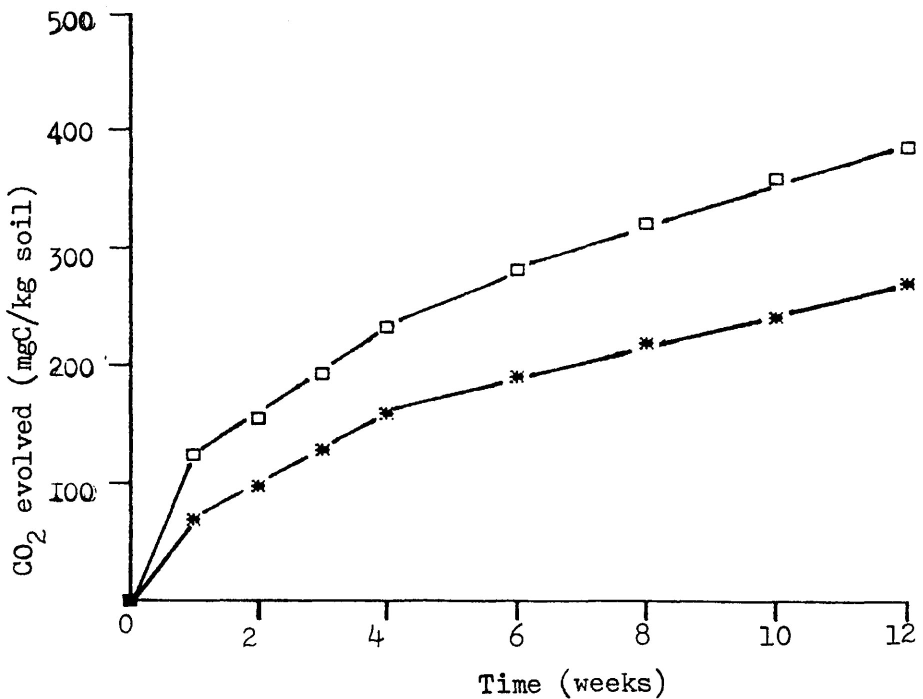


Fig. 6.2 CO_2 evolution by Alluvial soil
Fresh sample (*), Air dried sample (□)

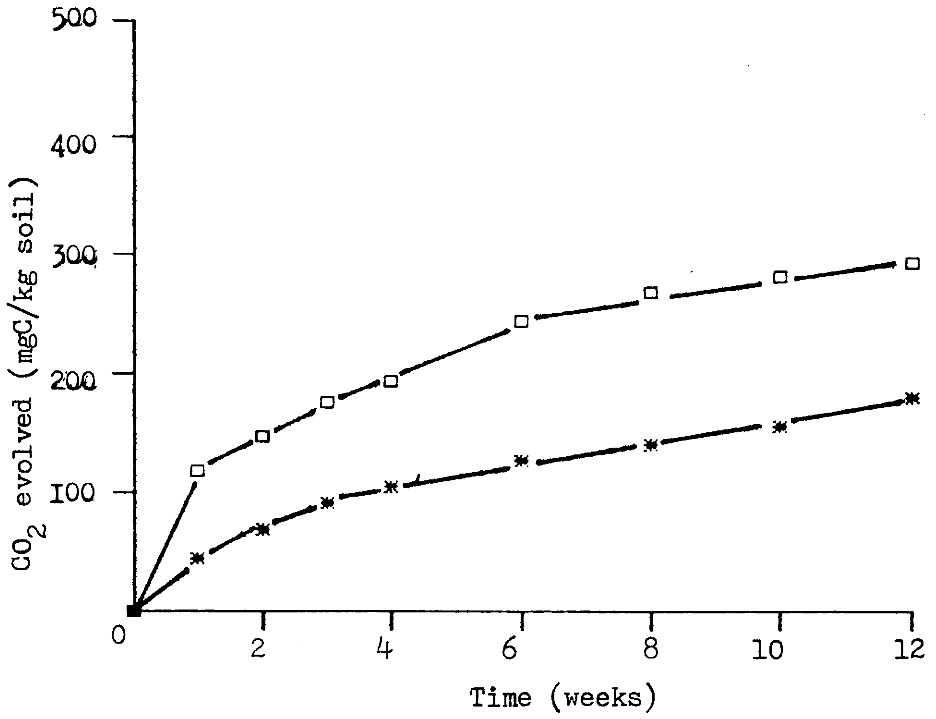


Fig. 6.3 CO_2 evolution by Darvel soil
Fresh sample (*), Air dried sample (□)

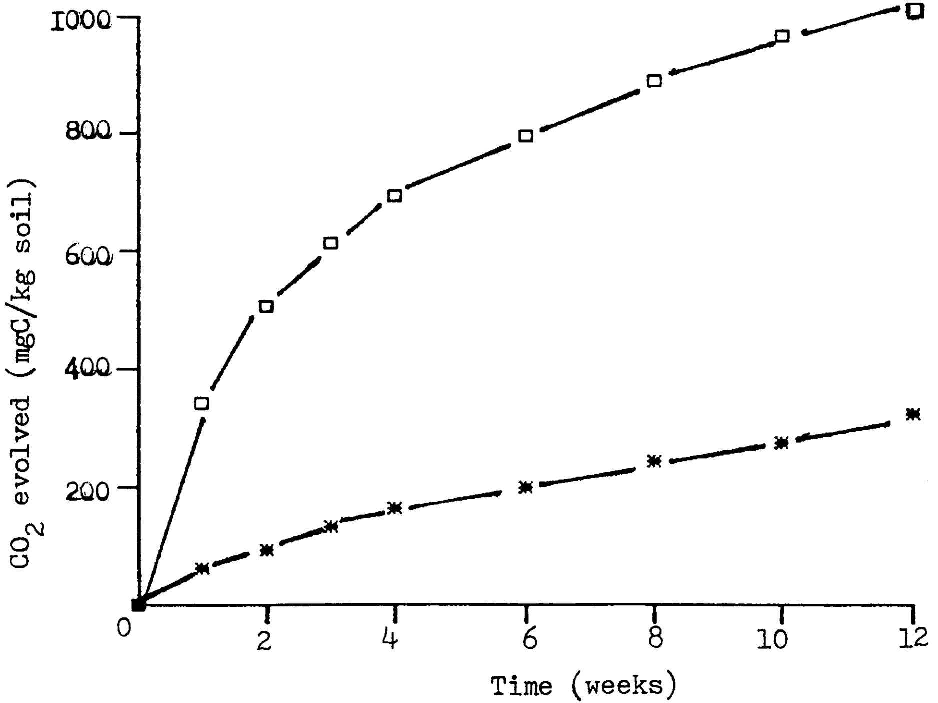


Fig. 6.4 CO_2 evolution by Darleith 2 (Carbeth) soil
Fresh sample (*), Air dried sample (□)

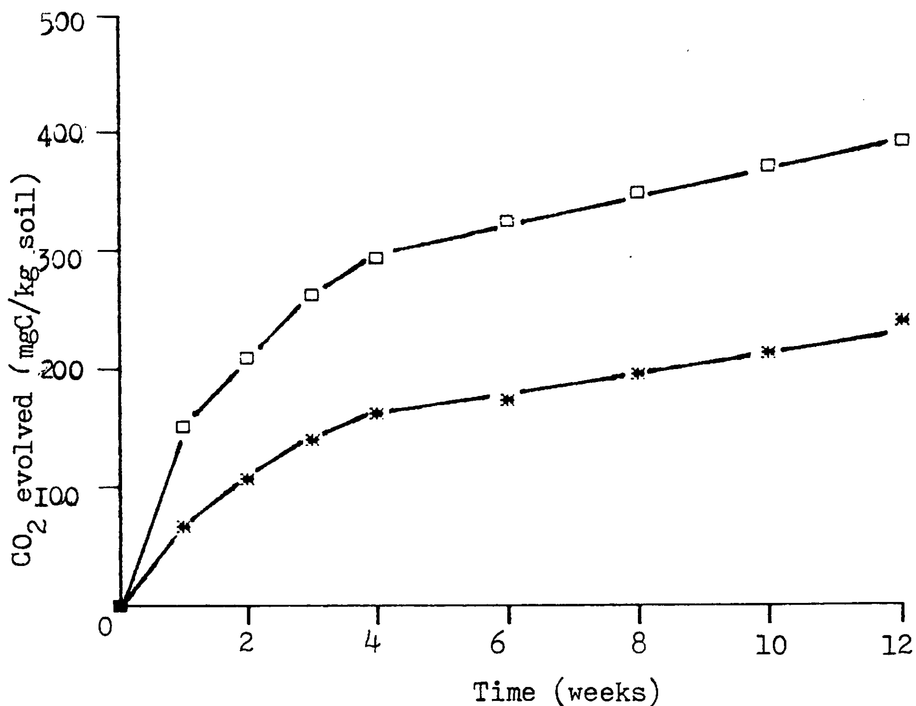


Fig. 6.5 CO_2 evolution by Midelney 2 (Arable) soil
Fresh sample (*), Air dried sample (□)

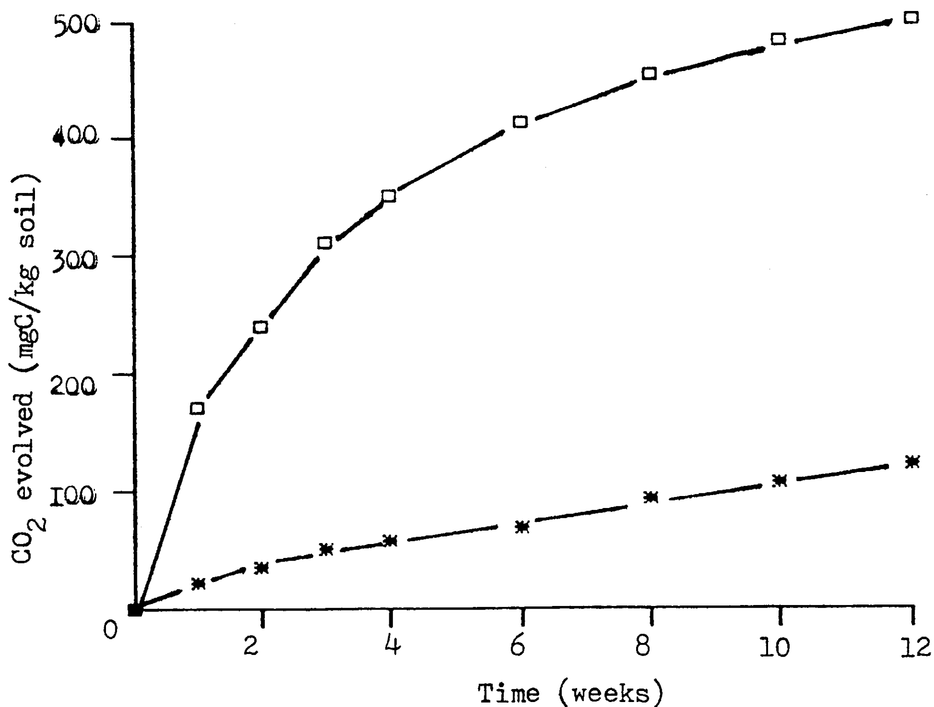


Fig. 6.6 CO_2 evolution by Dreghorn 1 (Ayr) soil
Fresh sample (*), Air dried sample (□)

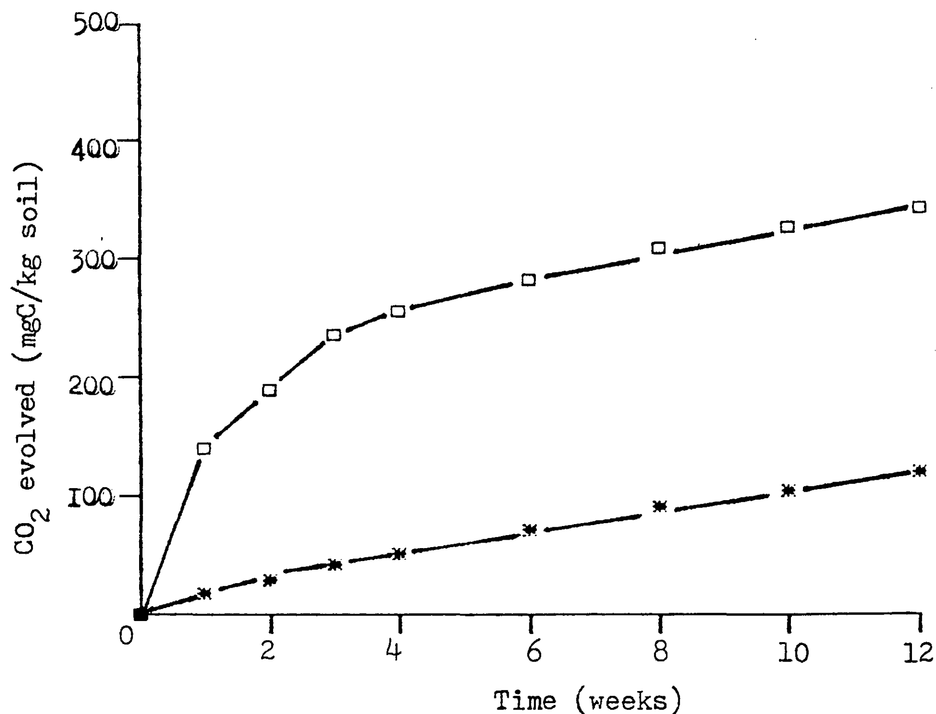


Fig. 6.7 CO_2 evolution by Dreghorn 2 (Arkleston) soil
Fresh sample (*), Air dried sample (□)

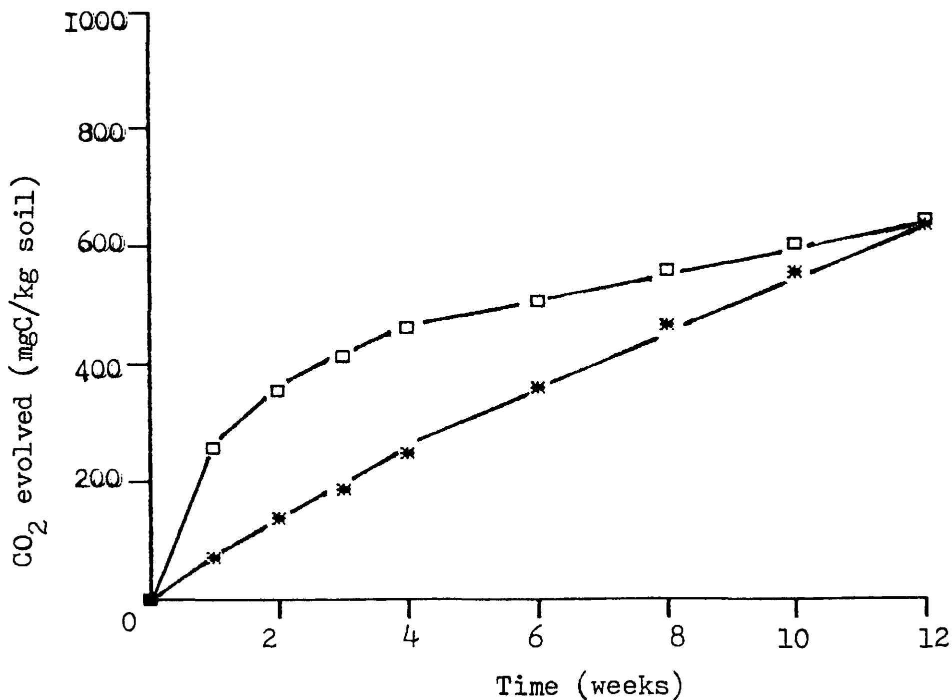


Fig. 6.8 CO_2 evolution by Caprington soil
Fresh sample (*), Air dried sample (□)

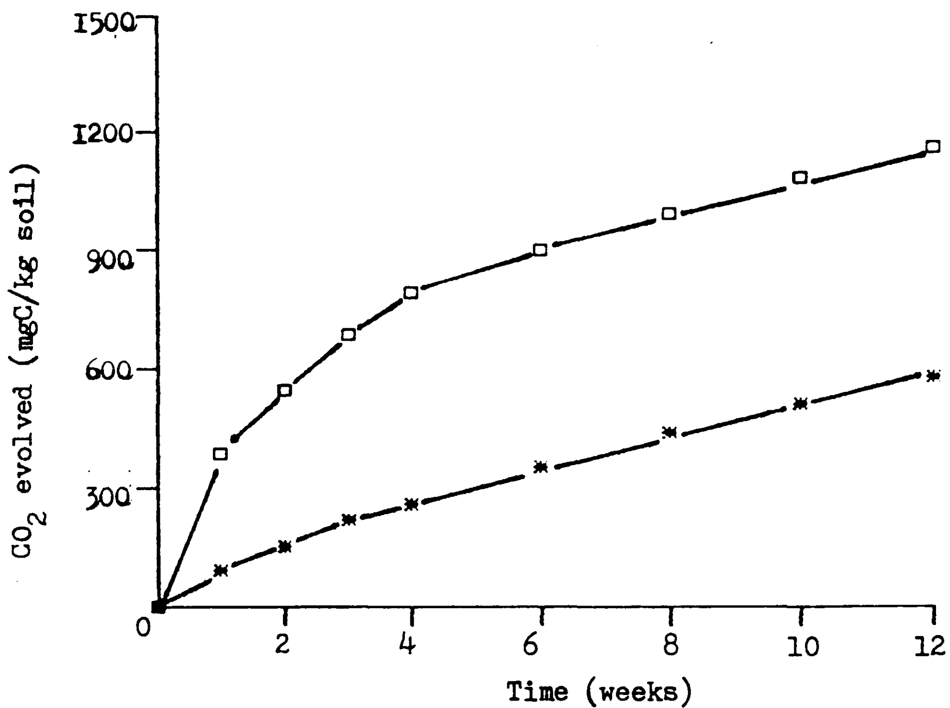


Fig. 6.9 CO_2 evolution by Dunlop soil
Fresh sample (*), Air dried sample (□)

Soil Series	Nitrogen (mgN/kg/week)		Carbon (mgC/kg/week)	
	Mean	S.D	Mean	S.D
Midelney 1	1.28	0.20	23.20	0.47
Alluvium	0.98	0.14	14.40	0.69
Darvel	1.11	0.26	9.40	0.33
Darleith 2	1.08	0.17	20.75	0.37
Midelney 2	1.93	0.26	8.77	0.83
Dreghorn 1	1.03	0.02	8.87	0.76
Dreghorn 2	0.87	0.06	8.63	0.30
Caprington	1.43	0.63	50.63	1.10
Dunlop	0.96	0.09	42.43	1.78

Table 6.1 Nitrogen and carbon dioxide carbon mineralization rates in fresh soils.
Mean and S.D. of 4 replicates

Soil Series	N Mineralized (mg/kg)		C Mineralized (mg/kg)	
	Mean	S.D	Mean	S.D
Midelney 1	148.3	1.50	730.80	3.90
Alluvium	69.1	0.51	375.70	8.70
Darvel	36.9	0.28	300.60	6.30
Darleith 2	136.7	0.23	1024.00	10.80
Midelney 2	44.7	1.94	390.80	6.30
Dreghorn 1	72.8	0.43	502.70	5.90
Dreghorn 2	39.9	0.26	340.80	2.50
Caprington	138.5	0.73	642.60	2.50
Dunlop	210.8	0.52	1162.60	2.20

Table 6.2. Total inorganic nitrogen and carbon dioxide carbon mineralized in air dried soils during 12 weeks aerobic incubation.
Mean and S.D. of 4 replicates

6.3.2. Nitrogen mineralization.

The changes in the ammonium, nitrate, nitrite and total inorganic nitrogen during 12 weeks incubation at 10 ^{o}C in fresh and air dried samples are shown in fig. 6.10 to 6.27.

There was a small initial increase in total inorganic nitrogen of fresh samples taken from Midelney 1 (Grass), Alluvium, and Dreghorn 2 (Arkleston) Series during the first week of incubation due to an initial flush of mineralization of soil organic nitrogen. The rate of mineralization was then linear from 1 to 12 weeks in these soils. There was a lag period in fresh samples of Darvel, Caprington and Dunlop during the first few weeks of incubation and then the increase in total inorganic nitrogen was linear with time of incubation. There were slight decreases in the total inorganic nitrogen levels of fresh samples of Darleith 2 (Carbeth) and Midelney 2 (Arable) during the first or second week of incubation possibly due to immobilization but the increase was linear during later periods of incubation.

The levels of ammonium nitrogen were less than 1 mg/kg in most of the fresh samples except Darleith 2 (Carbeth) and Dunlop soils where it was slightly more than 1 mg/kg of soil. The levels of nitrite nitrogen were too small to measure in all the fresh soil samples. Mineral nitrogen was accumulated as nitrate in the fresh samples and mineralization was linear after the first week except where there was a lag period or immobilization. For

total inorganic nitrogen in fresh soils, regression lines were fitted to the linear regions in order to calculate a mineralization rate constant which was expressed as mg/kg/week and presented as means and standard deviation of the replicates (see Table 6.1).

In the air dried soils, the rate of total inorganic nitrogen accumulation was high during the initial 2 weeks incubation period and was strongly curved throughout the 12 week period. There were initial flushes in the ammonium nitrogen production except for Midelney 2 (Arable) soil where it remained below 1 mg/kg of soil. The ammonium nitrogen disappeared in all soils at the end of the 12 weeks incubation period. There was nitrite nitrogen production in some of the soils (Midelney 1 (Grass) and Alluvium) during the first few weeks but it disappeared after 6 weeks incubation.

Nitrate nitrogen accumulation gave smoothly S shaped curves. The mineralization in air dried samples were tabulated as total net nitrogen mineralization after 12 weeks which are shown as means and standard deviation of 3 replicates (Table 6.2).

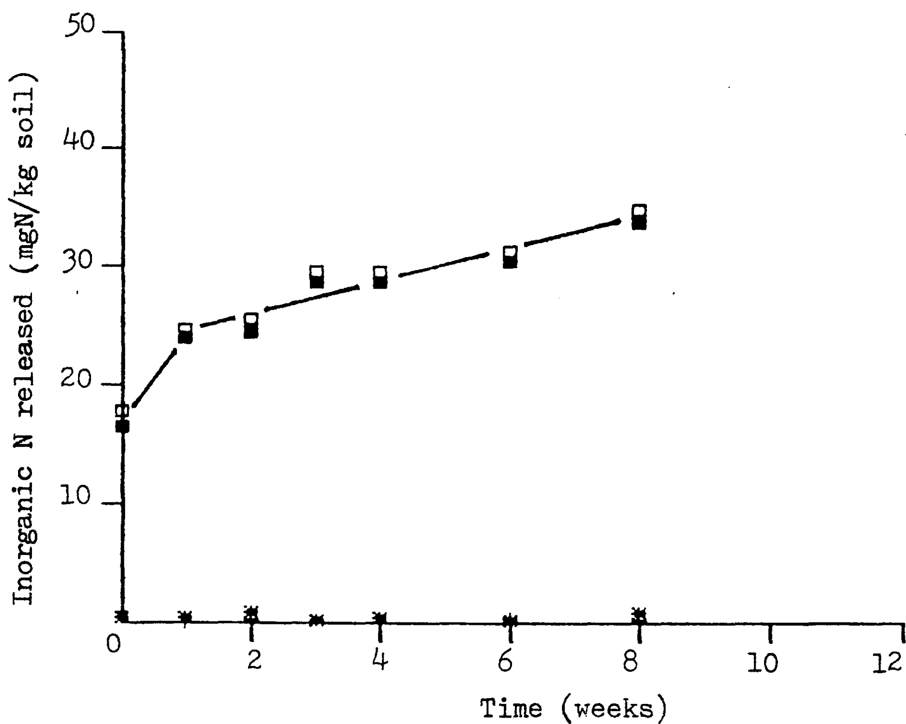


Fig. 6.10 N mineralized by Midelney 1 (Grass) soil (Fresh)
Total inorganic N (□), NO_3-N (■), NH_4-N (*)

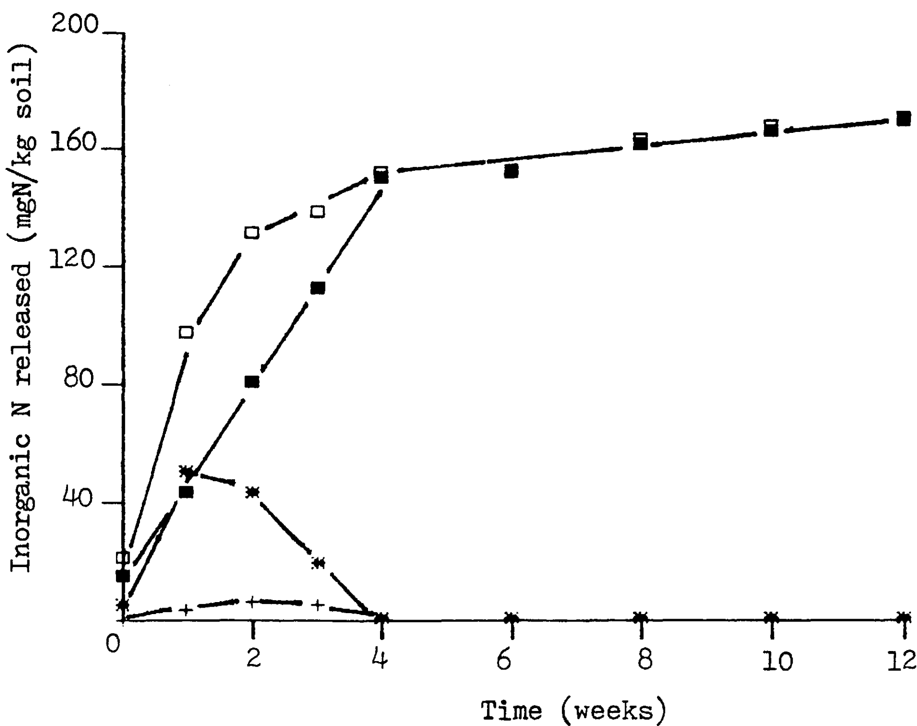


Fig. 6.11 N mineralized by Midelney 1 (Grass) soil (Air dried)
Total inorganic N (□), NO_3-N (■), NH_4-N (*), NO_2-N (+)

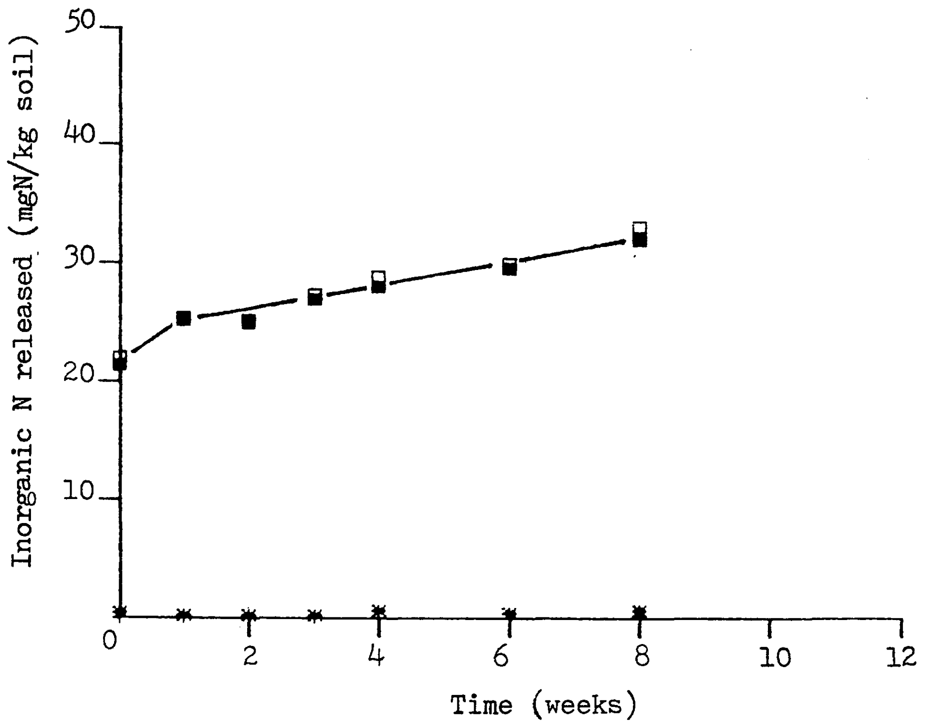


Fig. 6.12 N mineralized by Alluvial soil (Fresh)
Total inorganic N (□), NO_3-N (■),
NH_4-N (*)

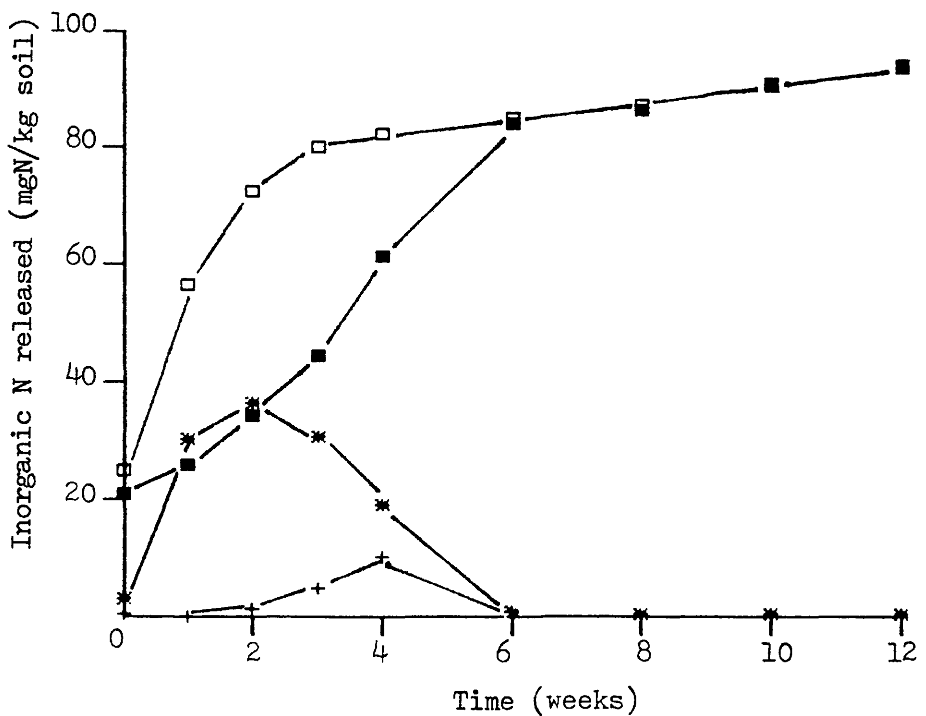


Fig. 6.13 N mineralized by Alluvial soil (Air dried)
Total inorganic N (□), NO_3-N (■),
NH_4-N (*), NO_2-N (+)

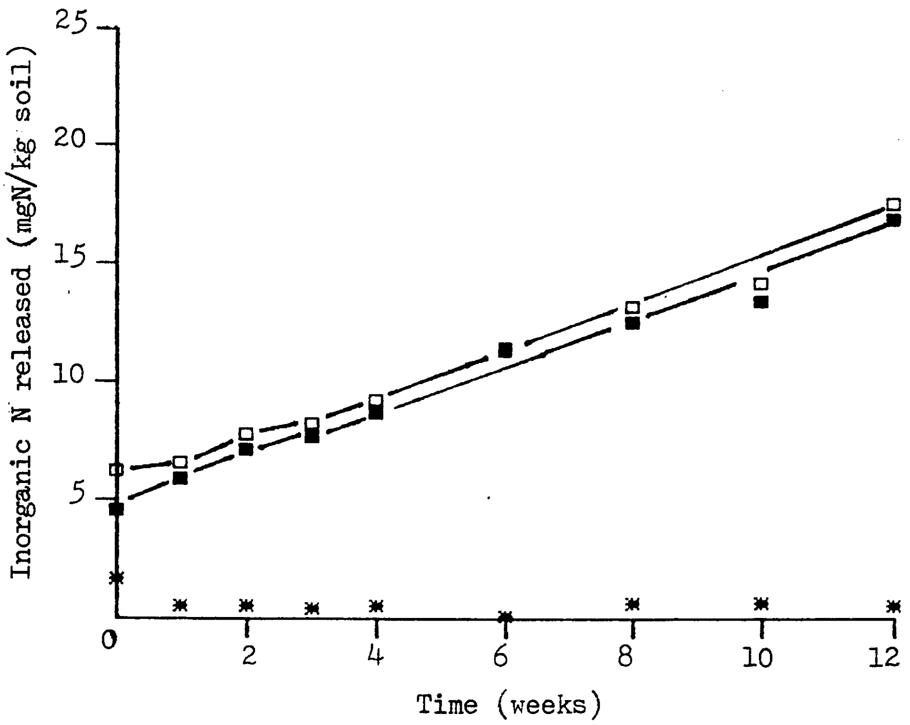


Fig. 6.14 N mineralized by Darvel soil (Fresh)
Total inorganic N (□), NO_3-N (■),
NH_4-N (   )

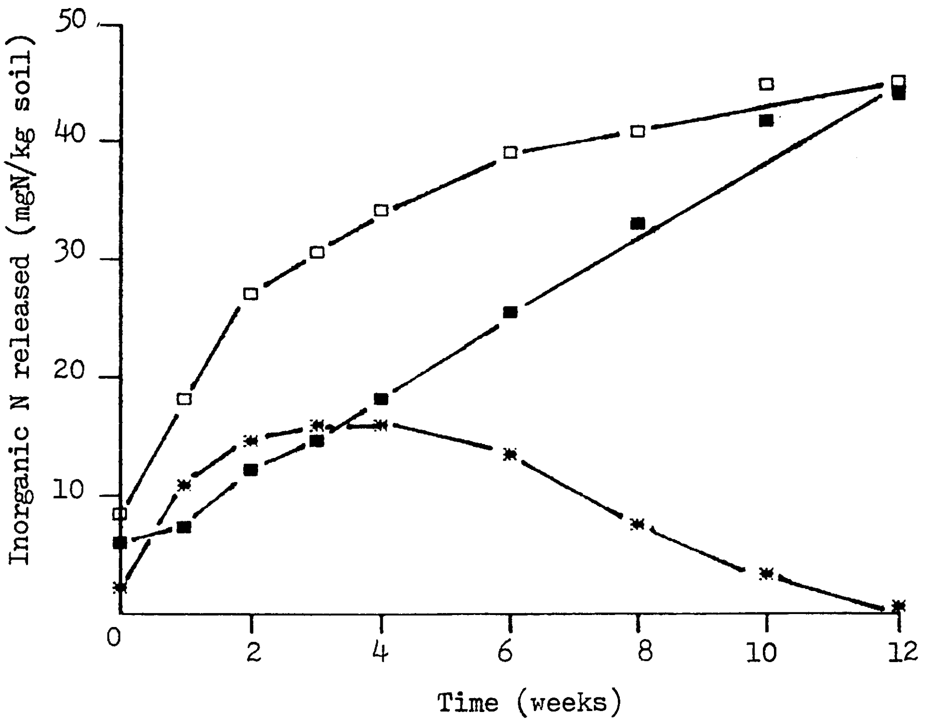


Fig. 6.15 N mineralized by Darvel soil (Air dried)
Total inorganic N (□), NO_3-N (■),
NH_4-N (*)

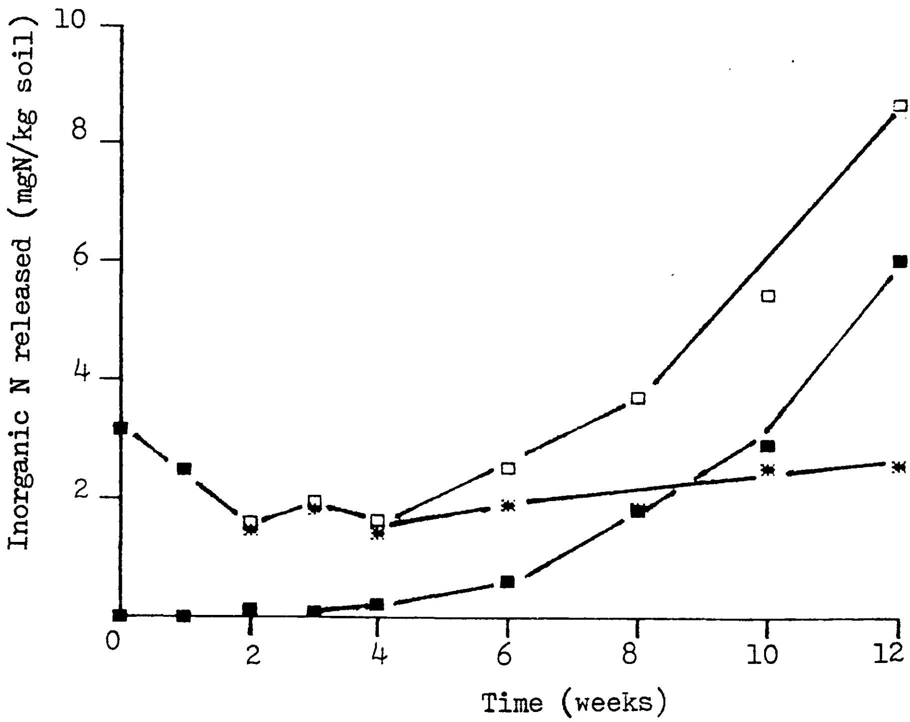


Fig. 6.16 N mineralized by Darleith 2 (Carbeth) soil (Fresh)
Total inorganic N (□), NO_3-N (■),
NH_4-N (*)

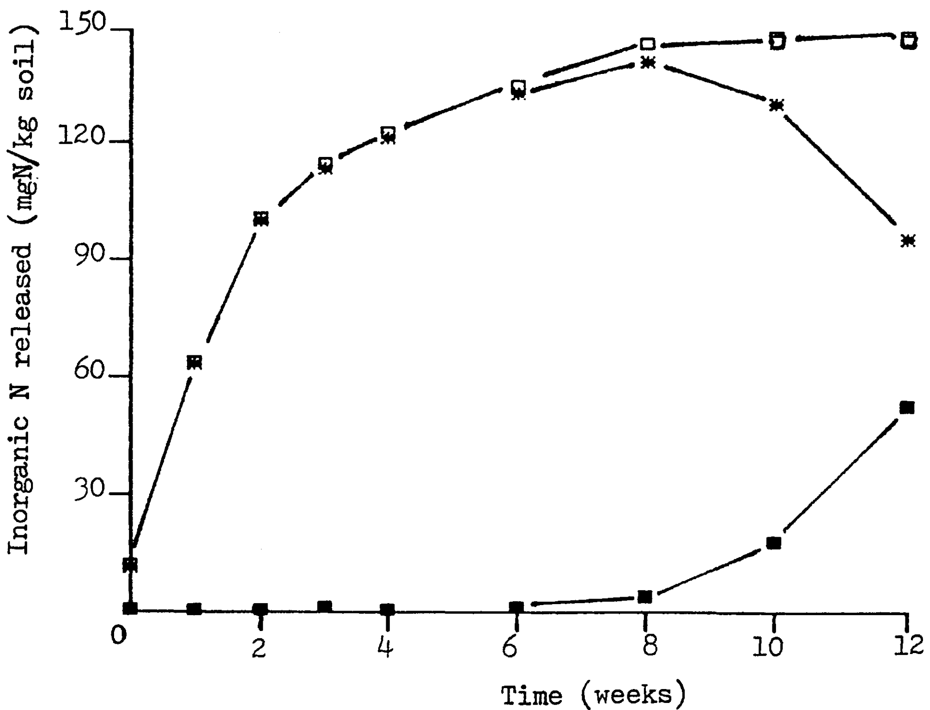


Fig. 6.17 N mineralized by Darleith 2 (Carbeth) soil (Air dried)
Total inorganic N (□), NO_3-N (■),
NH_4-N (*)

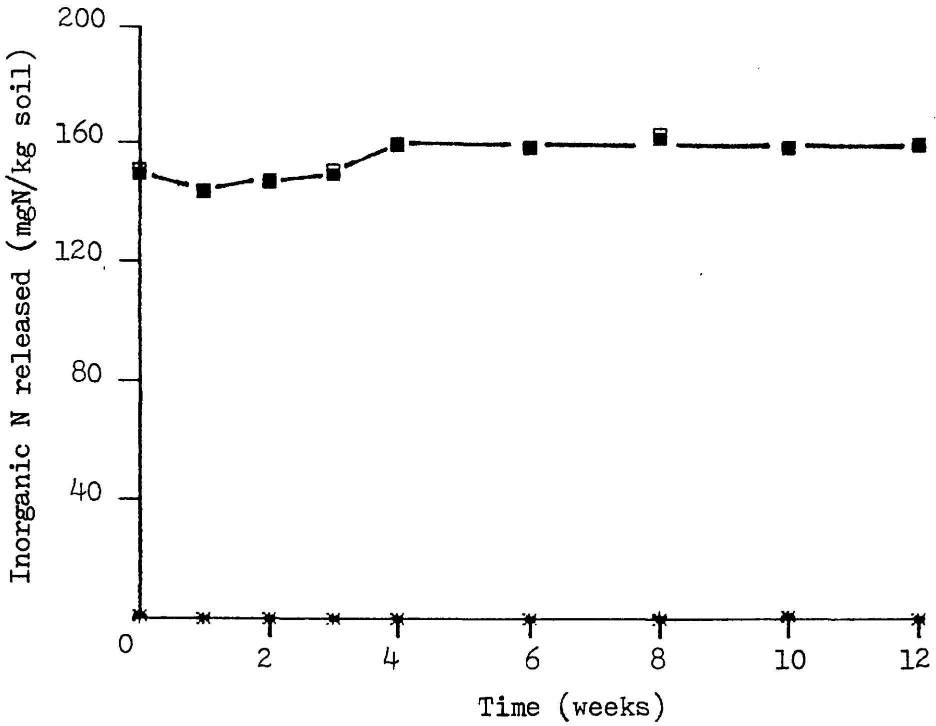


Fig. 6.18 N mineralized by Midelney 2 (Arable) soil (Fresh)
Total inorganic N (□), NO_3-N (■), NH_4-N (*)

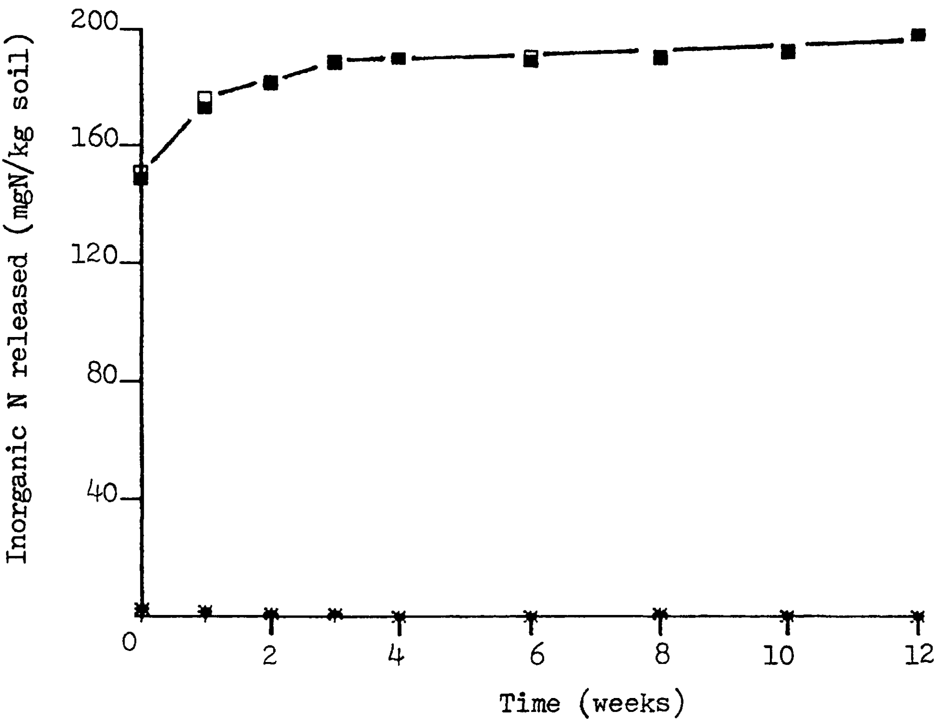


Fig. 6.19 N mineralized by Midelney 2 (Arable) soil (Air dried)
Total inorganic N (□), NO_3-N (■), NH_4-N (*)

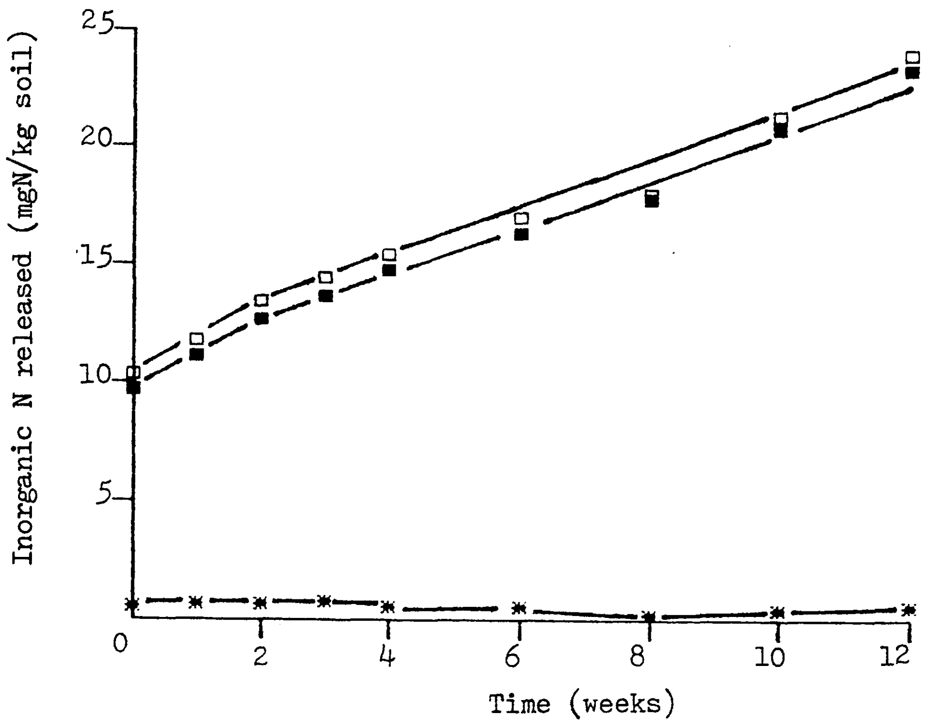


Fig. 6.20 N mineralized by Dreghorn 1 (Ayr) soil (Fresh)
Total inorganic N (□), NO_3-N (■), NH_4-N (*)

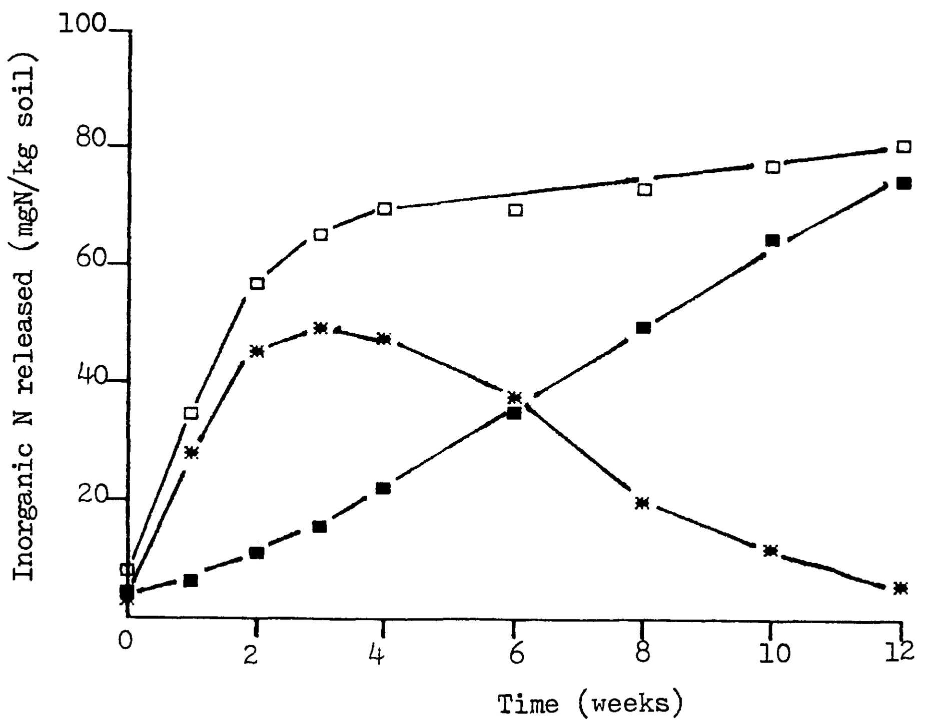


Fig. 6.21 N mineralized by Dreghorn 1 (Ayr) soil (Air dried)
Total inorganic N (□), NO_3-N (■), NH_4-N (*)

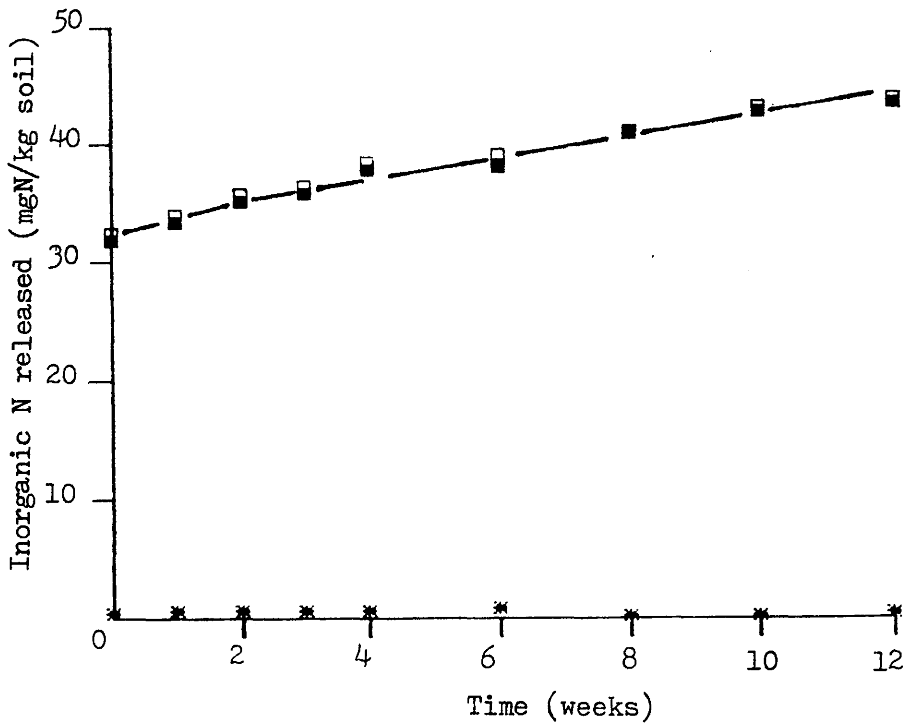


Fig. 6.22 N mineralized by Dreghorn 2 (Arkleston) soil (Fresh)
Total inorganic N (□), NO_3-N (■), NH_4-N (*)

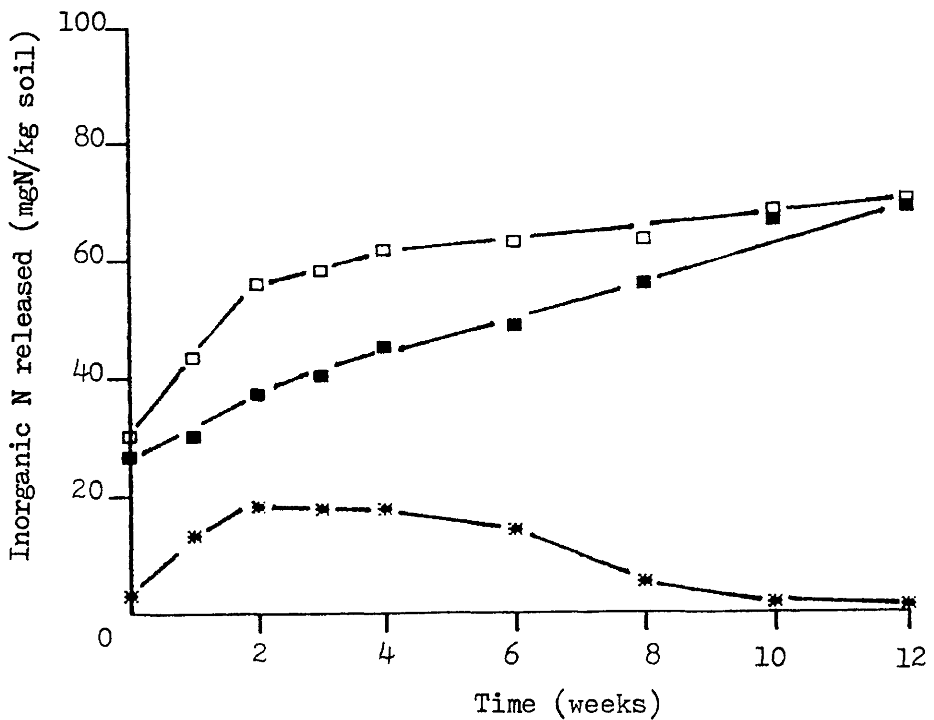


Fig. 6.23 N mineralized by Dreghorn 2 (Arkleston) soil (Air dried)
Total inorganic N (□), NO_3-N (■), NH_4-N (*)

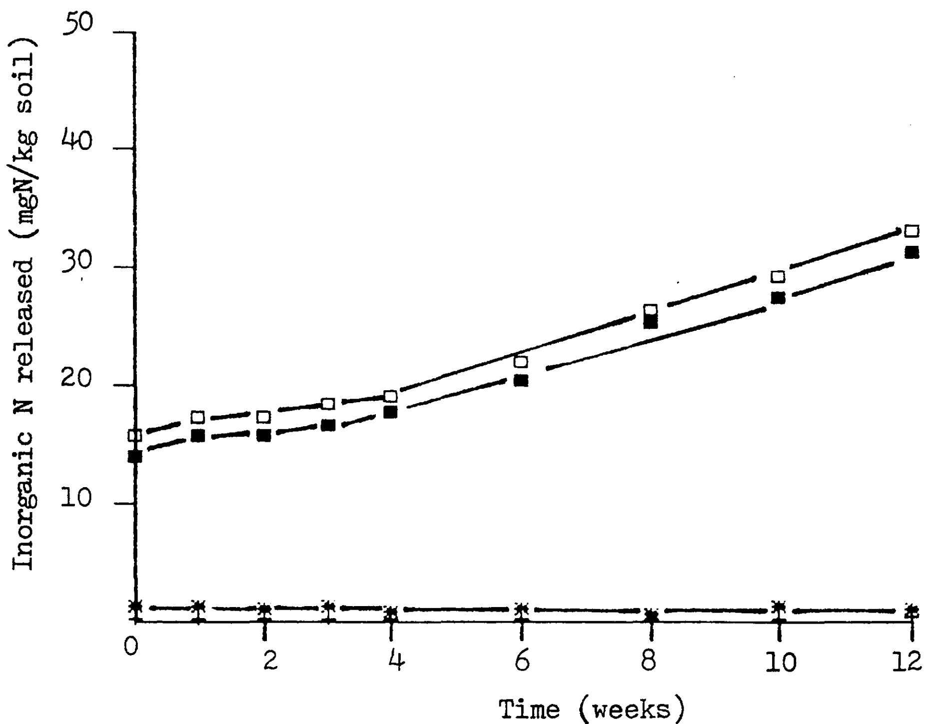


Fig. 6.24 N mineralized by Caprington soil (Fresh)
Total inorganic N (□), NO_3-N (■),
NH_4-N (*), NO_2-N (+)

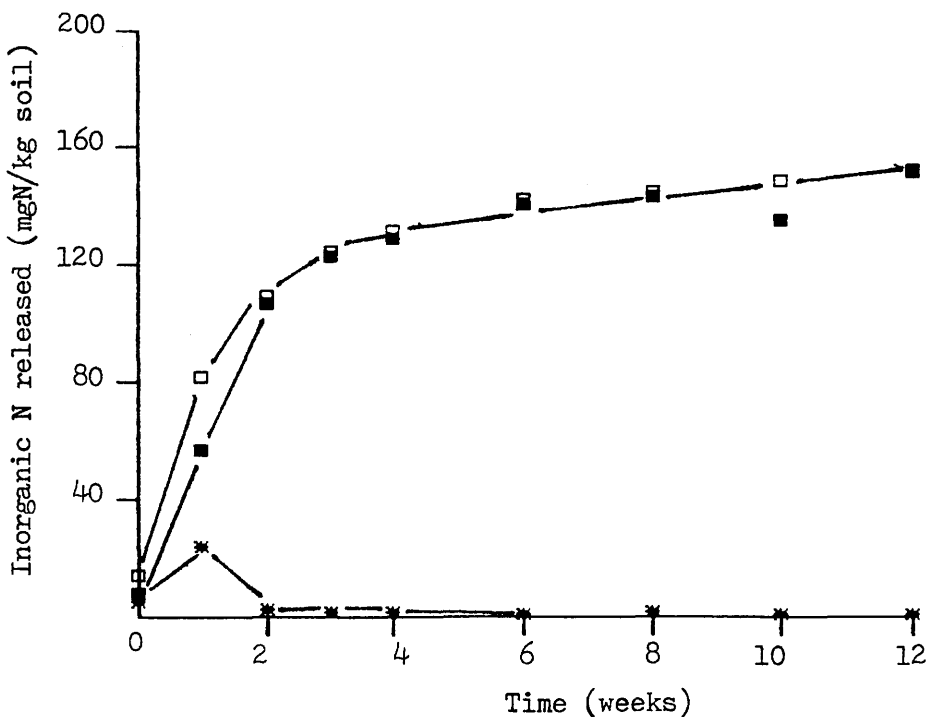


Fig. 6.25 N mineralized by Caprington soil (Air dried)
Total inorganic N (□), NO_3-N (■),
NH_4-N (*)

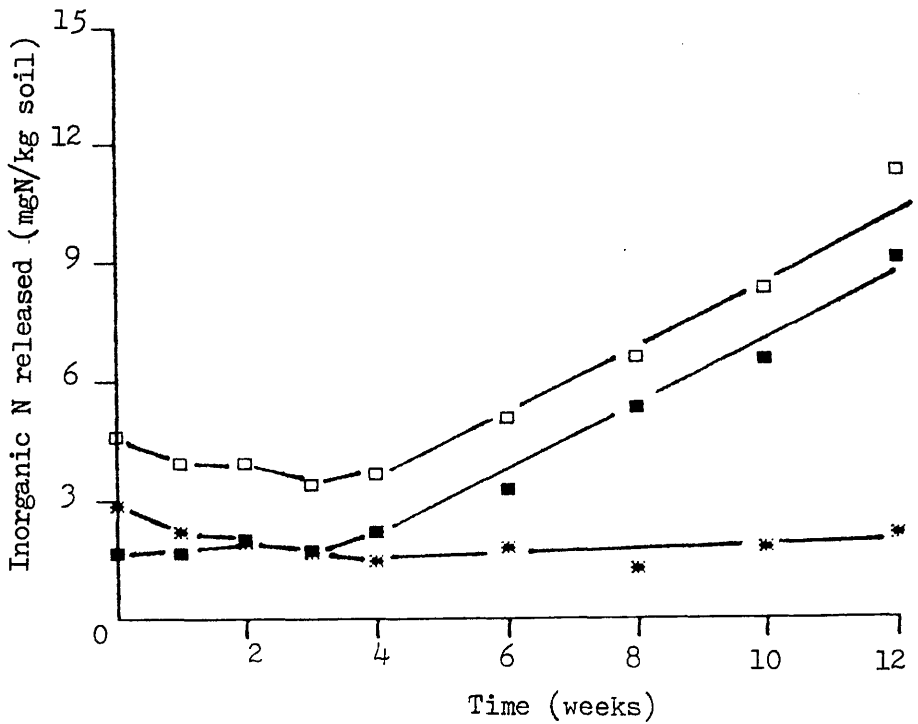


Fig. 6.26 N mineralized by Dunlop soil (Fresh)
Total inorganic N (□), NO_3-N (■),
NH_4-N (*)

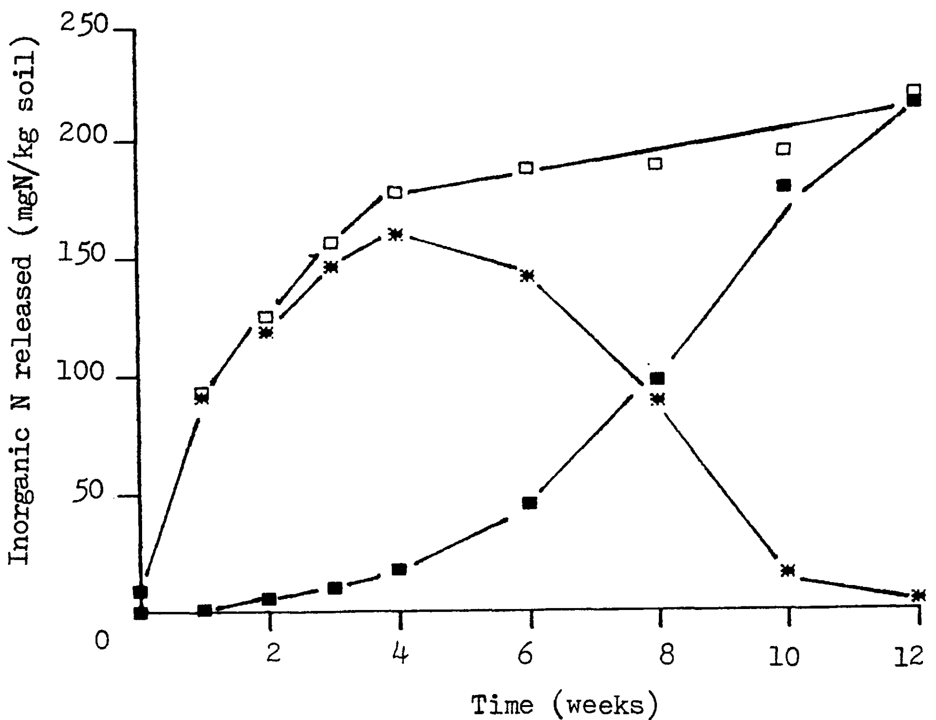


Fig. 6.27 N mineralized by Dunlop soil (Air dried)
Total inorganic N (□), NO_3-N (■),
NH_4-N (*)

6.4. DISCUSSION

The quantity of nitrogen mineralized in a given time under laboratory conditions depends upon a variety of factors like moisture, temperature, rate of oxygen replenishment and the condition of the soil (air dried or fresh) etc. (Stanford and Smith 1972). Soils in the present study were incubated at optimum moisture levels (soil water potential -0.5 bar). The quantity of oxygen in a 1.5 litre Kilner jar containing 50 g of soil was calculated and compared with maximum rate of carbon dioxide evolution. It was found that the maximum rate would correspond to a fall in percent oxygen from 21 to 18 in the atmosphere within the jar. Therefore, the supply of oxygen was not a limiting factor. Moreover, it was replenished each week during the 12 weeks incubation period.

Most research workers have used air dried samples for aerobic incubations for organic nitrogen mineralization studies, fresh samples have also been used. Stanford and Smith (1972) studied net mineralization of nitrogen in 39 air dried soil samples by incubating them at 35 ^{o}C over a 30 week period. Tabatabai and Alkhafaji (1980) incubated field moist samples at 20 and 30 ^{o}C for 25 weeks and studied mineral nitrogen and sulphur content every 2 weeks. Sahrawat (1982) compared the nitrogen mineralized by air dried samples during anaerobic incubation with that of chemical soil tests. Richter et al. (1982) studied nitrogen mineralization in air dried loess soils at 35 ^{o}C. Reddy (1982) used fresh samples during incubation

experiments to study the effect of fluctuating seasonal temperatures on the release of inorganic nitrogen. Flowers and Arnold (1983) incubated fresh soil samples at different temperatures and moisture contents for the study of mineralizable nitrogen status of soils. Magdoff et al. (1983) also incubated fresh samples under aerobic condition at 25 ^{o}C for 17 weeks and determined mineral nitrogen content at 2 week intervals. Farooqi et al. (1983) studied nitrogen mineralization under aerobic conditions using air dried samples at a constant temperature of 35 ^{o}C. Griffin and Laine (1983) incubated air dry samples at 35 ^{o}C for 40 weeks and the mineral nitrogen was determined after different intervals of time. Hussain et al. (1984) and Darah et al. (1985) also used air dry soils during aerobic experiments. Nordmeyer and Richter (1985) incubated soils in the fresh and air dried state at different temperatures (20 and 25 ^{o}C) and compared the nitrogen mineralization after 16 weeks period of incubation. Macduff and White (1985) studied nitrogen mineralization and nitrification in field moist clay soils in laboratory incubation experiments at 4, 10 and 20 ^{o}C. It was decided to compare fresh and air dried soil samples in the present study of aerobic incubation method.

In the present study temperature was maintained near that prevailing under the field conditions. Although in the past most research workers have used temperatures from 25 to 37 ^{o}C for soil incubations, recent literature shows that the relationship between the soils is different at the higher temperature in the laboratory than at the

lower temperatures found in the field. If the increase in nitrogen mineralization with temperature is simple and the same for different soils then the temperature is not very important but if the increases are different for different soils, then it becomes important to use a realistic temperature.

Stanford et al. (1973) studied mineralization of organic nitrogen in 11 soils by incubating them at 5, 15, 25 and 30 ^{o}C and they reported variable results for different soils. But due to a large experimental error it was not possible to calculate Q10 values for individual soils. Tabatabai and Alkhafaji (1980) studied nitrogen and sulphur mineralization in soils which were incubated in a field moist state for a total of 26 weeks at temperatures of 20 or 35 ^{o}C. They observed the expected marked effect of temperature on organic nitrogen mineralization but the Q10 values were different for different soils. The Q10 values ranged from 2.5 to 4.1. Reddy (1982) studied the effect of fluctuating seasonal temperatures on the release of inorganic nitrogen from soils which were collected from various locations in Florida. He observed a significant relationship between mineralization rates and daily ambient air temperature (9.4 to 28.5 ^{o}C). However, the Q10 values were different for different soils varying from 1.4 to 1.9. Similarly Flowers and Arnold (1983) investigated nitrogen mineralization and immobilization in 2 soils which were incubated in the fresh condition over a range of temperatures (5, 10, 15, and 30 ^{o}C) and moisture

conditions. They showed that the increase in mineralization rate was different in these two soils when the temperature was increased. For example there was no increase in the Rivington soil over the range of temperatures 5 to 10 ^{o}C. Addiscott (1983) incubated three different soils in fresh condition at a range of temperatures (5, 10, 15, and 25 ^{o}C). The mineralization of organic nitrogen was studied after periods of 1, 3, 6, 10, 14, and 20 weeks. He found that the response of soils was different at these temperatures. The Q10 values of all soils varied at different temperature. Macduff and White (1985) also reported variable results at different temperatures. It seemed, therefore, unreasonable to carry out incubation at 25 to 30 ^{o}C instead of a realistic temperature nearer to that prevailing under field conditions. The general idea is that the rate of nitrogen mineralization approximately doubles for every 10 ^{o}C rise in temperature, but the results of the above mentioned authors clearly indicate that the soils would not show the same relative nitrogen mineralization at laboratory temperature as at field temperature and choosing of a temperature close to reality would be more appropriate .

There is no detailed information about the annual average daily mean temperature for soils of Scotland in the literature. However, there is more detailed information about the agricultural climate of England and Wales (which includes the soil temperature at a depth of 30 cm) in Technical Bulletin 35 (1941-1970) published by the Ministry of Agriculture, Fisheries and Food. There

is information available about the average daily mean air temperature of Scotland in the "Averages of Temperature for the United Kingdom (1941-1970)". According to this the annual average daily mean air temperature 4 feet above ground at the West of Scotland (College of Agriculture, Auchincruive Meteorological Station) during the summer months (April to September) is 12.0 ^{o}C. According to the "Averages of Earth Temperature for the British Isles" (1921 to 1950) published by the Meteorological Office, the monthly average mean soil temperature at Auchincruive during April to September is 12.1 ^{o}C and the annual average mean temperature is 9.0 ^{o}C. Therefore, it seems quite reasonable to assume that a 10 ^{o}C incubation temperature would represent the actual field condition of soils in the South West of Scotland. The lower the temperature the lower will be mineralization of nitrogen in the soils used. Measurement of low levels of nitrogen needs a more sensitive analytical technique to reduce the levels of experimental error.

Tabatabai and Alkhafaji (1980) stated that the mineralization of soil nitrogen follows one of the following patterns during aerobic incubation:-

(i) Immobilization of nitrogen during the initial period of incubation, followed by mineralization of nitrogen in the later period.

(ii) A rate of release that decreases with time.

(iii) A steady, linear, release with time over the whole period of incubation.

(iv) A rapid release of nitrate during the first few weeks

followed by a slower, linear rate of release.

It is generally accepted that only a part of the nitrogen present in soil organic matter is susceptible to decomposition. This has been termed potentially mineralizable nitrogen by Stanford and Smith (1972) who suggested that the rate of mineralization would be proportional to the potentially mineralizable nitrogen and fitted their data to first order reaction kinetics. They obtained data from a long term aerobic incubation of 39 air dried soil samples. Stanford et al. (1973) incubated 11 air dried soil samples at different temperatures and calculated nitrogen mineralization rates using first order kinetics. Similarly, Campbell et al. (1974), Paul and Van Veen (1978), Juma and Paul (1981) and Griffin and Laine (1983) used first order chemical kinetics for the nitrogen transformations in air dried soil samples. An exponential decay rate has also been used frequently to describe the decomposition of added plant material in soil. Jenkinson (1977) fitted the results of a 10 years study of the decomposition of plant material to a double exponential model which predicted that approximately 70 percent of the plant material decayed with a half life of 0.25 year and the remainder with a half life of 8 years.

Zero order rate constants have also been used by several workers. For example Tabatabai and Alkhafaji (1980) using fresh soil samples during aerobic incubation studies on nitrogen mineralization, disagreed with Stanford and Smith (1972) and therefore, they used zero order rate constants. Flowers and Arnold (1983)

used zero order rate constants for comparing the nitrogen mineralization rates in the fresh soils. Addiscott (1983) measured mineralization of organic nitrogen in laboratory incubation experiments. He used fresh soil samples and expressed the nitrogen mineralization by zero order kinetics. He concluded that the first order kinetics could not be applied to these data. Similarly Macduff and White (1985) studied net mineralization and nitrification in fresh soil samples and applied zero order kinetics to the data between 24 and 168 hours incubation.

The cumulative amounts of carbon and nitrogen mineralized in 9 different soils (fresh and air dried) as a function of time at 10 ^{o}C are shown in fig. 6.1 to 6.9 and 6.10 to 6.27. Results for fresh soils were fitted to a straight line relationship after the initial effects due to disturbance of the soil had subsided. Zero order rate constants calculated from the slopes of the fitted regression lines were used as the basis for comparing mineralization rates.

Table 6.1 shows the rate constants calculated from the regression of the linear relationship between cumulative carbon and nitrogen mineralized with time in the fresh soils studied. The slopes of regression equation indicate that the rate mg/kg soil/week of mineral nitrogen released from soil organic matter at 10 ^{o}C was lowest for Dreghorn 2 (Arkleston) soil 0.87 mg/kg/week and highest for Midelney 2 (Arable) soil 1.93 mg/kg/week. Similarly the rate of carbon mineralization was lowest for Dreghorn 2 (Arkleston) 8.63 mg/kg/week but highest for Caprington

soil 50.63 mg/kg/week. The relationship obtained for cumulative carbon and nitrogen mineralized in the fresh soil with time of incubation do not support the finding of Stanford and Smith (1972). The results, however, support the finding by Tabatabai and Alkhafaji (1980), Richter et al. (1982), Flowers and Arnold (1983), Addiscott (1983) and Nordmeyer and Richter (1985) who applied zero order rate constants for comparing mineralization rates in fresh soils.

Potentially mineralizable nitrogen in air dried soils was estimated by the method used by Stanford and Smith (1972) to obtain the first estimation of potentially mineralizable nitrogen. It was not possible to use their method of successive approximation to refine the estimate. The expression

$$1/Nt = 1/No + b/t$$

was used to calculate potentially mineralizable nitrogen.

Where Nt = Total nitrogen mineralized during time t.

No = Potentially mineralizable nitrogen

b = a constant

t = time in weeks

No was obtained as the reciprocal of the intercept of the best fit straight lines for a plot of 1/Nt against 1/t excluding data for 0 and 1 weeks. The first order rate constant was calculated using the expression

$$\log (No - Nt) = \log No - kt/2.303.$$

k was calculated from the slope and the best fit straight lines from plot of log (No - Nt) against t.

Soil Series	Rate constant ($weeks^{-1}$)	No (mgN/kg soil)
Midelney 1	0.17	181.0
Alluvium	0.20	97.0
Darvel	0.16	50.0
Darleith 2	0.13	168.0
Midelney 2	0.16	194.0
Dreghorn 1	0.17	85.0
Dreghorn 2	0.23	72.0
Caprington	0.14	166.0
Dunlop	0.11	253.0

Table 6.3. Estimates of soil nitrogen mineralization potential (No) and mineralization rate constant (k) in the air dried samples according to Stanford and Smith (1972).

Table 6.3 shows No and k values in the air dried soils. Large amounts of nitrogen was released from all soils during the first 2 to 3 weeks incubation period. These results are similar to those obtained by Stanford and Smith (1972) and Griffin and Laine (1983). They reported that cumulative nitrogen mineralization is related to the square root of time and therefore, the data was analysed by same non-linear least squares procedure.

Fig. 6.1 to 6.27 compare fresh and air dried and rewetted soils for their organic carbon and nitrogen

mineralization. The differences in the fresh and air dried samples could partly be due to the fact that the fresh soil remained undisturbed and air dried samples were physically disturbed during drying operation which might have killed some of the soil biomass and this could explain enhanced carbon dioxide and nitrogen release (Powlson 1980). Another plausible explanation could be that the extra carbon dioxide and nitrogen released from the air dried samples corresponds to the mineralization of a non biomass organic fraction which was made susceptible to decomposition after drying or decay of dead microorganisms killed by the process of air drying.

Birch (1958) working with soils that were dried and rewetted, found that each drying cycle increased the rate of mineralization, the amount of nitrogen liberated proved to be a function of the log of the time the soil was kept in the air dried state prior to moistening. Birch (1960) considered that the increased carbon and nitrogen mineralization that takes place after drying and rewetting of a soil is due to an enlargement of the organic surfaces exposed. He suggested that the cracking of organic colloids on drying could expose a greater surface to solution or microbial attack. It is possible that decomposable material, trapped in the pores of soil colloids, is released during drying and not immediately readsorbed on rewetting because of the hysteresis effects accompanying the drying and rewetting of these colloids. Lynch and Panting (1980) reported that the disturbance of the soil i.e. sieving and drying would be expected to

cause more surfaces of previously inaccessible substrates to become available for microbial growth and also to kill part of the biomass.

The flush in air dried samples could be attributed partly to the decomposition of killed organisms and partly the non biomass substrate liberated on drying (Bottner, 1985). Jenkinson and Powlson (1976) concluded that the essential effect of soil drying at room temperature is a partial sterilization and liberation of non biomass organic matter. They observed that after air drying and remoistening, the carbon and nitrogen mineralization ratio was higher than that resulting from chloroform treatment.

Harada and Hayashi (1968), Ahmad etal. (1973) and Marumoto et al. (1977) demonstrated that cell cytoplasmic compounds become easily mineralized when active organisms are dried at low temperatures, whereas cell walls are more resistant to degradation.

Sparling and cheshire (1979) showed that drying as well as prolonged storage decreased the microbial population in soils. On rewetting, the dead biomass mineralized rapidly.

Richter et al. (1982) compared the amounts of nitrogen mineralized from air dried and fresh samples and they attributed the flush of nitrogen in air dried soils mainly to nitrogen mineralized from autolysed microorganisms killed by drying.

It is, therefore, clear that with incubation of dried soils at least 3 fractions of organic nitrogen contribute to mineralization. i.e. (i) resistant organic material

which decomposes very slowly (ii) decomposable organic material produced by air drying and (iii) autolysed microorganisms killed by drying.

The ratio of carbon dioxide-C evolved : N mineralized in the fresh soil samples was calculated from the total C and N released during the incubation period of 0-1 week and from the zero order rate constants of C and N mineralization. (see table 6.4).

The overall carbon dioxide-C evolved : N mineralized ratio in the fresh soil samples was high ranging from 8:1 to 44:1 which demonstrates the range of substrates in these soils. There was a negative carbon dioxide-C evolved : N mineralized ratio during 0-1 week period of incubation in some of the fresh soils which resulted from nitrogen immobilization. In others carbon dioxide-C evolved : N mineralized was wider in the first week than later in the incubation. Disturbance of fresh soils caused the presence of a carbon rich substrate.

Soil Series	C:N Ratio	
	0-1 weeks	rate constants
Midelney 1	16.0	18.1
Alluvium	22.0	14.7
Darvel	-2200.0	8.5
Darleith 1	-96.0	19.2
Midelney 2	-12.0	4.5
Dreghorn 1	15.0	8.6
Dreghorn 2	17.0	9.9
Caprington	51.0	35.4
Dunlop	-118.0	44.2

Table 6.4. The ratio C : N mineralized in fresh soils at different stages of incubation.

In the air dried soils carbon dioxide-C evolved : N mineralized was calculated from the total C and N mineralized during incubation periods of 0-1, 1-2, 2-12 and 0-12 weeks time (table 6.5)

Soil Series	period of incubation (weeks)			
	0-1	1-2	2-12	0-12
Midelney 1	4.2	2.6	7.8	4.9
Alluvium	4.0	2.3	10.1	5.4
Darvel	11.1	3.7	8.6	8.1
Darleith 1	6.5	4.5	10.8	7.4
Midelney 2	7.9	7.6	12.4	8.7
Dreghorn 1	6.4	3.2	10.6	6.9
Dreghorn 2	10.1	4.1	10.2	8.5
Caprington	3.8	3.5	6.6	4.6
Dunlop	5.1	5.2	6.0	5.5

Table 6.5. The ratio total carbon dioxide-C : total N mineralized in air dried soils at different stages of incubation.

The initial C:N ratio in the air dried samples (0-1 week) was narrow, ranging from 4:1 to 11:1, which suggests that the substrate was rich in nitrogen, and might have come from the dead cells of the killed microorganisms. Powlson and Jenkinson (1976) have reported that air drying of soil samples reduces the carbon dioxide-C evolved : N mineralized ratio compared to control treatments but not to the same extent as treatments like fumigation or autoclaving.

The carbon dioxide-C evolved : N mineralized ratio in the air dried samples declined in the second week of

incubation (table 6.5) but it became higher during the 2-12 week period. The changes in the carbon dioxide C evolved : N mineralized ratio during the 1-2 weeks period indicates that the soils may have exhibited changes in the active population of microorganism or the type of available substrate. One possible explanation could be that bacteria were dominant during the 0-1 week period and later fungi became dominant during the 1-2 week period. Fungi need less nitrogen and therefore, a greater portion of the nitrogen is mineralized (Jenkinson, 1981). There are contrasting reports about the kind of microorganisms in the soils during different periods of time. McGill et al. (1975) found that a fungal population developed initially during soil incubation and reached maximum by 5 days, then declined and was replaced by a population dominated by bacteria and actinomycetes. Nannipieri et al. (1978) have shown a high peak of bacteria in soils during the first 20 to 40 hours of incubation but in the later stage it was dominated by fungi.

The high coefficient of correlation between biomass C (Biomass is considered in detail in chapter 8) and the total amount of C and N released in air dried soils (see table 6.7) suggests that the flush in the first few weeks may be due to the biomass killed during the air drying process. However, as the flush of C and N is also strongly correlated to total %C and total %N, the role of the biomass could be either direct or indirect.

Table 6.6 shows the correlation between rate of carbon dioxide and nitrogen mineralization in fresh soil

with other soil parameters. Only the rate of carbon dioxide mineralized is highly correlated to the biomass C.

Soil properties	C rate constant	N rate constant
C rate constant	---	0.57
N rate constant	0.57	---
% total N	0.47	0.17
% total C	0.51	0.09
% Clay	0.23	0.42
pH (water)	-0.01	0.30
pH ($CaCl_2$)	-0.04	0.33
Biomass C	0.82 **	0.33

Table 6.6. Pearson product-moment correlation coeffient between rate of Carbon dioxide and nitrogen mineralization in fresh soil with other soil properties.

** significant at $p<0.01$ level

Soil properties	Total C released	Total N released
Total C released	---	0.93 ***
Total N released	0.93 ***	---
% total N	0.81 **	0.79 *
% total C	0.89 **	0.81 **
% Clay	0.30	0.36
pH (water)	-0.26	-0.05
pH ($CaCl_2$)	-0.30	-0.11
Biomass C	0.87 **	0.86 **

Table 6.7. Pearson product-moment correlation coefficient between total C and N mineralized in air dried soils with other soil properties

** significant at $p<0.01$

*** significant at $p<0.001$

Soils	CO_2-C evolved week 1 (mg/kg)	total (mg/kg)	biomass C (mg/kg)	CO_2-C Evolved (mg/kg)
Midelney 1	30.5	73.1	68	31
Alluvium	12.8	37.6	16	7
Darvel	12.1	30.3	30	13
Darleith	34.2	102.4	100	44
Midelney 2	15.2	39.1	70	31
Dreghorn 1	17.4	50.3	42	18
Dreghorn 2	14.1	34.1	30	13
Caprington	25.9	64.3	111	49
Dunlop	38.4	116.3	144	64

Table 6.8. Comparison of flush of carbon in air dried soils with the carbon released from the soil biomass.

Biomass-C = total carbon in the biomass

Carbon dioxide C evolved = carbon dioxide evolved following fumigation with the chloroform

Table 6.8 compares the flush in the air dried soils with the biomass C and the carbon dioxide evolved due to chloroform fumigation. The chloroform fumigation experiment was, however, carried out at a higher

temperature (20 ^{o}C). The biomass C was not high compared with the total carbon dioxide evolved by the air dried samples. Moreover, only part of the carbon is released on decomposition of the killed biomass, approximately 45% as shown in the final column of table 6.8.

Bottner (1985) reported that an air drying cycle kills 1/3 to 1/4 of soil biomass. If this is the case then only a small portion of the flush of carbon dioxide in the incubation has come from the killed soil biomass and the rest must have come from the soil organic matter fraction. Although the substrate was rich in nitrogen even in the first two weeks, biomass could not contribute more than 5 to 25 % of the carbon dioxide evolved. Much of the carbon dioxide and nitrogen mineralized must have come from the soil organic matter.

The present study brings out the importance of the use of fresh samples for predicting nitrogen availability under Scottish climatic conditions. However, great care is needed to ensure reproducible results. The weather conditions of Scotland are such that one would rarely find that the soil has become air dried to the extent where biological activities are completely inhibited and major changes in organic matter and organic nitrogen availability occur. Incubation of air dried samples, therefore, does not seem reasonable for nitrogen mineralization studies.

CHAPTER SEVEN

CHEMICAL INDEX OF NITROGEN AVAILABILITY

7.1. INTRODUCTION

The need for a satisfactory laboratory method of obtaining an estimate of the amount of nitrogen likely to be made available for crop growth by mineralization of soil organic matter during the growing season has long been evident and numerous biological and chemical methods have been proposed. The biological methods which involve aerobic or anaerobic incubation for various times are more reliable but they are not rapid enough to be used in soil testing laboratories. There is an urgent need for a rapid chemical test of assessing potentially available soil organic nitrogen that is suitable for use in such laboratories.

Stanford (1982) classified chemical methods of assessing soil organic nitrogen into (i) Intensive extraction methods which include boiling of soil samples in 6 M hydrochloric acid, boiling in 4.5 M sodium hydroxide or oxidation of organic matter by the Walkley and Black method. (ii) Extraction methods of intermediate intensity which involve various modifications of the alkaline permanganate method, oxidative release of ammonium by acid dichromate, room temperature extraction in dilute acids or 0.25 M sodium carbonate or 1 M sodium

hydroxide. (iii) Relatively mild extraction methods involving extraction by boiling water, autoclaving in calcium chloride solution, room temperature extraction for 1 hour in acid permanganate or extraction of soil samples in 0.01 M sodium bicarbonate.

The most common chemical methods for nitrogen availability are those that involve oxidative release of ammonium nitrogen. Stanford (1978) extracted ammonium nitrogen by distilling a soil sample with alkaline permanganate solution for 15 minutes. The ammonium nitrogen was determined by titration with 0.025 M sulphuric acid. Stanford and Smith (1978) used acid permanganate solution. Soil samples were shaken in 0.2 M potassium permanganate and 0.5 M sulphuric acid for 1 hour. The ammonium nitrogen was determined by steam distillation with sodium hydroxide. Nommik (1976) extracted soil with acid dichromate and then steam distilled with sodium hydroxide for the determination of ammonium nitrogen. Sahrawat (1982) treated soil with hydrogen peroxide for the oxidation of organic matter and determined ammonium nitrogen by steam distilling with magnesium oxide. Sahrawat (1982) extracted soil samples by shaking in dilute sulphuric acid for 1 hour. The ammonium nitrogen released was determined by distilling the soil suspension with 50 % sodium hydroxide solution. The sodium bicarbonate UV method was proposed by Fox and Piekielek (1978) whereby soil samples were shaken in 0.01 M sodium bicarbonate solution for 15 minutes, the suspension filtered and the absorption of the filtrate

measured at 260 nm. Autoclaving of soil samples in 0.01 M calcium chloride for 16 hours at 121 °C was proposed by Stanford and Smith (1976). The ammonium nitrogen was determined by steam distillation with magnesium oxide or by the Conway microdiffusion method.

In recent years some new methods have been proposed. Oien and Selmer-Olsen (1980) evaluated nitrogen status in soils by heating soil samples in 2 M potassium chloride solution for 20 hours at 80 °C and determining ammonium and nitrate nitrogen content of the filtrate by automatic colorimetric methods. Gianello and Bremner (1986b) developed 2 rapid methods of assessing potentially available organic nitrogen. One method involves determination of ammonium nitrogen produced by steam distillation of the soil sample with pH 11.2 phosphate-borate buffer for 8 minutes. The other involves determination of the ammonium nitrogen produced by heating the soil sample with 2 M potassium chloride in a stoppered tube at 100 °C for 4 hours. The results of these methods were compared with those obtained by other chemical and incubation methods. The chemical methods tested included the acid permanganate method, the alkaline permanganate method, the calcium chloride autoclaving method and the sodium bicarbonate UV method. The incubation methods included determination of ammonium nitrogen produced after one week anaerobic condition or the ammonium, nitrate and nitrite nitrogen produced under aerobic conditions for 2 and 12 weeks. They showed that the results of the two new methods were highly correlated with those obtained by

aerobic or anaerobic incubation methods.

The chemical method that has received most attention is the procedure proposed by Stanford and Smith (1976) which involves determination of the ammonium nitrogen produced by autoclaving the soil sample in 0.01 M calcium chloride at 121 ^{o}C for 16 hours. The method seemed to be too complicated because it required several extractions and a time consuming microdiffusion technique for determination of ammonium. Stanford (1968) used sodium hydroxide distillation for ammonium nitrogen determination following autoclaving soil samples in 0.01 M calcium chloride solution. This might include alkali labile amino nitrogen and would give higher results than other methods. The autoclaving method recommended in the recent revision of Methods of Soil Analysis Part II Chemical and Microbiological Properties, second edition edited by Page et al. (1982) is a modification of the calcium chloride method in which ammonium nitrogen present after autoclaving is determined by steam distillation of the autoclaved soil-calcium chloride suspension with magnesium oxide for 4 minutes. The modified calcium chloride method seems to be much simpler than the original one. However, Whitehead et al. (1981) has further simplified this method by determining ammonium nitrogen in the calcium chloride autoclaved soil extract by means of Technicon AutoAnalyzer system. The later 2 methods measure only ammonium nitrogen and there is no interference from alkali labile amino nitrogen.

In the present study a reliable chemical extraction

method of assessing potentially available nitrogen in soil was required to compare it with other biological nitrogen indexes. It was, therefore, decided to use the modified calcium chloride autoclaving method recommended by Whitehead et al. (1981) which involves determination of ammonium nitrogen in the filtered autoclaved soil extract by the automatic colorimetric method.

7.2. MATERIALS AND METHODS

This was essentially the method described by Whitehead et al. (1981). see section 2.7.

10 g (oven dry equivalent) of fresh or air dried samples were weighed into 2 ounce glass bottles to which was added 25 cm^3 of 0.01 M calcium chloride solution. The bottles were capped with plastic tops and were heated in the autoclave at 121 ^oC for 16 hours but in 2 periods of 8 hours each. They were then allowed to cool and the contents of the bottles were adjusted to their original weight with deionized water and then filtered through Whatman filter paper No 40. The filtrates were analysed for their ammonium , nitrate and nitrite nitrogen content with the Technicon AutoAnalyzer (see section 2.9). Blanks were also included in the autoclaving and the samples were replicated 4 times.

7.3. RESULTS AND DISCUSSION

The initial 0.5 M potassium sulphate extractable inorganic nitrogen content at 2 ^{o}C of fresh and air dried samples are shown in tables 7.1 and 7.2. The initial nitrogen contents of the fresh soil samples were greater than those of air dried soils with the exception of Darleith 2 (Carbeth) soil where it was almost the same in both air dried and fresh samples. The increase in total inorganic nitrogen levels of fresh samples may be attributed to mineralization of soil organic nitrogen which might have taken place although these samples were stored in a refrigerator at 2 ^{o}C.

The levels of inorganic nitrogen in fresh and air dried soils after autoclaving in 0.01 M calcium chloride at 121 ^{o}C for 16 hours are shown in tables 7.3 and 7.4.

Soil Series	Inorganic N (mg/kg)			
	NH4-N	NO3-N	NO2-N	Total
Midelney 1	0.8	63.8	0.6	65.2
Alluvium	0.6	51.7	0.0	52.3
Darvel	0.8	14.0	0.0	14.8
Darleith 2	9.5	15.0	0.0	24.5
Midelney 2	0.6	64.4	0.2	65.2
Dreghorn 1	0.7	24.4	0.0	25.1
Dreghorn 2	0.9	40.3	0.0	41.2
Caprington	1.4	60.0	0.1	61.5
Dunlop	5.7	18.2	0.0	23.9

Table 7.1. Initial 0.5 M Potassium sulphate extractable inorganic nitrogen content of fresh soils.
Mean of 3 replicates

Soil Series	Inorganic N (mg/kg)			
	NH4-N	NO3-N	NO2-N	Total
Midelney 1	9.0	15.5	0.3	24.8
Alluvium	4.9	20.7	0.1	25.7
Darvel	5.5	4.2	0.2	9.8
Darleith 2	11.5	15.0	0.0	26.5
Midelney 2	4.5	60.5	0.0	65.0
Dreghorn 1	4.3	3.9	0.0	8.2
Dreghorn 2	3.9	27.1	0.0	31.0
Caprington	5.7	7.5	0.0	13.2
Dunlop	12.5	0.0	0.0	12.5

Table 7.2. Initial 0.5 M Potassium sulphate extractable inorganic nitrogen content of air dried soils.
Mean of 3 replicates

Soil Series	Inorganic N (mg/kg)			
	NH4-N	NO3-N	NO2-N	Total
Midelney 1	73.0	39.0	0.2	112.2
Alluvium	29.0	44.6	0.0	73.6
Darvel	67.3	13.8	0.7	81.8
Darleith 2	131.3	14.3	0.0	145.6
Midelney 2	59.4	40.2	0.0	99.6
Dreghorn 1	72.5	21.8	0.0	94.3
Dreghorn 2	62.2	44.8	0.0	107.0
Caprington	71.0	37.5	0.0	108.5
Dunlop	125.9	16.1	2.6	144.6

Table 7.3. Mineral nitrogen content of fresh soils extracted by autoclaving in 0.01 M calcium chloride solution.
Mean of 4 replicates

Soil Series	Inorganic N (mg/kg)			
	NH4-N	NO3-N	NO2-N	Total
Midelney 1	83.9	7.9	0.2	91.8
Alluvium	28.3	20.0	0.3	48.3
Darvel	69.1	8.0	1.3	77.1
Darleith 2	149.0	18.1	2.8	167.1
Midelney 2	65.1	49.9	0.4	115.0
Dreghorn 1	59.7	27.1	0.0	86.8
Dreghorn 2	72.3	5.2	0.0	77.5
Caprington	80.4	3.4	0.4	83.8
Dunlop	142.4	11.9	3.0	154.3

Table 7.4. Mineral nitrogen content of air dried soils extracted by autoclaving in 0.01 M calcium chloride solution.
Mean of 4 replicates

Soil Series	Ammonium N (mg/kg)	
	Fresh	Air dried
Midelney 1	72.3	74.9
Alluvium	28.4	23.4
Darvel	66.6	63.6
Darleith 2	121.8	137.5
Midelney 2	58.7	60.7
Dreghorn 1	71.7	55.4
Dreghorn 2	61.3	68.3
Caprington	69.6	74.7
Dunlop	120.1	129.9

Table 7.5. Net increase in ammonium nitrogen content of fresh and air dried soils after autoclaving in 0.01 M calcium chloride solution.
Mean of 4 replicates

The levels of ammonium nitrogen after autoclaving increased in both air dried and fresh soil samples. There were also changes in the levels of nitrate and nitrite nitrogen both of which decreased in most of the fresh and air dried samples. However, in some of the air dried samples (Darvel, Dreghorn 1 (Ayr) and Dunlop) the levels of nitrate nitrogen increased after autoclaving. There were high nitrite nitrogen levels after autoclaving in some of the soils (Dunlop and Darvel fresh and Darleith 2 (Carbeth) and Dunlop air dried). On the whole there were changes in the levels of nitrate and nitrite as well as ammonium nitrogen after autoclaving. There is no explanation for the changes in nitrate and

nitrite nitrogen but the method is based on ammonium nitrogen production and therefore, changes in the nitrate and nitrite nitrogen were not studied further.

The net increase in ammonium nitrogen levels of air dried and fresh samples are shown in table 7.5. For most soils there was no real difference in the ammonium nitrogen released between the fresh and air dried samples. The results are similar to those reported by Gianello and Bremner (1986a). They found that the values of ammonium nitrogen obtained after heating soil samples in 2 M potassium chloride solution at 100 ^{o}C are not affected by air drying or air dry storage of the soil samples. The largest differences were noted in Darleith 2 (Carbeth) soil where the level of ammonium nitrogen released was higher in air dried sample and in the Dreghorn 1 (Ayr) soil where it was higher in the fresh soil than in the air dried soil. The total inorganic nitrogen after autoclaving in the fresh soils ranged from 73.6 to 145.6 mg/kg of soil and from 43.8 to 154.3 in the air dried soil samples. Gianello and Bremner (1986b) obtained values for ammonium nitrogen ranging from 8 to 158 mg/kg of soil after autoclaving in calcium chloride solution.

Table 7.6 compares the results of available nitrogen obtained by calcium chloride autoclaving method (fresh and air dried samples) with different soil properties. It shows that the results of fresh and air dried samples were correlated with % total nitrogen, % total C and total biomass C. However, the high correlation seems to be due to the two soils (Darleith 1 (Carbeth) and Dunlop)

which had high available nitrogen values after autoclaving in 0.01 M calcium chloride (see fig. 7.1 and 7.2). The correlation with % clay was poor and was negative with soil pH in water and in 0.01 M calcium chloride.

Soil properties	Calcium chloride autoclaving	
	fresh soil	air dried soil
autoclaving (fresh)	--	0.98 ***
autoclaving (dry)	0.98 ***	---
% Total N	0.72 *	0.74 *
% Total C	0.86 **	0.89 **
% Clay	0.21	0.28
pH in water	-0.63	-0.57
pH in $CaCl_2$	-0.64	-0.58
Biomass C	0.79 *	0.81 **

Table 7.6. Pearson product-moment correlation coefficient between the available nitrogen values obtained by the calcium chloride autoclaving method and other soil properties.

* significant at $p < 0.05$

** significant at $p < 0.01$

*** significant at $p < 0.001$

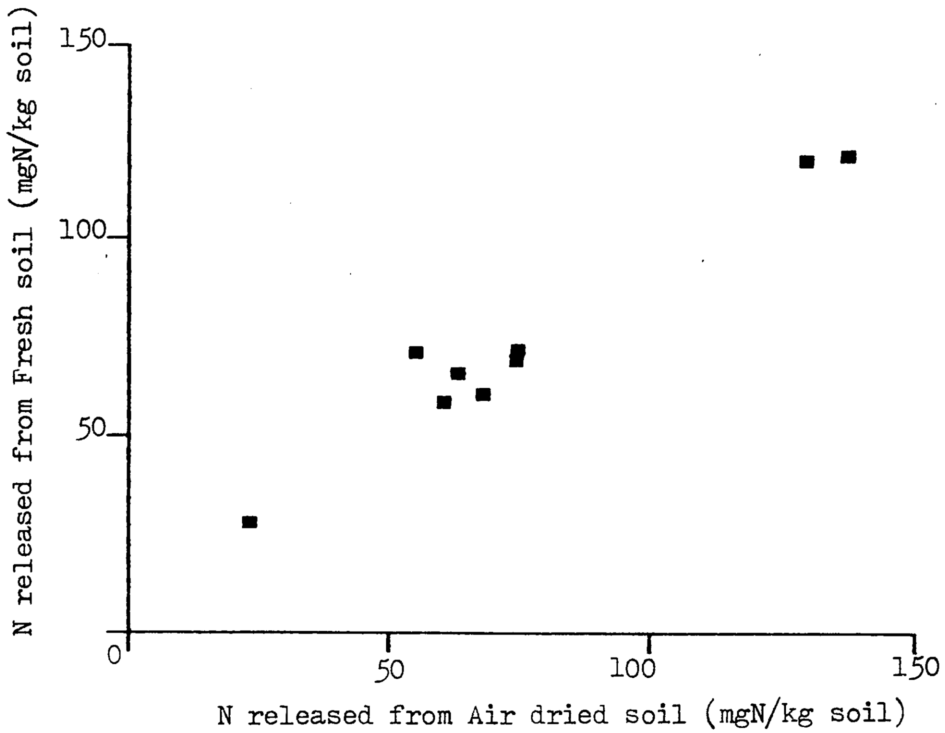


Fig. 7.1 Relationship between the amount of N released from Fresh and Air dried soils by autoclaving in 0.01M calcium chloride

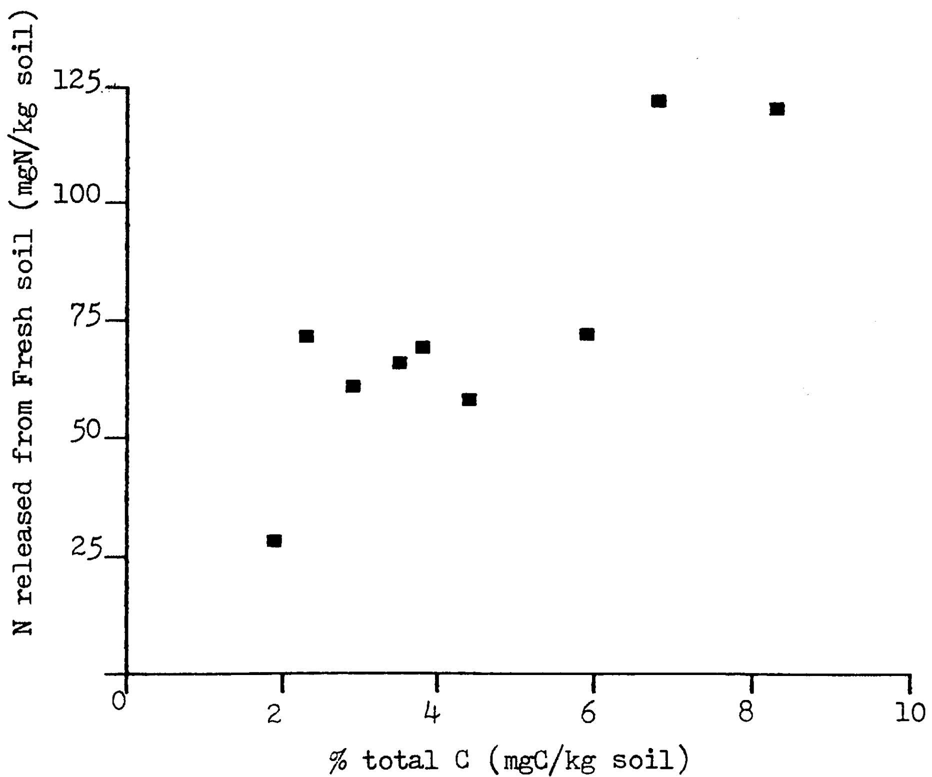


Fig. 7.2 Relationship between the amount of N released by autoclaving Fresh soils in 0.01M calcium chloride and % total C

CHAPTER EIGHT

MICROBIAL BIOMASS IN SOILS

8.1. INTRODUCTION

The important role of soil biomass concerns the conversion of essential elements from organic into inorganic compounds in which form these elements are normally assimilated by higher plants. This applies particularly to elements such as carbon and nitrogen which occur in large quantities in living organisms and their debris. The large size of microbial biomass in the soil implicates it as a major nutrient sink during carbon and nitrogen immobilization and as a source during mineralization.

The soil biomass contains 3 to 4 percent of the soil nitrogen and the top 25 cm of an arable soil contains approximately 200 kg per hectare of nitrogen in the microbial biomass (Jenkinson 1982). It has been reported by Anderson and Domsch (1980) that nitrogen in a single generation of the microbial biomass could supply many crops with much of the nitrogen they require to complete growth. Because the organic carbon that enters the soil is sufficient to allow the cells of the microflora to divide several times per year and because there is no evidence that the biomass of soils continuously increases in size, it is likely that the greater part of the nitrogen and

also the other plant nutrients contained in the microflora undergo several transformations each year.

The importance of microorganisms in ecosystem functioning has led to an increased interest in determination of soil microbial biomass. There are several simple methods for the measurement of biomass in soils. The chloroform fumigation method was developed by Jenkinson and Powlson (1976b). Anderson and Domsch (1978b) measured respiratory rate and related that to total microbial biomass in soils. Jenkinson and Oades (1979) and Oades and Jenkinson (1979) developed a method to measure adenosine triphosphate content in soil which reflects the total biomass content in soil. Sparling (1981) measured heat output from soils using microcalorimetry as a method for biomass determination. With the development of these methods, it has now become feasible to evaluate the soil microflora as both a sink and a possible source of nutrients to the plants. The chloroform incubation method proposed by Jenkinson (1976) was considered to be the most useful for soil studies. Jenkinson and co-workers introduced and then provided detailed analysis of a method for the estimation of the quantity of living microbial biomass in soils (Jenkinson, 1976; Jenkinson and Powlson, 1976a&b; Powlson and Jenkinson, 1976). In this method, the living biomass is made susceptible to mineralization by fumigating the soil with chloroform vapour and the soil is then reinoculated with small quantities of the original non-fumigated soil and incubated for 10 days, during which

time the freshly killed biomass is estimated by dividing the flush of carbon dioxide caused by fumigation by an experimentally determined fraction (k) equal to the fraction of the microbial biomass mineralized to carbon dioxide during the first 10 days following fumigation. Two major conditions are essential for the accuracy of this method. First, the fraction k, should approximately be equal to a constant value. Second the preparation of the samples should not alter carbonaceous residues associated with soil organic matter.

The value of k has been derived experimentally by various reasearch workers. Jenkinson (1966) initially proposed a value of 0.30 for k (i.e 30 % mineralization during the first 10 days following incubation). Since this value was derived from experiments conducted with a single bacterial species, it was considered provisional, and was later corrected to 0.5 (Jenkinson 1976) using data from experiments with 7 bacterial species, 4 fungal species and one earth worm species. The incubation of samples was carried out at 25 ^{o}C. Anderson and Domsch (1978a) proposed k factor of 0.41 at 22 ^{o}C which was later used by Voroney and Paul (1984). Adams and Laughlin (1981) reported a value of 0.50 for k at 25 ^{o}C based on 10 different organisms including both bacteria and fungi. A k value of 0.45 has been used by many research workers in their experiments. Anderson and Domsch (1978b) recommended that 0.45 would be probably the better estimation for 10 days aerobic incubation at 22 ^{o}C. Oades and Jenkinson (1979) used k value of 0.45 and

incubated samples at 25 ^{o}C. Shen et al. (1984) William and Sparling (1984), Ross and Tate (1984), and Chapman (1987) all used k to be 0.45 at 25 ^{o}C.

The use of fresh soil samples instead of air dried has been considered essential for measurement of microbial biomass (Jenkinson, 1976; Powlson and Jenkinson, 1976) because air drying before fumigation renders some non-biomass C decomposable as well as killing an appreciable fraction of the biomass. Thus, if an air dried soil is remoistened, one portion fumigated and incubated and a second portion incubated without fumigation, the carbon dioxide from both fumigated and unfumigated soils contains a component from the killed organisms and a component from the non-biomass organic matter rendered decomposable. The situation is analogous with that caused by mechanical disturbance, except that the effects of air drying are much greater than those of the rather gentle mechanical disturbance.

Jenkinson (1982) stated that it is most unlikely that any chemical method can measure the organic nitrogen about to be mineralized as this is distributed through various fractions of soil organic matter, the microbial biomass, partially decomposed plant material, the various humic materials etc. Since microorganisms are responsible for the production of nitrogen in forms usable by plants and to a less extent also for usable forms of phosphorus and sulphur, he suggested that the most successful tests are likely to be those that reflect the quantity of microbial mass present.

8.2. METHOD AND MATERIALS

50 g of (oven dry equivalent) fresh soil samples were taken in glass beakers. Half the samples were fumigated with chloroform for approximately 18 hours and half were left unfumigated. After fumigation, the samples were inoculated with 1 g of fresh soil. All the beakers containing fumigated and unfumigated samples were placed in 1.5 litre Kilner jars together with 25 cm^3 of 1 M sodium hydroxide. Blank incubations were also included. The samples were incubated in quadruplicate for a period of 20 days at 25 ^oC . Carbon dioxide evolved was measured two times (0 to 10 and 10 to 20 days) during the incubation period (see section 2.3). The biomass was calculated from the expression.

$B = (X-y)/k$ where

B is the soil biomass ,

X is the carbon dioxide-C evolved by the fumigated soil over the period 0 to 10 days,

y is the carbon dioxide-C evolved by unfumigated soil over the 10 to 20 days period. k was taken to be 0.45.

8.3. RESULTS AND DISCUSSION

In the present study soil biomass carbon was measured in a set of contrasting soils (9 different soils). They were freshly collected and taken with the minimum of soil disturbance and then fumigated with chloroform according to the method of Powlson and Jenkinson (1976b). Powlson and Jenkinson (1976) reported that samples should be used in the fresh state as air drying makes some non biomass organic material decomposable and killing a larger proportion of the soil biomass. Therefore, air dried samples were excluded from the test. Fumigation with chloroform caused the usual flush of decomposition (increases in carbon dioxide evolution) in all soils. Carbon dioxide evolution was markedly less during the 10 to 20 day period than during the 0 to 10 day. In general, the more organic matter in a soil the bigger the flush, although the relation is not exact. The rate of carbon dioxide evolution by fumigated soils was usually 2 to 3 times that of an unfumigated soils.

Table 8.1 shows the amounts of biomass carbon in the nine soils. The values for the amount of biomass in soil ranged from 150.9 to 1430.9 mg C/Kg of soil. These values are similar to the biomass estimated by Jenkinson and Powlson (1976b). They estimated the microbial biomass of a set of nine soils from long term field experiments at Rothamsted Experimental Station and the amount of biomass ranged from 170 to 1180 mg C/Kg of soil.

Table 8.2 shows how biomass C is related to other soil properties. The relationship with % total N and %

total C was good (r = 0.78 and 0.84 respectively). Biomass carbon increased with increase in the organic matter content of the soil (see fig 8.1) and with increase in percent total nitrogen.

Soil Series	Biomass C (mg/kg)
Midelney 1	682.9
Alluvium	159.3
Darvel	305.3
Darleith 2	998.0
Midelney 2	698.3
Dreghorn 1	416.9
Dreghorn 2	302.2
Caprington	1106.6
Dunlop	1438.5

Table 8.1. Total biomass carbon in the soils.

Soil properties	Biomass C
% Total N	0.78 *
% Total C	0.84 **
% Clay	0.44
pH (water)	-0.22
pH ($CaCl_2$)	-0.23

Table 8.2. Pearson product-moment correlation coefficient between soil biomass C and soil properties.

* significant at $p < 0.05$

** significant at $p < 0.01$

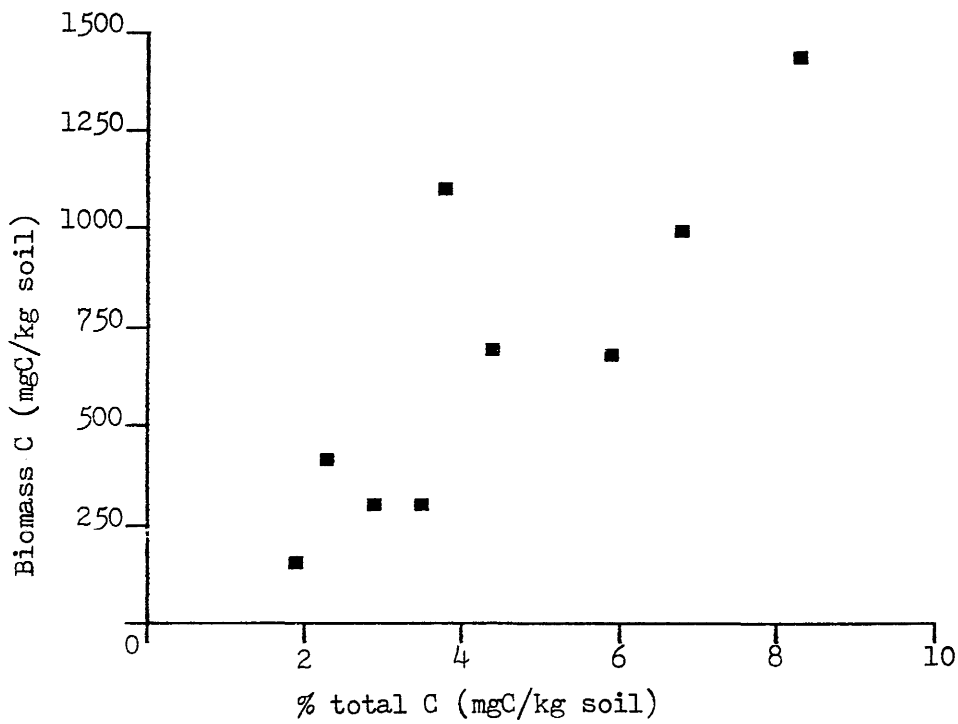


Fig. 8.1 Relationship between Biomass C and % total C in the soil

CHAPTER NINE

CHEMICAL/BIOLOGICAL MINERALIZATION METHOD FOR MEASURING AVAILABLE NITROGEN IN SOIL

9.1. INTRODUCTION

It would be of great interest to have a reliable and rapid method of evaluating the nitrogen status in soil. The best correlation between the nitrogen index and the yield or the uptake of nitrogen in plants has been found when the index includes both the inorganic nitrogen in the soil and the nitrogen being released from the organic compounds by incubation or by chemical methods (Jenkinson 1982).

Incubation of the soil is found to be one of the most reliable methods of evaluating the nitrogen reserves in soil organic matter. It is, however, difficult to get satisfactory reproducibility, because the analytical results are dependent on both pretreatment and storage of the soil samples. Moreover, incubation of soil samples is a time consuming and laborious task which requires controlled laboratory conditions.

Soil scientists have long been searching for a chemical method that would provide a reliable index of soil nitrogen mineralization. Of the various chemical indices that have been proposed, few have been shown to correlate well with nitrogen released by biological

methods for a broad range of soils and none have been put to general practical use in assessing the nitrogen supplying capacities of soils.

Most of the proposed chemical methods have involved measuring ammonium nitrogen or total organic nitrogen extracted by acid or alkaline oxidising reagents. Methods involving boiling of soil samples in water and then measuring total nitrogen extracted were proposed by Keeney and Bremner (1966b). Smith and Stanford (1971) proposed autoclaving in 0.01 M calcium chloride and determining the ammonium nitrogen released.

In a preliminary study of methods of extraction (see chapter 5) it was observed that the levels of inorganic nitrogen increased when the soil was shaken for an extended period of time at room temperature. The increase was attributed to biological mineralization of soil organic nitrogen. The release of nitrogen from soil organic matter was influenced by salinity and the nature of the cations present. Some of the cations like sodium or potassium disperse the soil organic matter and therefore, may render it more easily decomposable by the soil microorganisms. On the other hand cations like calcium flocculate the soil organic matter and render it less decomposable by the soil organisms.

Selmer-Olsen et al. (1974) stored 2 M potassium chloride soil suspensions in a refrigerator and at 20 ^{o}C room temperature and compared the inorganic nitrogen content with that produced after only 1 hour of shaking at room temperature. They observed that following storage in

a refrigerator for 17 days both ammonium and nitrate content were nearly the same as produced after 1 hour of shaking. However, the ammonium nitrogen content of the suspension increased several hundred percent during a storage period at room temperature, whereas the nitrate content was nearly the same. The results were attributed to biological activities which take place when the suspension is left at room temperature.

This chapter describes the investigation of a simple Chemical/Biological measurement of soil nitrogen availability. It involves shaking of soil samples in a salt solution (0.5 M potassium sulphate, 1 M potassium chloride, 1 M sodium chloride and 0.5 M calcium chloride) for 24 hours at 30 ^{o}C and determining inorganic nitrogen content (ammonium, nitrate and nitrite nitrogen) of the extracts with a Technicon AutoAnalyzer.

9.2. MATERIALS AND METHODS

2.5 g (oven dry equivalent) of fresh or air dried soil samples were added into 100 cm^3 plastic bottles. 50 cm^3 of the salts solutions (0.5 M potassium sulphate, 1 M potassium chloride, 1 M sodium chloride or 0.5 M calcium chloride) were then added. The salts solutions were cleaned of ammonium nitrogen contamination by raising the pH to 11.0 with an alkali solution and then boiling and stirring for 15 minutes to release ammonia gas from the solutions. The pH was readjusted to original pH with dilute acids. Half of the samples were shaken for 2 hours at 2 ^oC and half were shaken for 24 hours at 30 ^oC. The suspensions were filtered through Whatman filter paper No. 40 which were previously washed with 50 cm^3 of the salt solutions in 2 lots and then finally washed with deionized water. The filtrates were then analysed for their ammonium, nitrate and nitrite nitrogen content (see section 2.9). In order to avoid any chloride ion interference in the nitrate nitrogen analysis, the hydrazine content of the reducer was increased from 0.15 to 0.30 when using chloride salt solutions as an extractant. To stop the formation of precipitation due to calcium ions in the ammonium nitrogen analysis, the extracts with calcium chloride were diluted 5 times with a dilution system. Blanks were also included by shaking the salts solutions only. Replicates were done 4 times for each soil and treatment.

9.3. RESULTS

Details of inorganic nitrogen (ammonium, nitrate and nitrite) extracted from fresh and air dried soils with 0.5 M potassium sulphate and 1 M potassium chloride solutions at 2 ^{O}C, 2 hour shaking and 30 ^{O}C and 24 hours shaking are given in tables 9.1 to 9.4. Details are not given for 1 M sodium chloride and 0.5 M calcium chloride as there were no major differences in the results.

There were real changes in the inorganic nitrogen levels of all soils when the shaking period and temperature were increased. There was, overall, more ammonium nitrogen when the soils were extracted at 30 ^{O}C and 24 hours shaking compared with 2 ^{O}C and 2 hours shaking. The nitrite nitrogen levels also increased in some of the soils (Midelney 1 (Grass), Midelney 2 (Arable), Caprington and Alluvium) when extracted with 0.5 M potassium sulphate at 30 ^{O}C. The trend of increase was more in the air dried soils than in the fresh soils. Some significant decreases in the levels of nitrate nitrogen were also noted in some of the soils particularly those extracted with 0.5 M potassium sulphate solution when the extraction was done at 30 ^{O}C and 24 hours shaking for example Darvel fresh, Alluvium and Caprington air dried.

Soil Series	2 h at 2 °C			24 h at 30 °C		
	NH_4-N	NO_3-N	NO_2-N	NH_4-N	NO_3-N	NO_2-N
Midelney 1	0.3	30.2	0.8	0.6	49.6	15.1
Alluvium	0.9	27.7	0.2	10.1	31.6	0.4
Darvel	0.9	13.8	0.0	6.8	13.3	0.0
Darleith 2	6.1	3.1	0.0	14.8	3.7	0.0
Midelney 2	0.4	161.4	0.8	4.6	168.7	5.4
Dreghorn 1	1.2	15.7	0.0	9.0	15.8	0.1
Dreghorn 2	1.3	37.1	0.0	9.5	36.7	0.1
Caprington	1.6	23.1	0.3	22.1	28.4	0.9
Dunlop	4.3	4.7	0.0	24.8	4.6	0.1

Table 9.1. Extraction of mineral nitrogen from fresh soils with 0.5 M potassium sulphate at different temperatures and shaking periods. (mg N / kg soil).

Soil Series	2 h at 2 °C			24 h at 30 °C		
	NH_4-N	NO_3-N	NO_2-N	NH_4-N	NO_3-N	NO_2-N
Midelney 1	5.9	15.1	0.6	30.9	23.5	6.2
Alluvium	3.3	21.3	0.7	12.0	20.0	0.9
Darvel	2.3	6.2	0.0	5.9	6.1	0.0
Darleith 2	10.9	1.1	0.0	18.5	0.0	0.0
Midelney 2	2.9	145.5	0.4	11.9	143.4	1.5
Dreghorn 1	3.5	4.7	0.0	7.2	4.2	0.1
Dreghorn 2	3.2	27.6	0.1	7.6	26.6	0.1
Caprington	5.9	8.9	0.1	50.8	7.9	0.3
Dunlop	9.2	0.1	0.0	21.1	0.0	0.1

Table 9.2. Extraction of mineral nitrogen from air dried soils with 0.5 M potassium sulphate at different temperatures and shaking periods. (mg N / kg soil).

Soil Series	2 h at 2 °C			24 h at 30 °C		
	NH_4-N	NO_3-N	NO_2-N	NH_4-N	NO_3-N	NO_2-N
Midelney 1	0.9	34.0	0.0	20.2	35.1	0.1
Alluvium	0.8	31.1	0.1	8.3	32.0	0.3
Darvel	0.5	13.5	0.0	2.3	14.7	0.0
Darleith 2	4.6	2.0	0.0	12.3	2.7	0.0
Midelney 2	0.5	160.2	0.2	6.2	168.8	0.5
Dreghorn 1	0.7	15.9	0.0	4.0	17.2	0.0
Dreghorn 2	0.6	40.0	0.0	4.1	40.0	0.0
Caprington	1.1	21.4	0.0	27.8	23.3	0.1
Dunlop	4.2	6.4	0.0	19.5	7.2	0.0

Table 9.3. Extraction of mineral nitrogen from fresh soils with 1 M potassium chloride at different temperatures and shaking periods. (mg N / kg soil).

Soil Series	2 h at 2 °C			24 h at 30 °C		
	NH_4-N	NO_3-N	NO_2-N	NH_4-N	NO_3-N	NO_2-N
Midelney 1	5.6	20.0	0.2	29.8	19.9	0.6
Alluvium	3.3	25.2	0.6	9.9	26.1	0.7
Darvel	2.1	6.3	0.0	2.9	6.9	0.0
Darleith 2	10.0	0.2	0.0	14.4	0.0	0.0
Midelney 2	2.2	154.7	0.2	9.6	155.4	0.4
Dreghorn 1	3.2	5.3	0.0	4.4	5.3	0.0
Dreghorn 2	2.9	30.2	0.0	5.9	29.2	0.0
Caprington	5.0	9.6	0.1	43.1	9.5	0.1
Dunlop	8.8	1.9	0.0	14.4	1.7	0.0

Table 9.4. Extraction of mineral nitrogen from air dried soils with 1 M potassium chloride at different temperatures and shaking periods. (mg N / kg soil).

In order to summarize the values for all four extractants (0.5 M potassium sulphate, 1 M potassium chloride, 1 M sodium chloride and 0.5 M calcium chloride) the total inorganic nitrogen extracted from fresh and air dried soils at 2 ^{o}C and 30 ^{o}C and the increase are presented in tables 9.5 to 9.12. Total inorganic nitrogen was calculated as the mean of 4 individual replicates and the increase was calculated from the difference between the two means. Increases in total inorganic nitrogen were shown by all soils with the different salt solutions. All the soils showed a similar pattern, Midelney 1 (Grass) and Caprington showed higher total inorganic levels while Darvel and Dreghorn 1 (Ayr) soils showed lower values. The general trend for both fresh and air dried samples for the various salt solutions was potassium sulphate > sodium chloride > potassium chloride > calcium chloride. For all the salt solutions, the fresh soils gave higher values than air dried ones, although there were exceptions, for example Caprington soil.

Soil Series	Inorganic N (mg/kg)		
	2 °C	30 °C	Increase
Midelney 1	31.1	65.3	34.2
Alluvium	28.8	42.1	13.3
Darvel	14.7	20.1	5.4
Darleith 2	9.3	18.6	9.3
Midelney 2	162.6	178.7	16.1
Dreghorn 1	16.9	24.8	7.9
Dreghorn 2	38.4	46.3	7.9
Caprington	25.0	51.4	26.4
Dunlop	9.0	29.4	20.4
		mean	15.6

Table 9.5. Total inorganic nitrogen content of fresh soils after different shaking periods and temperatures in 0.5 M potassium sulphate solution and the increase due to organic nitrogen mineralization.

Mean of 4 replicates

Soil Series	Inorganic N (mg/kg)		
	2 °C	30 °C	Increase
Midelney 1	21.6	54.6	33.0
Alluvium	25.3	32.9	7.6
Darvel	8.6	12.0	3.4
Darleith 2	12.0	18.5	6.5
Midelney 2	148.9	156.9	8.0
Dreghorn 1	8.2	11.5	3.3
Dreghorn 2	30.8	34.2	3.4
Caprington	14.9	59.0	44.1
Dunlop	9.3	21.1	11.8
		mean	13.5

Table 9.6. Total inorganic nitrogen content of air dried soils after different shaking periods and temperatures in 0.5 M potassium sulphate solution and the increase due to organic nitrogen mineralization.

Mean of 4 replicates

Soil Series	Inorganic N (mg/kg)		
	2 °C	30 °C	Increase
Midelney 1	34.9	55.5	20.6
Alluvium	32.0	40.5	8.5
Darvel	14.0	17.0	3.0
Darleith 2	6.6	15.0	8.4
Midelney 2	160.8	175.5	14.7
Dreghorn 1	16.5	21.2	4.7
Dreghorn 2	40.6	44.0	3.5
Caprington	22.5	51.3	28.8
Dunlop	10.6	26.7	16.1
		mean	12.3

Table 9.7. Total inorganic nitrogen content of fresh soils after different shaking periods and temperatures in 1 M potassium chloride solution and the increase due to organic nitrogen mineralization.

Mean of 4 replicates

Soil Series	Inorganic N (mg/kg)		
	2 °C	30 °C	Increase
Midelney 1	25.6	50.3	24.7
Alluvium	28.6	36.7	8.1
Darvel	8.4	9.9	1.5
Darleith 2	10.2	14.4	4.2
Midelney 2	156.9	165.3	8.4
Dreghorn 1	8.5	9.7	1.3
Dreghorn 2	33.1	35.2	2.1
Caprington	14.6	52.7	38.1
Dunlop	10.8	16.1	5.3
		mean	10.4

Table 9.8. Total inorganic nitrogen content of air dried soils after different shaking periods and temperatures in 1 M potassium chloride solution and the increase due to organic nitrogen mineralization.

Mean of 4 replicates

Soil Series	Inorganic N (mg/kg)		
	2 ^{o}C	30 ^{o}C	Increase
Midelney 1	35.9	59.4	23.5
Alluvium	31.6	41.4	9.8
Darvel	14.3	17.6	3.3
Darleith 2	8.6	14.5	6.0
Midelney 2	173.9	185.2	11.3
Dreghorn 1	15.9	21.7	5.8
Dreghorn 2	39.7	43.9	4.2
Caprington	25.1	53.4	28.3
Dunlop	9.2	25.3	16.1
		mean	12.3

Table 9.9. Total inorganic nitrogen content of fresh soils after different shaking periods and temperatures in 1 M sodium chloride solution and the increase due to organic nitrogen mineralization.

Mean of 4 replicates

Soil Series	Inorganic N (mg/kg)		
	2 ^{o}C	30 ^{o}C	Increase
Midelney 1	24.2	50.4	26.2
Alluvium	27.7	35.2	7.5
Darvel	8.4	10.0	1.7
Darleith 2	11.2	14.3	3.2
Midelney 2	158.7	163.6	4.9
Dreghorn 1	7.2	10.4	3.2
Dreghorn 2	31.1	36.1	5.0
Caprington	15.5	50.9	35.4
Dunlop	9.6	17.1	7.5
		mean	10.5

Table 9.10. Total inorganic nitrogen content of air dried soils after different shaking periods and temperatures in 1 M sodium chloride solution and the increase due to organic nitrogen mineralization.

Mean of 4 replicates

Soil Series	Inorganic N (mg/kg)		
	2 ^{o}C	30 ^{o}C	Increase
Midelney 1	34.3	48.7	14.4
Alluvium	30.7	38.9	8.2
Darvel	14.2	18.0	3.8
Darleith 2	9.1	18.3	9.3
Midelney 2	155.3	162.2	6.9
Dreghorn 1	16.6	20.8	4.2
Dreghorn 2	37.0	41.1	4.1
Caprington	21.1	45.6	24.5
Dunlop	8.6	26.6	18.0
		mean	10.4

Table 9.11. Total inorganic nitrogen content of fresh soils after different shaking periods and temperatures in 0.5 M calcium chloride solution and the increase due to organic nitrogen mineralization.

Mean of 4 replicates

Soil Series	Inorganic N (mg/kg)		
	2 °C	30 °C	Increase
Midelney 1	24.8	43.2	18.4
Alluvium	27.8	31.4	3.6
Darvel	8.4	10.3	2.1
Darleith 2	12.0	14.1	2.1
Midelney 2	149.4	156.1	6.7
Dreghorn 1	7.8	10.4	2.6
Dreghorn 2	30.0	32.1	2.1
Caprington	13.2	59.1	45.9
Dunlop	9.5	14.8	5.3
		mean	9.9

Table 9.12. Total inorganic nitrogen content of air dried soils after different shaking periods and temperatures in 0.5 M calcium chloride solution and the increase due to organic nitrogen mineralization.

Mean of 4 replicates

9.4. DISCUSSION

The technique adopted for assessment of the potentially mineralizable nitrogen in soil was based on the determination of the ammonium, nitrate and nitrite nitrogen released when the soils were shaken for 24 hours at 30 ^{o}C in various salt solutions. The method is, therefore, different from other chemical and incubation techniques as it involves both chemical and biological processes for the mineralization of soil organic nitrogen. Processes of mineralization, nitrification and denitrification were all found to occur in fresh and air dried samples. Nitrification was higher in the fresh soil samples while mineralization and denitrification were higher in the air dried samples. The high rate of mineralization in the dried samples was attributed to the effect of drying treatment on the organic matter decomposition. (see section 6.4)

In the present investigation all the 9 soils showed an increase in soil nitrogen mineralization after 24 hours of shaking at 30 ^{o}C. The rate of mineralization ranged from 5.0 to as high as 45.0 mg N / kg soil per day. These values seem to be much higher than those reported previously for the incubation of soil in the fresh state at temperatures in the range of 20 to 35 ^{o}C and have applied zero-order rate constant to calculate the rate of nitrogen mineralization. For example Tabatabai and Alkhafaji (1980) studied nitrogen and sulphur mineralization in 12 different fresh soil samples at 35

^{o}C. They reported rates of nitrogen mineralization ranging from 1.1 to 2.4 mg / kg soil per day. Similarly Flowers and Arnold (1983) used 2 different soils in their incubation experiments and reported nitrogen mineralization rates of 0.71 and 1.04 mg/kg soil per day at 30 ^{o}C. Addiscott (1983) used 3 soils which were incubated at 25 ^{o}C. The rate of nitrogen mineralization in the soils ranged from 0.21 to 0.40 mg / kg soil per day.

All the different salt solutions which were used during shaking / extraction had a stimulating effect on the formation of ammonium nitrogen although the effect was different for different extracting solutions. For example the potassium and sodium solutions yielded more ammonium than calcium solution. This was probably due to the fact that calcium salts flocculate the soil organic matter and thereby make it less susceptible to microbial decomposition. On the other hand the sodium and to a lesser degree potassium salts help in dispersion of the soil organic material thereby making it more susceptible to microbial decomposition.

The 0.5 M potassium sulphate salt solution released more total inorganic nitrogen from the soil organic matter compared with potassium chloride or sodium chloride. This was probably due to the effect of concentration of the extracting solution on the soil microorganisms (see section 5.4). The osmotic pressure of 0.5 M potassium sulphate would be slightly lower than that of M sodium chloride or M potassium chloride and would have, therefore, less effect on biological activities in the

soil. Selmer-Olsen et al. (1974) studied the effect of different concentrations of potassium chloride solution on the ammonium and nitrate nitrogen levels by incubating soils for 14 days in 0, 0.25, 0.5, 1.0, 2.0 and 2.5 M potassium chloride and found that smaller concentrations of potassium chloride (0.25 to 0.5 M) had a stimulating effect on the release of inorganic nitrogen whereas higher concentrations were, in general found to be inhibitory.

If it is desired to use a more dilute salt solution to increase biological activities, it is necessary to see whether it will reduce the chemical effect of the salt or whether the extractant would be as efficient. Bremner and Keeney (1966) showed that the amount of inorganic nitrogen extracted by shaking the soil at room temperature for one hour is the same whether 1 M, 2 M or 4 M potassium chloride is used provided that the amount of potassium chloride solution employed contains the equivalent of 20 meq of K per g of soil. It may be, therefore, possible to increase the solution : soil ratio to maintain the efficiency of the extractant but that will give lower concentration of nitrogen in solution for measurement which could provide a problem.

The automated colorimetric methods used for analysis are sensitive to various interfering ions. The chloride ion interferes in the nitrate nitrogen analysis. High concentrations of chloride ions affect the reduction of nitrate to nitrite. In order to overcome the chloride ion interference, the hydrazine concentration was increased from 0.15 to 0.30 g per litre and at this level there was

no obvious interference of chloride ions. The divalent cations like calcium, magnesium and iron in the extract produce precipitates in the ammonium nitrogen system. The citrate tartrate complexing reagent is intended to remove divalent ions from solution but is not strong enough to complex the calcium in M/2 solution. The calcium ion interference was eliminated by using the dilution system whereby the extracts were diluted 5 times giving M/10 calcium in solution which the citrate tartrate solution is able to cope with.

There was reduction in the nitrate nitrogen in some of the soils which was attributed to biological denitrification. Similar results were obtained in the case of Downholland soil (section 5.3.4) where the levels of nitrate nitrogen decreased after 24 hours shaking compared with 1 hour. Although the shaking bottles were only half full, the solution could become anaerobic toward the end of the shaking period. It might be occurring in other soils but would not be easily detectable. It can be easily controlled by using a nitrification inhibitor during the extraction process in which case only ammonium nitrogen would need be measured.

The correlations between the nitrogen mineralized in the various salt solutions are shown in table 9.13 (fresh soils) and table 9.14 (air dried soils). A high correlation is shown between the different salt solutions. Figures 9.1 and 9.2 show as examples the relationship between nitrogen mineralised in 1 M potassium chloride and 1 M sodium chloride in the fresh and air dried soils. The

points on the graphs for the fresh soils were in general, well scattered and the correlations were, therefore, reliable. But the points in the graphs for the air dried soils were not well scattered and were more or less found in two clusters. Therefore, the correlations were less reliable.

The good correlations between the salt solutions indicate that any of these might provide equally good information about available nitrogen in soils.

	potassium sulphate	potassium chloride	sodium chloride
potassium chloride	0.89	----	----
sodium chloride	0.94	0.98	----
calcium chloride	0.78	0.92	0.91

Table 9.13 Pearson product-moment correlation coefficients between the amounts of organic nitrogen mineralised from fresh soils in different salt solutions.

values are significant if > 0.67 (p<0.05), if > 0.80 (p<0.01) or if >0.90 (p<0.001).

	potassium sulphate	potassium chloride	sodium chloride
potassium chloride	0.99	----	----
sodium chloride	0.99	0.99	----
calcium chloride	0.95	0.97	0.95

Table 9.14 Pearson product-moment correlation coefficients between the amounts of organic nitrogen mineralized from air dried soils in different salt solutions.

values are significant if > 0.67 (p<0.05), if > 0.80 (p<0.01) or if >0.90 (p<0.001).

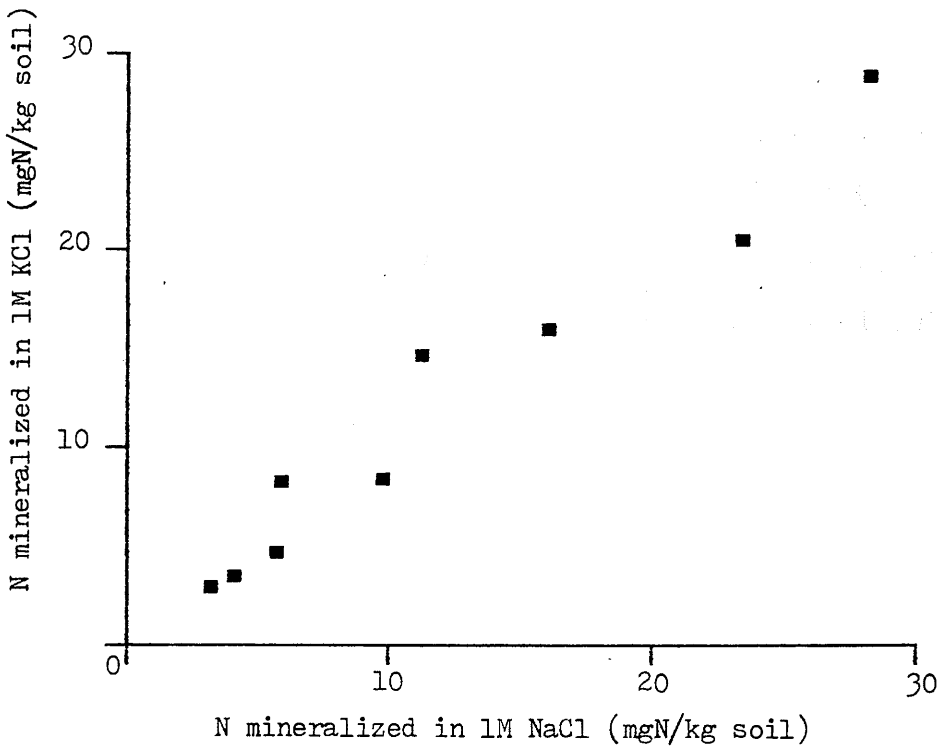


Fig. 9.1 Relationship between the amount of N mineralized in 1M KCl and 1M NaCl (Fresh soil)

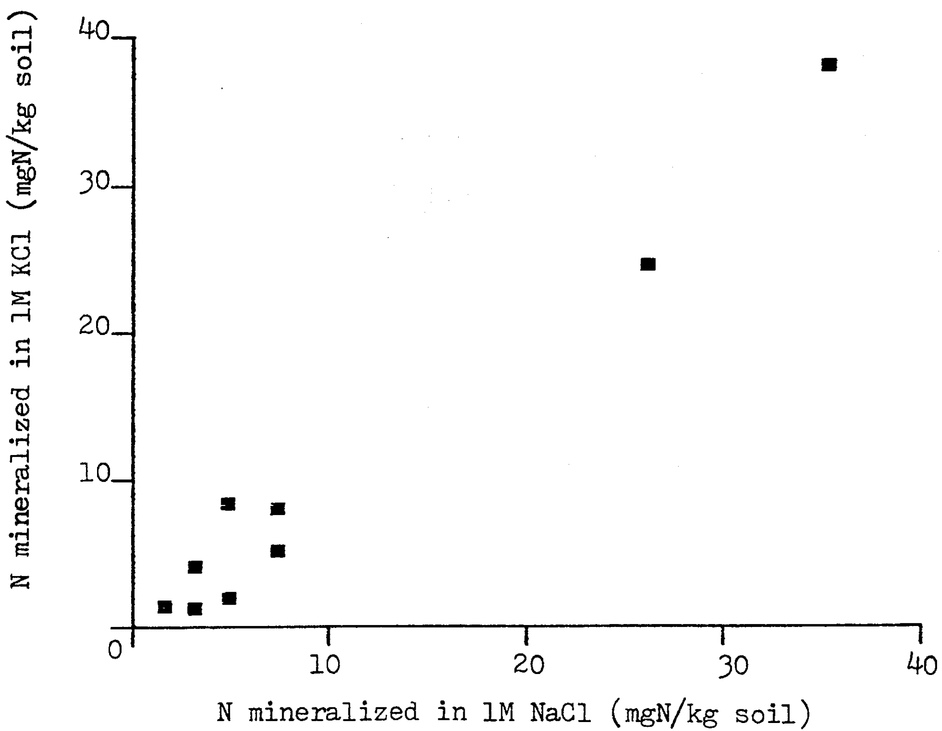


Fig. 9.2 Relationship between the amount of N mineralized in 1M KCl and 1M NaCl (Air dried soil)

After making all the necessary changes to overcome the chemical interference during analysis, it may be possible to develop the method further by looking at:-

(i) use of nitrification inhibitors which would solve the problem of denitrification and would simplify the analysis because only the ammonium nitrogen released would be measured.

(ii) using different salt concentrations to find a suitable salt concentration which could be used for routine study of nitrogen availability indices in soils and (iii) to compare different cations for their ability in releasing nitrogen from the soil organic matter.

The present study offers the potential to develop a method of measuring soil nitrogen availability in which both chemical and biological processes operate concurrently. It has a quick time scale of 24 hours shaking at 30 ^{o}C and could, therefore, be developed for routine use in the soil testing laboratories.

9.5. CONCLUSIONS

These conclusions are based on the work reported in chapters 6, 7, 8 and 9. The work was confined to a small number of soils which were selected from various locations in England and Scotland to obtain a wide range of properties. Correlations were, therefore, carried out with great caution. Results for the Midelney 2 (Arable) fresh soil were not included in the correlation studies because of its long period of immobilization, the difficulty in fitting regression lines to calculate mineralization rate constants and the very low values of the mineralization rate constant, which would have had excessive influence on the correlation results. Correlations involving zero order mineralization rate constants for fresh soils were, therefore, calculated using 8 soils while all other correlations were calculated using 9 soils.

The main measurement was considered to be the rate of nitrogen mineralization in the fresh soils as the actual field conditions are best represented by fresh soil samples and not air dried. Since the majority of workers have used air dried samples during their incubation experiments for comparison with the results of various soil tests; the rate constants in air dried soils, the total nitrogen mineralized by the air dried soils and potentially mineralizable nitrogen are also included for the comparison with the various soil tests. Mineralization rate constants and potentially mineralizable nitrogen in the air dried soils were calculated by the method of

Stanford and Smith (1972), but were based on their first approximation and not their method of successive approximation as no computer programme was available.

In the present work, correlation coefficients between various soil parameters were calculated and presented in tables 9.15 and 9.16.

The zero order nitrogen mineralization rate constant for fresh soils was poorly correlated with the nitrogen released by autoclaving of soil samples in calcium chloride, biomass carbon, % total C, % total N and other soil properties. Interestingly, there was also a poor correlation between nitrogen mineralization rate constants in the fresh and in the air dried soils. The nitrogen mineralization rate constant in the fresh soils was best correlated with the nitrogen released by shaking with the different salt solutions and with the total nitrogen mineralized by fresh soils.

Although there was a significant correlation between the mineralization rate constants (fresh soil) and the nitrogen mineralized from fresh and air dried soils on shaking with the various salt solutions, fig 9.3 and 9.4 show that the points are not well scattered and therefore, the relationship must be treated with caution. However, improvements to the method have been suggested earlier and it may be possible to develop the method further and obtain a better correlation with a wider range of soils.

Soil parameters	N rate constant
% total C	0.09
% total N	0.17
% clay	0.42
pH in water	0.31
pH in $CaCl_2$	0.33
Biomass C	0.33
C rate constant (fresh)	-0.57
C mineralized (dry)	0.10
N rate constant (fresh)	-
N mineralized (fresh)	0.75
N rate constant (dry)	0.38
N mineralized (dry)	0.31
Potentially mineralizable N	-0.14
Autoclaving in $CaCl_2$ (fresh)	-0.01
Autoclaving in $CaCl_2$ (dry)	0.0
K_2SO_4 shaking (fresh)	0.67
KCl shaking (fresh)	0.79
NaCl shaking (fresh)	0.78
$CaCl_2$ shaking (fresh)	0.67
K_2SO_4 shaking (dry)	0.89
KCl shaking (dry)	0.89
NaCl shaking (dry)	0.86
$CaCl_2$ shaking (dry)	0.87

Table 9.15. Pearson product-moment correlation coefficient for the zero order nitrogen mineralization rate constant in fresh soils with the various soil parameters.

Values > 0.71 are significant at $p< 0.05$
values > 0.83 at $p< 0.01$
values > 0.93 at $p< 0.001$.

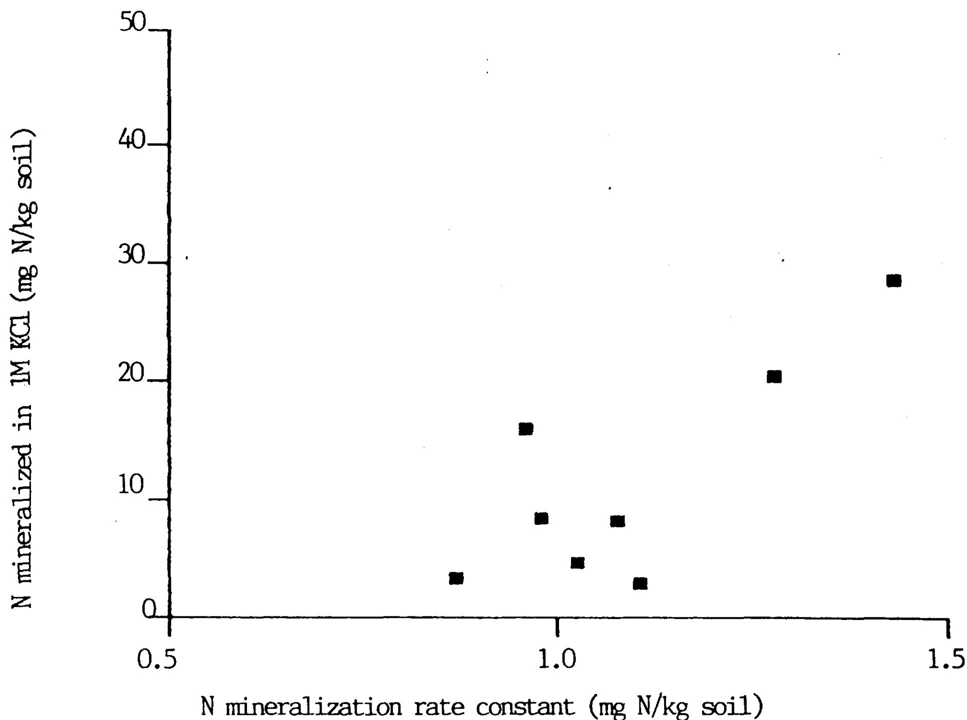


Fig. 9.3 Relationship between the N mineralization rate constant in fresh soil and the amount of N mineralized in fresh soil on shaking in 1M KCl.

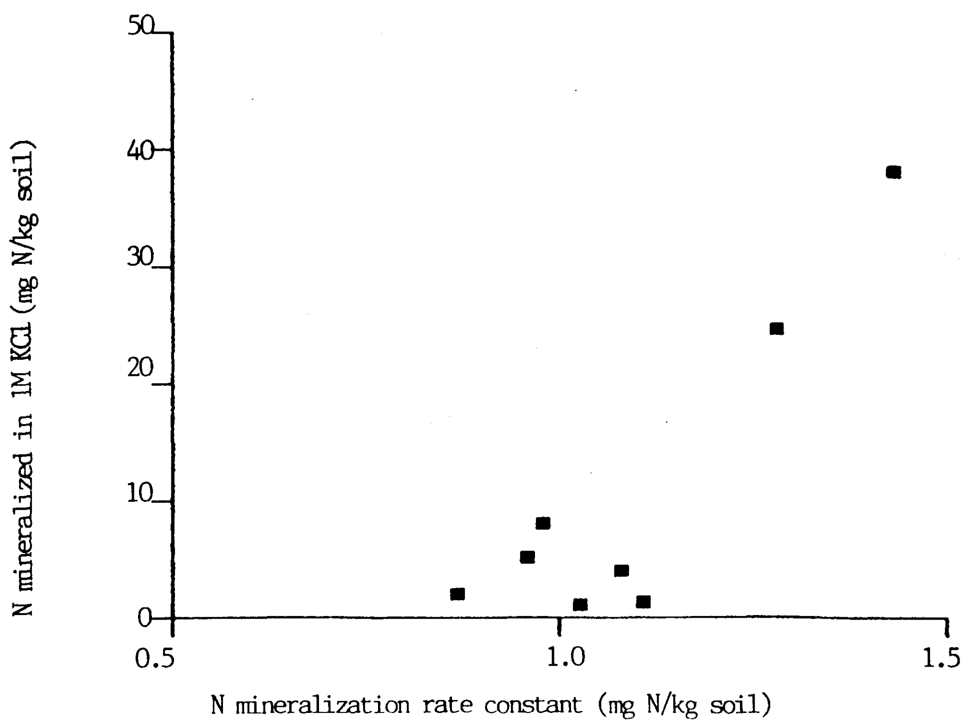


Fig. 9.4 Relationship between the N mineralization rate constant in fresh soil and the amount of N mineralized in air dried soil on shaking in 1M KCl.

Soil parameters	No	N rate constant	Total N mineralised
% total C	0.82	-0.75	0.81
% total N	0.87	-0.69	0.79
% clay	0.69	-0.39	0.36
pH in water	0.19	0.24	-0.05
pH in $CaCl_2$	0.19	0.26	-0.11
Biomass C	0.87	-0.85	0.86
C rate constant (fresh)	0.64	-0.63	0.82
C mineralized (dry)	0.77	-0.77	0.93
N rate constant (fresh)	-0.14	-0.15	0.44
N mineralized (fresh)	-0.16	0.19	-0.02
N rate constant (dry)	-0.70	-	-0.72
N mineralized (dry)	0.79	-0.72	-
Potentially mineralizable N	-	-0.70	0.79
Autoclaving $CaCl_2$ (fresh)	0.58	-0.75	0.71
Autoclaving in $CaCl_2$ (dry)	0.62	-0.71	0.71
K_2SO_4 shaking (fresh)	0.65	-0.28	0.63
KCl shaking (fresh)	0.69	-0.46	0.61
NaCl shaking (fresh)	0.61	-0.36	0.63
$CaCl_2$ shaking (fresh)	0.68	-0.57	0.79
K_2SO_4 shaking (dry)	0.42	-0.27	0.51
KCl shaking (dry)	0.35	-0.20	0.40
NaCl shaking (dry)	0.33	-0.16	0.45
$CaCl_2$ shaking (dry)	0.29	-0.25	0.36

Table 9.16. Pearson product-moment correlation coefficient for potentially mineralizable nitrogen (No),first order nitrogen mineralization rate constant in air dried soils and total nitrogen mineralized by air dried soils with the various soil parameters.

Values > 0.67 or < -0.67 are significant at $p < 0.05$
values > 0.80 or < -0.80 at $p < 0.01$
values > 0.90 or < -0.90 at $p < 0.001$.

The first order nitrogen mineralization rate constant and total nitrogen mineralized in the dried soils were correlated with % total C , % total N , Biomass C , Carbon mineralised and the nitrogen released by autoclaving in calcium chloride. However, the nitrogen mineralization rate constant in the air dried soils was poorly correlated with the nitrogen released by shaking of air dried or fresh soils with different salt solutions. Potentially mineralizable nitrogen was not correlated with the nitrogen released from soils after autoclaving in calcium chloride.

There is not much information available in the literature where the nitrogen mineralized during the incubation of fresh soil samples has been used for comparison with other soil parameters. Magdoff, et al. (1983) compared nitrogen mineralised from fresh soil with the nitrogen released by autoclaving in 0.01 M calcium chloride. They reported a high correlation between the two methods of nitrogen availability measurement. Their results disagree, therefore, with those reported in the present study. However, most of their soils were amended with sewage sludge which might have provided a source of mineralizable nitrogen for both methods much larger than the pool of mineralizable soil organic nitrogen.

Results of aerobic incubation of air dried samples have been compared by several workers with various chemical tests of soil nitrogen availability. Stanford and Smith (1976) found a high correlation between

potentially mineralizable nitrogen and the ammonium nitrogen extracted from soil by autoclaving in calcium chloride. Oien and Selmer-Olsen (1980) found a good correlation between the nitrate nitrogen obtained by aerobic incubation and the ammonium nitrogen released from the soil organic matter after heating in 2 M potassium chloride for 20 hours at 80 ^{o}C. Fox and Piekielek (1984) found a good correlation between the nitrogen mineralized anaerobically in air dried soil, total nitrogen and boiling 0.01 M calcium chloride extractable nitrogen. More recently Gianello and Bremner (1986b) compared the results of nitrogen mineralized during aerobic incubation of air dried soils with a list of 12 different chemical methods of assessing potentially available organic nitrogen in soils and they reported a good correlation of all the chemical soil tests with the biological test (aerobic incubation). Similar results have been obtained in the present work with air dried soils but not with fresh soils.

The method described in section 9.5 (Chemical/Biological method of measuring available nitrogen in soil) may be further improved for routine use in soil testing. It has the advantage that it involves both chemical and biological processes. If further studies were made and the method correlated well with the nitrogen supplying capability of a wide range of soils it could provide useful information on the mineralizable nitrogen in soils and could aid in making nitrogen fertilizer recommendations. The test is simple, quick and inexpensive

and would, therefore, not only increase the efficiency of nitrogen fertilizer use by farmers, but would also minimise possible water and air pollution by excess nitrogen fertilization.

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