TRANSFORMATIONS OF NITROGEN AND ITS AVAILABILITY TO PLANTS IN COAL MINE SOILS

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CONTENTS

SUMMARY

The work of this thesis is concerned with the study of some aspects of nitrogen cycling in coal mine soils. A survey of sites in Central Scotland was made to assess which parts of the N cycle function in coal mine soils. 90 samples of widely varying properties were taken and various properties measured. The mineral N status of the waste material was low. Carbon dioxide evolution and nitrogen mineralization rates showed that a high level of carbon, but little nitrogen was turned over. Nitrification was measurable only on about half of the sites studied and was highly pH dependent, being inhibited below pH 5.0. However, even on sites above this pH, nitrification was not always measured which suggests that the introduction of nitrifying bacteria into the spoil was also an important factor. Some nitrogen was lost due to the fixation of ammonium by the clay minerals and much more was lost during incubation due to immobilization. The urease and amidase activities measured were similar to those in agricultural soils which suggests that the use of urea or amide-N fertilizers may be possible on these coal mine soils. Significant correlations of carbon turnover, urease activity, amidase activity and nitrification rate with each other, suggest the importance of organic matter for both microbial activity and enzyme stabilization.

A laboratory incubation experiment was carried out to study the fate of nitrogen added as ammonium sulphate,

urea or chicken manure in five selected samples of coal mine soils with different properties. Two samples showed no net mineralization of N at any stage in either the control, ammonium sulphate or urea treated samples. Two samples which were collected from the plots of an established organic manure trial, showed N mineralization rates comparable to agricultural soils suggesting the favourable effect of organic amendments in the long term on the establishment of N cycling. There were large losses of N in the first weeks of incubation, which were generally greater in the manure treated samples, where up to 69 % of added N was lost. These losses were attributed to a combination of ammonium fixation and immobilization. In the later stages of the incubations there was a clear contribution to mineralization from the chicken manure. Mineralization and nitrification rates were significantly improved by manure addition in all samples, especially in the acid soil where these processes did not occur with ammonium sulphate or urea addition. The manure not only increased the pH of the acid soil, but may have also added nitrifying bacteria to the soil. In general however, it would seem that these transformations of nitrogen species can occur in coal mine soils if other conditions, particularly pH, are suitable.

A field experiment was set up to study nitrogen response on a reclaimed site for two years in 1986 and 1987. Seven rates of N fertilizer ranging between 0 and 150 kg N/ha as ammonium nitrate were added to triplicate plots. An attempt was also made to study the herbage

response to N both in the presence and absence of added P and K, and to compare urea with ammonium nitrate. Nitrogen fertilizer significantly improved the herbage yield in both years, which was also reflected generally in higher N, P, and K contents in the herbage. In addition P and K tended to increase the yield of herbage when applied with N but their effect was not significant. Vegetation responded equally to both N fertilizers with no significant differences in herbage yield.

CHAPTER 1 GENERAL INTRODUCTION

Land is one of our major natural resources and one of our most real assets. The most important element of this natural resource is the soil, for within it are held the nutrients and water for growth of plants, including those which provide us with materials for food and shelter. Although a stable fertile soil system is built up over centuries, it can be destroyed very easily when industrial processes produce derelict land. The land which was once biologically productive has been degraded or totally destroyed, and left in a state where it cannot be used even for leisure or recreation activities.

Dennington and Chadwick (1983) quoted a figure of 340,000 hectares of derelict land in UK, as a result of industry's need for materials extracted from the ground, such as coal, iron ore, slate, stone, sand, gravel and clay. The greatest area of dereliction in this country is associated with coal mining. Clouston and McLean (1980) estimated that twenty million tons of coal have been mined in the last 100 years, leaving 2,000 spoil heaps, each containing between 1 and 4 million tons of waste on 10,530 hectares of land.

The history of industrial revolution in Scotland is the history of the exploitation of the mineral resources of the Midland valley and its legacy is an area of dereliction extending from the Ayrshire coast to the Firth

of Forth. Mining for coal dates from the fourteenth century, but most mining occurred during the last two centuries to meet the demands of heavy manufacturing industries for steel, gas, and electricity. These industries are now in decline, but the spoil heaps remain. There was an estimated 18,000 hectares of derelict land in Scotland in 1975 (MacPherson, 1980). Of this total, about 8,000 hectares comprises mineral waste tips, mainly coal and oil shales (Luke and MacPherson, 1983).

Coal may be extracted either by removing the over burden from shallow seams or by sinking a shaft to deeper seams and thence removing the coal. Reclamation of land disturbed during opencast mining has become an integral part of the mining process and therefore poses far fewer problems compared to deep mining spoil. As most of the National Coal Board product in Great Britain comes from deep mining, reclamation theory and practice has been mainly involved with problems of waste materials resulting from deep mining of coal (Kimber, 1982).

1.1 COAL MINE WASTE

Coal mine waste (hereafter referred to as coal mine soil) is the waste material that is brought to the surface when coal is mined underground. This material is discarded direct from the mine and tipped to form mounds, known in Scotland as bings. These bings may contain spoil from a number of seams, and in many cases from more than one colliery. A bing, therefore, may contain a heterogeneous

mixture of materials with variable properties. This variation within a single site can be as great as the variation between different sites. The spoil consists of low-grade coal, dirt and material from associated mineral strata in the coal measure, usually gritstone, sandstone, siltstone, mudstone and shale. The dominant geological materials are shales, which often constitute over 90 percent of all waste.

Although the marketable coal is removed in mining, considerable amounts of carbonaceous material exist in the shales and may be sufficient to cause combustion in the spoil heaps, particularly if loosely tipped. Burnt spoil is usually red in colour, as opposed to the black unburnt material, and may amount to 20-25 % of the spoil in Central Scotland (Pulford, 1976). Burnt material may have certain advantages over unburnt material as a plant growth medium (Pulford and Duncan, 1976). One obvious advantage is the complete oxidation and therefore removal of pyrite and hence any potential acidity problem. Beside burnt colliery spoil, there are many oil shale bings in the Lothians and Fife. They are also composed of burnt shales.

1.1.1 RECLAMATION OF COAL MINE SOILS

Coal mine soils have caused a wide concern in Scotland and other countries, not only because of their unsightly and barren visual appearance, but also because they give rise to a degraded environment with sedimentation and water pollution as well as erosion problems. It is desirable to reclaim such material as

quickly as possible. It is also a social responsibility that succeeding generations should not inherit this dereliction.

Since 1975 the Scottish Development Agency has been working on various reclamation projects for the clearance of dereliction and improvement of the environment. On average 400 ha of derelict land are reclaimed each year which includes about 140 hectares per year of coal mining restoration (Hall et al., 1986).

Coal mine soils, like other derelict land, represent a degraded or ruined ecosystem in which normal biological processes are at a standstill. These processes need to be restored so that a normally functioning ecosystem of soil and plants is achieved, in which the natural processes of nutrient release, plant growth and nutrient cycling go on at a normal rate.

Revegetation and the creation of a satisfactory soil cover are the prime aims of most derelict land reclamation schemes. Revegetation not only blends the waste material into the surrounding landscape but also provides a rapid visual improvement. It helps in reducing the amount of wind and water erosion, by protecting the land surface from the direct effect of wind and rainfall. Vegetation cover not only prevents erosion but also reduces penetration of water through the waste material and therefore leaching is also reduced. This occurs because the plants intercept and evaporate back a substantial proportion of the rainfall. Water pollution is therefore reduced in two ways. Coal mine soil with a plant cover

absorbs less heat than barren material due to the heat dissipating abilities of plants, by reducing the amount of sunlight directly reaching the surface.

Organic matter added through roots and dead vegetation improves the soil physical condition with all the advantages that this brings.

1.1.2 RECLAMATION TECHNIQUES

The waste material of coal mining is usually dumped in large conical heaps. They are visually intrusive and their sides are often too steep to be sufficiently stable to support vegetation. As lowering of the height and a lessening of the slope are essential therefore, the first practical step in the process of reclamation is usually regrading to produce a more regular land form. After moving and levelling operations, there is usually some attempt to produce a surface suitable for seeding. The weathered material which has undergone some chemical and physical changes, and which may even support a natural vegetation, is buried during reshaping of the bing material. On regrading, relatively unweathered material is exposed which varies considerably in physical and chemical properties. Under such conditions some amendments are needed to make it a suitable medium on which to establish vegetation.

The regraded material is either treated with lime and varying proportions of N, P and K fertilizers or it is covered with top soil. The top soil can be used either as a blanket layer or mixed with the surface of the waste

material. Due to the increasing cost of inorganic fertilizers in recent years and the lack of top soil, organic waste materials, like farm-yard manure, chicken manure or sewage sludge may be used. The use of such types of organic waste material also depends upon their availability and transportation cost.

In order to reduce the cost of reclamation incurred during reshaping, direct seeding of a mixture of seeds containing trees, shrubs and some herbaceous plant species has also been tried. This method was reported to be a feasible technique for establishing scrub and woodland on coal and oil shale bings of Central Scotland (Luke and Macpherson, 1983).

Revegetation programmes commonly include the establishment of a mixed sward containing grasses and legumes. Legumes are an important component in almost all seeds mixtures, because they contribute and maintain adequate nitrogen supplies and ensure the build up of an adequate capital of organic nitrogen in the newly forming soil. Kent (1982) reported a list of the grasses and legumes most commonly used in the seed mixtures (Table 1.1).

There are a number of leguminous trees, for example black locust (Robinia pseudoacia) in cool climates and members of the genus Acacia in warm climates. They are very valuable because they can also fix nitrogen like herbaceous legumes. Some trees, for example species of alder which are not legumes also fix nitrogen with the help of nodule forming actinomycetes, Frankia. These

species can grow relatively quickly and are useful restoration tools (Bradshaw and Chadwick, 1980b).

Latin name	English name
Agrostis tenuis	Bent grass
Coronilla varia	Crown vetch
Dactylis glomerata	Cocksfoot
Festuca pratensis	Meadow fescue
Festuca rubra	Creeping red fescue
Festuca rubra commutata	Chewings fescue
Lolium multiflorum	Italian ryegrass
Lolium perenne	Perennial ryegrass
Phleum bertolonii	Small-leaved timothy
Phleum pratense	Timothy
Poa pratensis	Smooth-stalked meadow grass
Medicago lupulina	Black medock
Lotus corniculatus	Birdsfoot trefoil
Trifolium hybridum	Alsike clover
Trifolium pratense	Red clover
Trifolium repens	White clover

Table 1.1. Species of grasses and legumes most commonly planted in the reclamation of colliery spoil (Kent, 1980).

1.1.3 PHYSICAL AND CHEMICAL PROPERTIES OF COAL MINE SOILS

Once the regrading and cultivation operations have been completed, coal mine soils are considered to be ready to support a vegetation cover. However, the survival and growth of plants are hampered by a variety of physical and chemical features of such drastically disturbed material.

The physical factors that limit plant growth on coal mine soil include instability, high temperature and moisture stress. In the process of regrading a colliery bing, the surface is crossed and recrossed many times by heavy machines used for moving and levelling, which results in compaction of the waste material. Impermeability to moisture and air is a common feature of regraded coal mine soils (Doubleday, 1974). Moreover, the age of most bings is too low to have allowed any significant accumulation of organic matter and therefore the physical conditions of coal mine soils are generally very poor. Such structureless material dries up in summer and becomes waterlogged in winter because of poor water holding and drainage properties.

The high surface temperatures due to the dark colour of the shale can further accentuate the drought conditions during summer. A further consequence of the lack of structure is that the coal mine soil can easily become compacted, leading to problems of root penetration and erosion (Down, 1975a; Richardson, 1975; Ayerst, 1978).

The chemical factors reducing plant growth on coal mine soil include: low pH (Chadwick 1973a), high levels of

aluminium and manganese (Kimber et al., 1978; Pulford et al., 1982), high concentrations of salts (Doubleday, 1974) and low levels of availability of major nutrients especially nitrogen and phosphorus (Davison and Jefferies, 1966; Fitter and Bradshaw, 1974; Bloomfield et al., 1982; Pulford et al., 1988).

The occurrence of iron pyrites (FeS_2) is very common in colliery shales of Central Scotland. During weathering the pyrite is subjected to the action of air and water, and oxidizes to produce large quantities of acidity.

$$4\ FeS_2 + 15\ O_2 + 14\ H_2O \ \text{---}> \ 4\ Fe(OH)_3 + 8\ H_2SO_4$$

Apart from pyrites, shale may also contain carbonate minerals which can mitigate the effects of the acid to some extent (Caruccio, 1975; Costigan et al., 1981). If the balance is in favour of the carbonate minerals, the resulting product of weathering will be neutral or slightly alkaline. If pyrite content exceeds the carbonate content, then the acidification potential is greater than the neutralizing capacity and very low pH conditions prevail.

Beside the acidity problem, the sulphate released during oxidation of pyrite creates salinity problems for plant growth. It has been suggested that at pH values below 3.5, the total reserve of soluble salts, particularly of chlorides and sulphates which lead to toxic salinity levels, is probably of even greater

significance than the extreme acidity (Palmer,1970). Another adverse effect of low pH conditions is the reduction in the availability of other ions, and in particular to provide the conditions for fixation of any phosphate present or added to coal mine soil (Fitter, 1974; Pulford and Duncan, 1975).

The acid conditions may also tend to increase the release of metals, particularly Al and Mn into soil solution which can be toxic to plants growing on coal mine soils (Kimber et al., 1978; Pulford et al., 1982; Keefer et al., 1983). The effect of low pH is also to lower the cation exchange capacity, so that nutrient ions are held less well against leaching and to tip the balance of inorganic nitrogen form in the direction of ammonium ion dominance (Chadwick, 1987).

The acidic leachate increases contamination of water when it flows into streams, rivers, or lakes. Pulford et al. (1983) found high levels of metals (Fe, Al, Mn, Cu, Zn, Co, Ni and K) in stream water near a colliery bing in Scotland.

When brought to the surface the discarded coal mine waste has a pH value of around 7.0 or slightly above (Doubleday, 1974; Kent, 1982). However, in the presence of air and water pyritic oxidation takes place and the resulting acidity causes the pH of the material to fall. Low pH values of 2-3 can be found on highly pyritic sites (Pulford and Duncan, 1978a). This pH can destroy any plant species that have already colonized the material. Under such conditions the material is further attacked, exposing

fresh surfaces which may be oxidized, resulting in further acid production, depending on the reservoir of oxidizable pyrite and the carbonate: pyrite ratio, until the pyrite is weathered out. The salts and trace metals will be leached from the upper layers. The pH may then begin to rise again, often very slowly until an equilibrium position of about pH 5.0 is reached (Kent, 1982). The resulting medium may be less hostile but is generally deficient in plant nutrients. The whole process could take of the order of 20-100 years.

1.2 PLANT NUTRIENTS

White (1987) lists 16 elements as essential for the growth and reproduction of higher plants. These elements can be divided into two groups on the basis of their concentrations in plants: (1) the macronutrients, which occur at concentrations in the plant of greater than 0.1% include carbon, hydrogen, oxygen, nitrogen, phosphorus, sulphur, calcium, magnesium, potassium and chlorine and (2) the micronutrients, which are generally less than 0.01% in the plant, include iron, manganese, zinc, copper, boron and molybdenum.

The biological availability of nitrogen, phosphorus and potassium is of considerable importance because they are the major plant nutrients derived from the soil. Of the three, nitrogen stands out as the most susceptible to microbial transformations.

Nitrogen is probably the most important nutrient

element in soils, when it is considered in light of the total quantity required by plants and the frequency with which it is a growth limiting factor. It is a key building block of the protein molecule upon which all life is based, and it is thus an indispensable component of the protoplasm of plants, animals and microorganisms. It is a component of chlorophyll, each molecule of which contains four atoms of nitrogen. It is also a component of enzymes which play an important role in plant life (Olson and Kurtz, 1982).

Nitrogen undergoes a number of biological or chemical transformations that involve both soluble and volatile compounds which are easily lost. These transformations occur simultaneously, but individual steps often accomplish opposite goals. The reactions may be viewed in terms of a cycle in which the element is shunted back and forth at the discretion of the microorganisms.

In view of the important role of nitrogen in plant nutrition, nitrogen cycling has been extensively studied in well-developed ecosystems where surface soils represent the major storage pool of nutrients. In contrast, the processes acting and interacting during the development of nutrient cycles in new ecosystems where the soil pool may be severely depleted is unclear.

Although the establishment of a stable, self-sustaining ecosystem depends on the effective cycling of several major elements, the accumulation of nitrogen and the formation of effective cycling is particularly important as nitrogen is readily lost from the ecosystem.

1.2.1 NITROGEN CYCLING AND COAL MINE SOILS

Nitrogen is not a component of the rocks from which soils are derived. It is abundant in the earth's atmosphere where it constitutes about 78 % of the gases present. There is about 3 tons of nitrogen in the air above each square foot of the earth's surface (Stevenson, 1982). Although this nitrogen is not of direct significance to plants that do not form symbiotic associations with nitrogen fixing microorganisms, it is present in ample supply for both symbiotic and nonsymbiotic nitrogen fixation. Besides this biological fixation, some fixation takes place in the atmosphere as a result of lightning discharge, with the inorganic products being principally ammonium and nitrate ions, which can enter the soil via rainfall (Table 1.2).

Site	NH_4^+-N	NO_3^--N	Organic-N	Total-N	Rainfall
	(kg N/ha)				(cm)
Mitchell	4.58	2.98	0.90	8.46	52.96
Maltby	4.11	3.03	1.44	8.58	63.88
Bullcroft	4.63	3.43	1.52	9.58	63.38

Table 1.2. Mean annual nitrogen input from rain at three colliery spoil sites in industrial northern England. (Bradshaw and Chadwick, 1980a)

Of substantially greater significance than atmospheric nitrogen fixation is the biological fixation carried out by a variety of soil microorganisms. They may be free living bacteria or blue-green algae, which make use of N_2 by nonsymbiotic means, and by symbiotic associations composed of a microorganism and a higher plant. They are able to fix this atmospheric nitrogen and convert it to ammonium compounds which are available to plants. The most important nitrogen fixing bacteria include the bacterium Rhizobium, and the actinomycete Frankia which are nodule forming microorganisms symbiotically associated with the roots of leguminous and nonleguminous plants respectively.

Research workers have often recommended the use of leguminous species with symbiotic bacteria on their roots as a good way for improving the nitrogen status of the coal mine soil (Jefferies et al., 1981a; Palmer, 1982). Skeffington and Bradshaw (1980) also reported that up to 50 kg N per hectare can be applied to Trifolium repens without affecting its nitrogen fixation rate. Legume species which are important colonizers in the development of natural communities on abandoned mine spoils (Roberts et al., 1981), can subsequently enable non-fixing species to grow in the new ecosystem. Dancer et al. (1977) found that 125 to 250 kg N /ha/year can be accumulated on sand or mica waste with introduced legumes. Palaniappan et al. (1979) suggested that Lupinus arboreus should be considered as a tool in reclamation as it was capable of accumulating 180 kg N/ ha/year over its lifespan of 6

years on unameliorated mica waste, promoting vigorous growth of associated species after approximately 4 years of growth. Jefferies et al. (1981a) reported that legumes can result in nitrogen accumulation in mine soils with rates as high as 295 kg N/ha/year being measured for Lupinus perennis sown on sand waste. They also found that Trifolium repens sown on coal mine soil increased the nitrogen content of the companion grass by 76 kg/ha within two years of sowing. Palmer and Chadwick (1985) reported that the annual rate of accumulation of nitrogen in plots of coal mine soil sown with white clover, treated with ammonium sulphate or untreated over 7 years since establishment was 54, 39 and 8 kg N/ha respectively. They stated that during this time fertilizer plots had received 500 kg N/ha but the white clover plots received no nitrogen fertilizer.

Species of nonleguminous trees capable of forming the actinorhizal symbiosis are often pioneers, colonizing devastated or other nitrogen-deficient areas. Alder can add as much as 100 kg N/ha/annum to soil from leaf fall alone (Postgate, 1982) and is probably an important source of fixed nitrogen in reclaimed coal mine soils. Quantitative data on the nitrogen inputs of Frankia in coal mine soils are scarce, so their ecological and economic roles are less than clear.

Under ordinary conditions, nitrogen in soils begins its existence mainly in an organic form, which is considered as the main natural source of nitrogen to plants especially those which do not fix nitrogen in

symbiosis with microorganisms. In unfertilized soils, the mineral forms of nitrogen are derived almost entirely from decomposition of organic nitrogenous compounds. The mineralization of organic nitrogen takes place in two distinct microbiological steps, ammonification and nitrification. The first transformation is effected by heterotrophic microorganisms by which ammonium is formed from organic compounds. The second transformation, oxidation to nitrate, is brought about largely by autotrophic soil bacteria.

The ammonium released into the soil system by ammonification of organic nitrogen or that added in the fertilizer form may be (1) utilized by higher plants, (2) immobilized by heterotrophic organisms in the further decomposition of organic residues, (3) fixed in a biologically unavailable form in the lattice of certain expanding type clay minerals (4) lost by volatilization or (5) oxidized by nitrifying bacteria.

Nitrogen immobilization is the reverse of mineralization and it occurs when large quantities of low nitrogen containing crop residues begin decomposing in soil. The high amounts of carbohydrate in such residues cause the population of soil microflora to build up quickly. As new cells are formed, nitrogen and other essential elements are used to build protoplasm. Almost invariably this leads to a decrease in the levels of inorganic nitrogen for growing plants.

Although the ability of coal mine soils to retain added ammonium in a non-exchangeable (fixed) form has not

been studied, its possibility has been suggested (Reeder and Berg, 1977b). However, it has been shown that such materials may contain significant amounts of fixed ammonium, trapped in the layer lattice structure of clay minerals. Such ammonium-N cannot be extracted by the commonly used extractants for mineral nitrogen determination and is consequently termed as fixed ammonium. This form of ammonium-N probably remains unavailable to plants, except under very acid conditions. It has been claimed that nitrogen is available in abundance in the acid coal mine soils of Pennsylvania as a result of clay lattice destruction by acid (Cornwell and Stone, 1968 and 1973). However, these conditions are not otherwise favourable for plant growth. Power et al. (1974) measured 150 to 300 mg N/kg soil of fixed ammonium in the Paleocene shale. Palmer and Chadwick (1985) reported 632 mg N/kg soil as non exchangeable ammonium in coal mine soils. Palmer et al. (1985) found 579 mg N /kg soil as fixed ammonium in the vegetated coal mine soils at Thorne in South Yorkshire, England.

Ammonia volatilization can occur whenever free ammonia is present near the soil surface. Ammonia concentration in the soil is markedly increased by applying ammoniacal fertilizers or decomposable organic materials like animal manures to neutral or alkaline soil. Reeder and Berg (1977b) reported an ammonia loss of less than 5 mg N/kg soil volatilized from an ammonium treated alkaline coal mine soil (60 mg N/kg soil) during 168 days of incubation. Nitrification is a two-step process in

which the ammonia is first converted to nitrite and thence to nitrate by obligate autotrophic bacteria known as Nitrosomonas and Nitrobacter. Some heterotrophs are also known to produce nitrite and nitrate, but Nitrosomonas and Nitrobacter are usually referred to collectively as the Nitrobacteria. Under normal conditions, oxidation of ammonia proceeds more rapidly than ammonification in soils and consequently soil nitrification is substrate-limited. Similarly, nitrite is so rapidly oxidized that it rarely accumulates in soil (Macdonald, 1986).

Nitrogen, once in the nitrate form, may be lost from the soil in several ways. It is removed from the soil to satisfy the nutrient demand of the plant cover. Nitrate is completely mobile and within limits moves largely with the soil water. It moves downward out of the zone of root penetration under conditions of excessive rainfall. Under anaerobic conditions, nitrate may be readily lost due to denitrifying bacteria, resulting in the formation of volatile products, N_2, usually N_2O (nitrous oxide), and often NO (nitric oxide) which are lost into the atmosphere.

The overall changes in the amount of inorganic nitrogen, N_i, can be expressed by the following relationship.

$$N_i = \text{organic N mineralized} - (N_a + N_p + N_l + N_g)$$

Where N_a, N_p, N_l and N_g represent the nitrogen assimilated by microflora, removed by the plant, lost by leaching, and gaseous loss due to ammonia volatilization and denitrification respectively. The rate at which organic

nitrogen is converted to ammonium and nitrate is termed the mineralization rate.

Although some of these transformations have been studied by some researchers, most studies were limited to one specific transformation process. Power et al. (1974) found during an incubation study of exposed Paleocene shales that exchangeable ammonium was readily nitrified, but they did not observe a net increase in the inorganic nitrogen content of shale samples. Williams and Cooper (1976) reported that unfertilized soil released greater amounts of mineralized nitrogen than either unamended or amended coal mine soil, although the total nitrogen contents of the coal mine soils were higher than the normal soils. Reeder and Berg (1977b) showed that the nitrogen mineralization rate in exposed sedimentary rocks is much lower than in soils. Similarly, Pulford et al. (1988) who conducted an incubation study, reported that high levels of carbon, but little nitrogen were mineralized in the coal mine soils of Central Scotland.

1.2.2 ENZYME ACTIVITY IN COAL MINE SOILS

Soil is a living system, where all biochemical action is bound up with the presence of enzymes. Enzyme activities are an important index of the biological activity of a soil because enzymes are involved in the process of metabolism and energy transfer. While there is contradictory evidence on the correlation of soil fertility with the activity of individual enzymes, there is no doubt that enzyme processes are closely associated

with soil fertility. Extra-cellular enzymes, in particular, bring about the conversion of unavailable forms of nutrients to forms that are readily available to plants and microorganisms. The levels of enzyme activities can be used as an indicator of soil fertility (Nannipieri et al., 1978; Skujins, 1978).

Due to their importance in the soil nitrogen cycle enzyme activities have been studied extensively in agricultural soils. Limited information about enzyme activities in coal mine soils is available, although this approach has been advocated by previous researchers (Cundell, 1977; Fresquez and Lindemann, 1982).

Stroo and Jencks (1982) measured respiration rates and activities of some enzymes (amylase, urease, phosphatase) as indices of microbial activity in relation to time and fertilizer amendments. They found that microbial activity in mine soils was generally lower than in adjacent native soils. With added fertilizer, higher microbial activities were observed in mine soils, but organic matter formation was retarded. In another study, Stroo and Jencks (1985) studied the effect of lime, fertilizer, and sewage sludge upon microbial activity and growth of vegetation on an acidic minesoil. They concluded that microbial populations exist in such soils and that their activity can be increased by liming and fertilizing. They further stated that amylase activity could be used as an indicator of microbial activity and that urease and phosphatase activities appear to be of little value for evaluating mine soil microbial activity.

Any nitrogen fertilizer added to coal mine soils in the form of nitrate may be rapidly lost by leaching. However, ammonium nitrogen can be retained on cation exchange sites. To obviate problems arising from the extreme solubility of many nitrogen fertilizers, and the susceptibility of nitrate nitrogen to leaching, various sparingly soluble nitrogen compounds, including natural organic materials, have been developed. Although these are usually inferior, per kg of nitrogen, to the soluble forms, they may be useful for the establishment of vegetation on difficult sites, such as reclaimed coal mine soils, where a slow but assured release of nitrogen over several seasons is required. Another alternative solution to the leaching problem is to use an organic fertilizer like urea or an amide. Urea is the most concentrated commercially available solid nitrogen fertilizer. It contains 46% N compared with 35% N in ammonium nitrate which is the most common form of nitrogen fertilizer used in UK. There is little difference in the per tonne price of urea and ammonium nitrate. On the basis of cost per tonne of N, urea appears to be more economical than ammonium nitrate. Similarly amides, for example formamide seem to have potential as nitrogen fertilizers (Frankenberger and Tabatabai, 1982). Such types of organic fertilizers undergo hydrolysis by enzymic action before they can be used by plants. Studies by Stroo and Jencks (1982, 1985) indicate that enzyme inhibition should not be a problem on coal mine soils.

1.2.3 FACTORS AFFECTING NITROGEN TURNOVER

Mineralization rate can be affected by physical and chemical features of an ecosystem such as moisture, pH, aeration, temperature and the inorganic nutrient supply. Ammonification is generally less sensitive to environmental change compared to nitrification.

The ammonifying population include aerobes as well as anaerobes. Organic nitrogen is mineralized at moderate or at excessively high moisture levels. Although soils differ in the precise moisture values that are optimal for the conversion, the optimum for nitrogen mineralization generally falls in the area ranging from -0.1 to -0.5 bar soil moisture potential (Miller and Johnson, 1964; Stanford and Epstein, 1974). As a rule, soils active in aerobic mineralization also form ammonium readily in the absence of oxygen, and those slow in this process in aerated conditions likewise generate ammonium slowly under anaerobic conditions (Waring and Bremner, 1964). However, oxygen is an obligate requirement for the nitrification process. Because of this nutritional characteristic, poor soil structure and compaction of coal mine soils may affect the accumulation of nitrate through its influence on aeration. Physical conditions such as waterlogging and compaction of the soil may establish more or less anaerobic conditions, generally hampering microbial activity, stimulating ammonium or ammonia formation and accumulation, and causing nitrate losses by denitrification (Jansson and Persson, 1982).

Drying and wetting cycles may also affect the

nitrogen mineralization rate by making inaccessible substrates more easily available to microbial action. Drying may cause cell disintegration. Laboratory experiments with soil have shown that alternate drying and rewetting cycles result in an extra release of carbon dioxide, and frequently an extra production of mineral nitrogen (Bottner, 1985).

Mineralization is influenced by the pH of the environment. All other factors being equal, the production of inorganic nitrogen (ammonium plus nitrate) is greater in neutral than in acid environments (Ishaque and Cornfield, 1972). In acid conditions, nitrification proceeds slowly even in the presence of an adequate supply of ammonium, and the responsible species are rare or totally absent at low pH. Although an exact limiting pH cannot be ascertained, the rate falls off markedly below pH 6.0 and becomes negligible below 5.0 (Dancer et al., 1973).

It has been found that incubation of an acid coal mine soil with lime not only resulted in increased nitrification of added ammonium-N, but also increased the quantity of mineralizable nitrogen (Williams, 1975; Williams and Cooper, 1976). The acidity of some coal mine soils results in the accumulation of ammonium nitrogen due to inhibition of nitrifying bacteria. It has been found that mineral nitrogen concentrations are generally greater in acid coal mine soils, (pH < 5.0) than in limed or neutral coal mine soils and that in acid coal mine soil mineral nitrogen is mainly found as ammonium ions

(Williams and Cooper, 1976).

Temperature may affect the mineralization sequence as each biochemical step is catalyzed by temperature sensitive enzymes produced by microorganisms whose growth is in turn conditioned by temperature. Thus, at 2 ^{o}C, the microflora slowly mineralize the organic complexes, but there is no ammonium or nitrate formed when soil is frozen. Nitrification is markedly affected by temperature, and many investigations have confirmed the fact that below 5 ^{o}C and above 40 ^{o}C the rate is very slow (Alexander, 1977).

It has been reported that addition of nitrogen fertilizer sometimes enhances the mineralization rate of native organic nitrogen in soils (Wickramasinghe et al., 1985). On the other hand, such amendments appear to have no influence or adverse effects on the mineralization process in other soils. For example, Williams (1975) found that addition of nitrogen (50 mg N/kg soil) as ammonium sulphate decreased the quantity of mineralizable nitrogen in incubated coal mine soils. He suggested that this may be due to the increase in acidity that results from the addition of ammonium sulphate.

Under ordinary conditions the rate of nitrogen mineralization is closely correlated with the total nitrogen, and therefore sites rich in nitrogen liberate more inorganic nitrogen than those deficient in total nitrogen in a given time interval. Coal mine soils, which are usually comparable in total nitrogen content to normal soils are usually deficient in mineralizable nitrogen

(Williams and Cooper, 1976; Reeder and Berg, 1977b).

The C:N ratio in organic matter present in, or added to, soil is of primary importance to the course of mineralization. Only materials with a C:N ratio of 20:1 or lower generally can provide mineral nitrogen. Material with higher C:N ratios initially allows only the liberation of CO_2, and the mineralized nitrogen immediately again becomes bound in the protoplasm of the developing microbes. At this stage a lag period must be expected until the C:N ratio is narrowed to 20:1 or thereabouts. It has been found that the C:N ratios of coal mine soils are generally higher than agricultural soils. For example, Reeder and Berg (1977b) found a wider C:N ratio ranging from 19 to 35 in coal mine soils as compared to 11 in a normal soil. Schafer et al. (1980) who studied soil genesis in mine soils of Montana, reported C:N ratios from 17 to 46. Pulford et al. (1988) reported mineralized C:N ratios in coal mine soils of Central Scotland ranging from 24 to 58. The values reported indicate that coal mine soils have wider C:N ratios compared to normal soils. Therefore significant amounts of inorganic nitrogen can also be assimilated into the organic fraction of the coal mine soils (Reeder and Berg, 1977b).

1.2.4 NITROGEN AVAILABILITY INDEX IN COAL MINE SOILS

It is generally accepted that coal mine soils are comparable in total nitrogen with agricultural soils, but they are usually deficient in plant available nitrogen. The behaviour of nitrogen in coal mine soil may be markedly different from its behaviour in normal agricultural soils. More than 90 % of the total nitrogen in the surface layer of normal soils is organically combined (Stevenson, 1982). It is generally the case for soils that the higher the value of total nitrogen (Kjeldhal method), the greater the fertility with regard to its nitrogen content. Such determinations are often made to predict the nitrogen status of coal mine soils. Cornwell and Stone (1968) found a high total nitrogen content ranging from 0.30-0.69 % with an average value of 0.54 % in the black shales of Pennsylvania coal mine soils. Pulford (1976) measured 0.1 to 0.8 % total nitrogen in the coal mine soils of Central Scotland. Williams and Cooper (1976) and Reeder and Berg (1977b) obtained comparable values of total nitrogen in coal mine soils to adjacent normal soils, but very little was plant available.

Values obtained from total nitrogen analysis for agricultural soils may be quite useful, provided that their pH is close to neutrality. When this condition does not prevail, as in most of the acid coal mine soils the determination can be misleading. The values obtained might be high, but the inhibition of the soil microflora may be

so great that very little of the organic nitrogen becomes mineralized and hence available to the vegetation. Furthermore, the organic matter in the coal mine soils may be relatively inert to microbial decomposition. For example some coal mine soils may contain appreciable quantities of fossil nitrogen due to the the presence of inert organic nitrogen in the coal and associated shales (Power et al., 1974). It is generally accepted that values for total nitrogen are inadequate for assessing the nitrogen supplying power of coal mine soils (Williams and Cooper, 1976).

Some soil scientists have tried to determine the need for nitrogen fertilization by analysis of the soil for mineral nitrogen. The most important biologically available forms of inorganic nitrogen include exchangeable ammonium ions adsorbed on the exchange sites of the clay particles and water soluble nitrite and nitrate. The mineral nitrogen occurs largely as ammonium and nitrate. With the exception of neutral or alkaline soils receiving ammonium or ammonium-producing fertiliser, nitrite is seldom present in detectable amounts. The total quantity of mineral nitrogen present is usually a very small proportion of the total nitrogen (< 2%) in the soil, but is of great importance because it is in these forms that plants take up their nitrogen.

The content of mineral nitrogen in soil fluctuates over a very wide range and is influenced by many external factors (season, weather conditions, plant growth, fertilizer etc.). The quantities of mineral nitrogen that

occur in a soil are a reflection of the interactions of a number of processes of consumption and production. Agencies that increase the level of mineral nitrogen include mineralization of organic matter and input in rainfall. Mineral nitrogen levels are decreased by plant and microbial uptake, leaching and denitrification. So the content of mineral nitrogen found at a given time does not provide helpful information about the rate or magnitude of nitrogen transformations in the ecosystem, and hence the ability of a soil to provide nitrogen for plant growth cannot easily be assessed from such data.

It is generally accepted that the only property that may be expected to correlate with the availability of soil nitrogen to plants is the rate of mineralization of organic nitrogen in the soil. Reeder and Berg (1977a) suggested that laboratory incubation tests could be useful in estimating the plant available nitrogen potential of certain derelict lands before initiating an extensive revegetation programme. They found a high positive correlation between mineral nitrogen content of laboratory-incubated coal mine soil and total nitrogen uptake by plants grown under greenhouse conditions.

Palmer and Chadwick (1985), who determined the various nitrogen fractions in coal mine soils, found that mineralizable nitrogen was the fraction most highly correlated with herbage nitrogen.

1.2.5 RESPONSE OF VEGETATION TO NITROGEN APPLICATION

In most instances during reclamation, fertilizers and lime if necessary are applied at seeding after regrading the colliery spoil. This usually provides immediate benefit in the initial establishment of vegetation. However, many of the reclamation schemes are just for cosmetic purposes and do not include provision for management after the establishment period. This can lead to serious regression of vegetation which was looking very promising at an early stage. Under such circumstances, if proper aftercare is not given, all the money and effort expended on the reclamation can be wasted.

The depletion of nitrogen reserves is often the main factor in the regression. It has been widely accepted that coal mine soils supply little nitrogen for plant growth, even though some shales may contain appreciable quantities of total nitrogen. Several workers have commented on the low levels of available nitrogen in these materials and they have found that plants responded to nitrogen fertilizer added to coal mine soils (Davison and Jefferies 1966; Bloomfield et al., 1982 and Pulford et al., 1988). Such results indicate the need to ensure an adequate supply of nitrogen to plants growing in coal mine soil.

Bradshaw (1983) has estimated that a nitrogen capital of about 1600 kg N/ha is needed to sustain a vegetation cover. This is based on a requirement of 100 kg N/ha/year and an organic matter decomposition rate typical of temperate soils. Although legumes may be an effective way

of accumulating nitrogen in coal mine soils, there is, however, a necessity to apply fertilizer nitrogen at the outset to promote rapid establishment of the grass component. Although expensive, this may be the most effective means of stabilising the slopes of the waste materials against erosion.

Moreover, it is observed that if fertilizer nitrogen is withheld from a young mixed sward on reclaimed coal mine soils, the grass tends to disappear and the clover becomes dominant; there appears to be insufficient transference of nitrogen from legume to grass to keep the grass productive. This situation could change when the sward and soil become more mature, which needs some time in terms of years.

Pulford et al. (1988) studied the response of established vegetation to N, P and K fertilizer treatments on reclaimed Scottish coal mine soils. They found a significant ($p<0.05$) increase in yield when 100 kg N/ha was applied but observed no effect by additions of 50 kg N/ha, or by P or K alone.

1.3 AIMS OF THESIS

The major nutrient factor limiting plant growth on reclaimed coal mine soils is nitrogen deficiency. An understanding of nitrogen cycling is important for successful reclamation and proper maintenance of plant growth on such drastically disturbed material.

The aim of the work described in this thesis is to study the supply and turnover of nitrogen in coal mine soils. This will be carried out by means of

1. A survey of the nitrogen status and some nitrogen transformations (mineralization, immobilization, nitrification, ammonium fixation, urease and amidase activities) in a range of coal mine soils of differing properties.

2. Incubation studies under controlled laboratory conditions to investigate the transformations of nitrogen in coal mine soils amended with ammonium nitrate , urea or chicken manure.

3. A field trial to measure the response of established vegetation to added nitrogen fertilizer.

4. In addition, attempts have also been made to assess and modify some of the methods followed during this study of nitrogen cycling in coal mine soils.

CHAPTER 2 METHODS AND ASSESSMENTS

This chapter is intended to describe the various methods and procedures adopted during the study of coal mine soils. In spite of the different land management objectives, and soil materials that often are not soil in that they have not developed over a long period of time, most soil test procedures have proven useful on coal mine soils. However, some soil test procedures have certain limitations due to which they cannot be used successfully on coal mine soils. Some attempts have been made to evaluate and assess the different methods and procedures followed for the study of some processes involved in the nitrogen transformations in coal mine soils.

2.1 STANDARD ANALYTICAL TECHNIQUES

2.1.1 MEASUREMENT OF COAL MINE SOIL pH

Coal mine soil pH was determined electrometrically by a combined glass/ reference electrode in a suspension of 1:2.5 soil in deionized water. The pH meter was first calibrated with buffer solutions of pH 7.0 and 4.0. Triplicate 10 g samples of fresh coal mine soil were weighed into 4 ounce glass screw cap bottles and 25 cm^3 deionized water were added to each bottle. The suspension was shaken for 30 minutes on an end over end shaker. The pH electrode was then immersed in the suspension, stirred

by swirling the electrode slightly and the pH measured.

2.1.2 MOISTURE DETERMINATION

Porcelain basins were washed, and dried in the oven at 110^{o}C for one hour, cooled in a desiccator and the weight was recorded. A suitable weight of fresh soil was placed in the basin and weighed. Then the basin containing fresh soil was left in a 110 ^{o}C oven overnight, cooled in a desiccator and reweighed. The percent moisture content of the soil was calculated on an oven dry basis.

% Moisture = (Weight of water/weight of oven dry soil)x100

2.1.3 MOISTURE DETERMINATION AT -0.5 BAR SOIL MOISTURE POTENTIAL

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

2.1.4 TITRATION METHOD FOR CARBON DIOXIDE DETERMINATION

The method of Jenkinson and Powlson (1976) was used for the measurement of carbon dioxide trapped in 1M sodium hydroxide during incubation experiments (see procedure described in section 2.4.1).

The sodium hydroxide was transferred from the bottle into a 250 cm^3 glass beaker, and the bottle was rinsed 3 times with carbon dioxide free water into the beaker to ensure that no sodium hydroxide had been left in the bottle. Five drops of carbonic anhydrase (1mg/cm^3) solution were also added to the beaker. The enzyme solution was prepared by dissolving 10 mg of the pure enzyme (Sigma Chemical Company) in 10 cm^3 deionized water. Immediately after adding the enzyme, the pH of the solution was brought down to 10 by slow addition of 1M hydrochloric acid and then to 8.3 by slow addition of 0.05M hydrochloric acid, the solution being stirred with a magnetic stirrer. Addition of carbonic anhydrase improved the pH 8.3 end-point by decreasing drift, so that the titration could be done more quickly (Underwood, 1961). The solution was finally titrated with 0.05 M hydrochloric acid to pH 3.7 and the amount of carbon dioxide evolved during incubation was calculated from the volume of the 0.05 M hydrochloric acid required to bring down 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. Decarbonated water was used to wash the pH electrodes

between titrations. It was prepared by boiling some deionized water to remove gases.

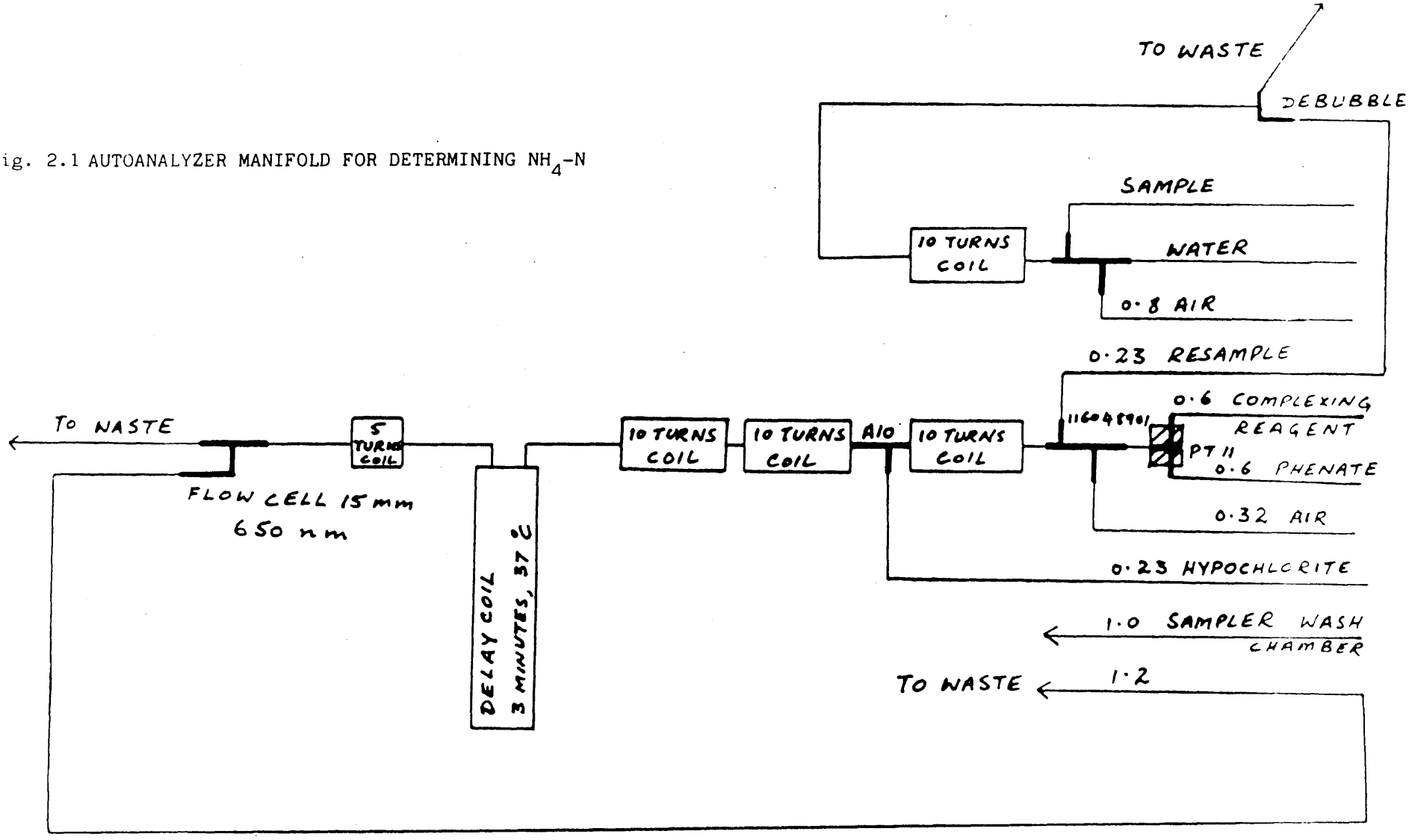
2.1.5 METHODS OF INORGANIC NITROGEN DETERMINATION

Colorimetric methods were used in this study for the analysis of inorganic forms of nitrogen (ammonium, nitrite and nitrate) in soil extracts and other test solutions because of their sensitivity, speed, and ease of use. The Technicon AutoAnalyzer II system used for this purpose comprised a sampler, pump, water bath with constant temperature 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.

2.1.5.1. Determination of Ammonium Nitrogen

Ammonium-N was determined by a modification of the indophenol green method using a complexing reagent to prevent interference 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 of 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.

Fig. 2.1 AUTOANALYZER MANIFOLD FOR DETERMINING NH_4-N



Reagents

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

1. Alkaline phenol

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

2. 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 water and the volume was made to 1 litre . 0.5 cm^3 of 30 % brij-35 solution was also added to this solution and thoroughly mixed .

3. Sodium hypochlorite solution (0.5 %)

50 cm^3 sodium hypochlorite solution (12% W/V available chlorine) was diluted to 1 litre with deionized water.

4. Ammonium-N 1000 mg/l Stock Solution

4.717 g dried ammonium sulphate was dissolved in deionized water and the volume made up 1 litre. This stock solution was stored at 2 ^oC. Working standards were prepared by dilution in the appropriate extracting solutions.

In addition to the above reagents required for measurement of ammonium-N in soil extracts, the following were also used for the analysis of ammonium-N in the Kjeldahl digest of plants.

(i). Wash chamber solution. 50 cm^3 concentrated sulphuric acid was diluted to 1 litre with deionized water.

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

Procedure

Filtered soil extracts and other test solutions were analysed directly on a Technicon AutoAnalyzer II. The samples were run at the rate of 50 per hour, but where dilution was required, the sampling rate was reduced to 40 per hour. Colour development was carried out at a constant temperature of 37 ^oC in the heating bath and the colour intensity was measured at 650 nm. The air was cleaned of atmospheric ammonia by bubbling through 5 % sulphuric acid solution. The calibration curve for the system was linear over the full range. Appropriate dilution was carried out for concentrations above 5 ppm.

For the analysis of Kjeldahl digests of plant material, the following modifications were made in the standard ammonium-N manifold.

(i) Sampler wash solution- 5% v/v sulphuric acid.

(ii) Dilution ratio 20:1

Sample 0.1 cm^3 per minute.

Diluent 2.0 cm^3 per minute.

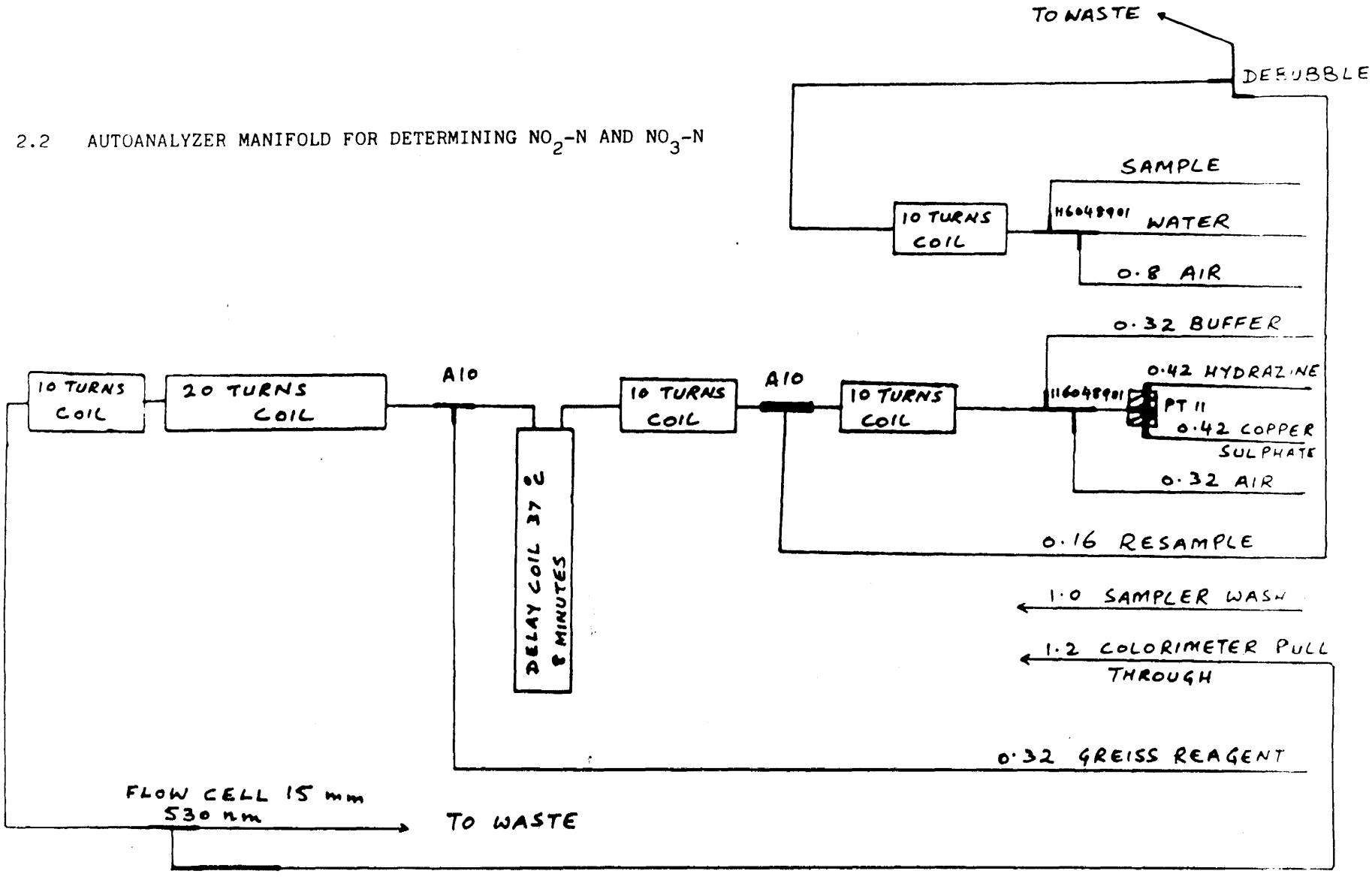
Air 0.8 cm^3 per minute.

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 (1M) was added to the neutralizing solution until the indicator just changed colour from red to yellow in the diluter mixing coil.

2.1.5.2. 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 nitrite plus nitrate nitrogen. 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.

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



Reagents

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

1. Buffer solution (pH 9.6)

22.5 g sodium tetraborate and 2.5 g sodium hydroxide were dissolved in water and the volume was made to 1 litre.

2. Greiss reagent

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

3. Hydrazine sulphate solution

0.30 g hydrazine sulphate was dissolved in 1 litre water. The hydrazine sulphate was dissolved by stirring with a magnetic stirrer, keeping the top of the flask closed in order to prevent access of oxygen. 0.5 cm^3 of 30 % brij-35 solution was then mixed with the solution.

4. Catalyst solution

1 cm^3 of 2.47 % copper sulphate pentahydrate solution was added into approximately 800 cm^3 water and the volume made to 1 litre. The solution was stored in a plastic bottle. 0.5 cm^3 of 30 % brij-35 solution was also added and thoroughly mixed with the solution.

For the determination of nitrite nitrogen, the reagents 3 and 4 were replaced with nitrogen free deionized water containing 0.5 cm^3 per litre of a 30 % brij-35 solution.

5. Nitrate-N 1000 mg/l Stock Solution

7.218 g dried potassium nitrate was dissolved in water and the volume made up to 1 litre. This stock solution was stored at 2 ^{o}C. Working standards were prepared by dilution in the appropriate extracting solutions.

6. Nitrite-N 1000 mg/l Stock Solution

4.926 g of dried sodium nitrite was dissolved in water the volume made up to 1 litre. This stock solution was stored at 2 ^{o}C. Working standards were prepared by dilution in the appropriate extracting solutions.

Procedure

The manifold shown in Fig. 2.2 was used for determination of nitrate and nitrite nitrogen in filtered soil extracts and other test 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 the constant heating bath at 37 ^{o}C (Best, 1976). Then, the nitrite nitrogen was 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 but it was curved at 0 to 5 ppm. Dilution was carried out for concentrations above above 5 ppm. Nitrite present in the sample was determined by the same procedure, except that the reduction step was omitted.

2.2 WASHING OF FILTER PAPERS TO REMOVE N CONTAMINATION

2.2.1 INTRODUCTION

Many procedures for the measurement of ammonium and nitrate nitrogen in soils and other materials call for extracting these ions with a salt solution and then filtering the extract through filter paper (Keeney and Nelson, 1982). Seldom, however, do these procedures caution about the possibility of the filter paper contributing to significant analytical errors.

In the past several research workers have reported that filter paper can cause errors in the measurement of ammonium or nitrate-N in filtered extracts. Leitch and Wells (1946) obtained erratic results in total and non-protein nitrogen determinations due to filtration through standard filter papers and have recommended the use of small sintered glass filters. O'Halmhain and O'Danachair (1974) reported variable amounts of ammonia content in analytical filter papers, and re-emphasized the necessity of carrying out blank determinations with all reagents, including filter papers, and of taking the blank determination through all the stages of the analysis. Muneta (1980) tested different grades of filter papers for nitrate-N. He found that some qualitative papers contained enough nitrate-N to cause significant errors in the analysis of cured meats, whereas quantitative (low-ash or ashless) grades contained very little or no nitrate-N. Hattori et al. (1983) tested different grades and batches

of filter papers for ammonium-N content by eluting them with 1.3 M (10 %) potassium chloride solution and analysing the leachate. They found a significant variation among grades, batches within grade, packages within batches, and papers within package and recommended the use of filter papers with low ammonium-N content. Sparrow and Masiak (1987) tested filter papers for ammonium and nitrate-N and reported a significant amount of ammonium or nitrate-N in many filter papers. They have recommended the prewashing of cellulose filter papers with water or 2 M potassium chloride solution. Khan (1987) has also found variable amounts of ammonium in various batches of filter papers and recommended the prewashing of Whatman No. 40 filter papers with 50 cm^3 of 0.5 M potassium sulphate solution in two equal successive portions, and then rinsing twice with deionized water and drying for 4 hours at 70 ^oC.

Since coal mine soils contain very low levels of inorganic nitrogen compared to normal soils, therefore, a very small amount of impurity in the filter paper can cause a considerable analytical error. So it was considered essential to wash the filter paper prior to filtration.

Washing of filter papers with 0.5 M potassium sulphate solution prior to use for removing sources of nitrogen contamination was one acceptable procedure in our laboratory as recommended by Khan (1987), but such a procedure was rather tedious and time-consuming due to preparation of ammonia-free potassium sulphate solution.

Moreover, the method seemed to be expensive as Whatman No. 40 filter papers are more costly compared with Whatman filter paper No. 2.

The objectives of this study were (i) to measure ammonium, nitrite and nitrate-N eluted from Whatman filter paper No.2. (ii) To compare M hydrochloric acid, 0.5 M sulphuric acid and 0.5 M potassium sulphate as washing solutions for filter papers prior to use for filtration.

2.2.2 MATERIALS AND METHODS

Two boxes of Whatman filter paper No.2 (W. & R. Balston Ltd.) having the same control No. (5141/131) and size (12.5 cm) were used during this study.

2.2.2.1. Reagents

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

1. Sulphuric acid (0.5 M)
2. Hydrochloric acid (1 M)
3. Potassium sulphate solution (0.5 M)

0.5 M sulphuric acid and M hydrochloric acid were made from the bottles of the corresponding concentrated acid. Ammonium-free potassium sulphate solution was prepared according to the method described in section 2.3.

2.2.2.2. Procedure

In order to compare acid washing of filter paper with that of potassium sulphate, 20 filter papers were selected

at random from a box and each one folded separately into a clean and dry plastic funnel. Ten filter papers were leached with 0.5 M sulphuric acid and another set of ten with 0.5 M potassium sulphate. 50 cm^3 of washing solution was filtered through each filter paper in two equal successive portions of 25 cm^3 each. The filtrate of each portion of washing solution was collected separately into clean dry plastic bottles and kept for ammonium, nitrite and nitrate-N determinations. Nitrate-N was determined only in potassium sulphate filtrates, because it was impossible to determine nitrate-N in the acid filtrates. The filter papers were rinsed with deionized water to remove any residue of washing solution. In the case of potassium sulphate washing, two rinsings with deionized water were given, but for acid washed filters 3-4 rinsings with deionized water were required until the filter papers were acid free. Litmus test paper was used for this purpose. Then the washed filter papers, along with funnels, were dried in a 70 ^oC oven for 4 hours. After drying each of the 10 filters were again leached with 50 cm^3 0.5 M potassium sulphate solution and the leachate was analysed for ammonium, nitrite and nitrate-N by a Technicon AutoAnalyzer II (see section 2.1.5) for any N contamination left after washing.

For comparing sulphuric acid washing of filter papers with that of hydrochloric acid, 20 filter papers were selected at random from the 2nd box. A set of 10 filters was used for each acid wash. 50 cm^3 of acid in two equal portions of 25 cm^3 were used for washing each filter paper

by following the above procedure. In this case the acid filtrates were not collected. After rinsing with deionized water and drying in the oven, each of 10 filters were leached with 50 cm^3 of 0.5 M potassium sulphate solution and the leachate was analysed for ammonium, nitrite and nitrate nitrogen.

2.2.3 RESULTS AND DISCUSSION

The amount of nitrite nitrogen extracted from each filter paper was very small. The ammonium and nitrate nitrogen eluted from filter papers by the washing solution was calculated and divided by the average weight of a filter paper to obtain the amounts of ammonium or nitrate nitrogen per unit weight of filter paper. Each value in the tables represents the mean of 10 replicate analyses. The data were analysed on a micro computer using a statistical program. A 'T test' was applied to determine the significance of differences between means with probability of 0.05, 0.01 and 0.001.

A summary of ammonium and nitrate nitrogen extracted by 0.5 M sulphuric acid or 0.5 M potassium sulphate solution from unwashed filter papers, and the nitrogen extracted from washed filters by 0.5 M potassium sulphate solution is given in Table 2.1. Whatman filter paper No. 2 used in this experiment contained a range of 3.1 to 5.3mg of ammonium-N and 12.1 mg of nitrate-N/kg of filter paper. This is large enough to cause significant errors in the analysis of coal mine soils which are already very low in such forms of nitrogen. As far as the efficiency of 0.5 M

sulphuric acid or 0.5 M potassium sulphate as washing solutions was concerned, the former also seemed to be a good washing solution. There was no significant difference in the amounts of ammonium nitrogen extracted from filters washed by either of the above two washing solutions. The amount of nitrate nitrogen left after washing in the filter paper was significantly more in potassium sulphate washed filters than sulphuric acid washed filter papers.

The results presented in Table 2.2 indicate the ammonium and nitrate nitrogen extracted by 0.5 M potassium sulphate solution from filter papers after washing with 0.5 M sulphuric acid or M hydrochloric acid from a 2nd box of Whatman filter paper No. 2. There was no significant difference in ammonium nitrogen content extracted from the washed filters with either of the two different acids, but in this case 0.5 M sulphuric acid proved to be a comparatively better washing solution than hydrochloric acid, due to the significant amount of nitrate nitrogen left in the filters washed with M hydrochloric acid. The higher content of nitrate nitrogen in the hydrochloric acid washed filter papers was probably due to chloride ion interference in the nitrate-N determination, because of some of the washing solution remaining in the filter paper and then being extracted with 0.5 M potassium sulphate solution.

The difference between 0 and 0.2 mg of nitrate-N/kg filter paper (Table 2.1) and between 0.7 and 0.8 mg/kg filter paper (Table 2.2) though statistically significant, seemed to be not more than the estimated random error of

the method. The estimated random error of the method for nitrate-N determination on the Technicon AutoAnalyzer II was from $\pm$0.1 to $\pm$0.2 ppm, therefore the difference was not big enough to matter.

The selection of 70 ^{o}C drying for 4 hours was based on the findings of Khan (1987), who reported that drying at this temperature for 4 hours will not affect the ammonium levels in the filter papers.

It is concluded that washing of the Whatman filter paper No. 2 with 0.5 M sulphuric acid prior to use could be an acceptable procedure for eliminating this source of error, but care must taken that acid washed filter paper must be rinsed at least 3 times with deionized water, in order to make it acid-free, otherwise this may result in acid leachates that could not be analysed accurately.

2.2.4 METHOD OF FILTER PAPER WASHING

0.5 M sulphuric acid was prepared from Analar grade concentrated acid using nitrogen-free deionized water.

Each filter paper was folded separately into a clean and dry plastic funnel. 50 cm^3 0.5 M sulphuric acid was filtered through each filter paper in two equal successive portions of 25 cm^3 each. Then the acid washed filter papers were rinsed 3 times with deionized water to wash away any acid left in the filter paper. Care was taken to make sure that the filter papers were made acid-free. Litmus test paper was used for this purpose. Then the washed filter papers along with funnels were dried for 4 hours in a 70 ^{o}C oven before using for filtration.

Washing Solution	NH_4-N (mg N/kg paper)		NO_3-N (mg N/kg paper)	
	unwashed	washed	unwashed	washed
0.5 M H_2SO_4	5.3	0.9	-	0.0
0.5 M K_2SO_4	3.1	0.7	12.1	0.2
		NS		***

Table 2.1. Effect of washing solution on the ammonium and nitrate nitrogen extracted from unwashed filter papers and on the nitrogen extracted from washed filter papers by 0.5 M potassium sulphate solution.

T Test on N content of Washed filters
NS Not Significant
* $p < 0.05$
** $p < 0.01$
*** $p < 0.001$

Washing Solution	NH_4-N (mg N/kg paper)		NO_3-N (mg N/kg paper)	
	unwashed	washed	unwashed	washed
0.5 M H_2SO_4	-	1.2	-	0.7
M HCl	-	1.4	-	0.8
		NS		**

Table 2.2. Ammonium and nitrate nitrogen extracted by 0.5 M potassium sulphate solution from filter papers washed with 0.5 M sulphuric acid or M hydrochloric Acid.

T Test on N content of Washed filters
NS Not Significant
* $p < 0.05$
** $p < 0.01$
*** $p < 0.001$

2.3 SELECTION OF A SUITABLE EXTRACTANT FOR THE EXTRACTION OF INORGANIC NITROGEN FROM COAL MINE SOIL

2.3.1 INTRODUCTION

Plant available nitrogen is usually a major plant growth limiting factor in coal mine soils. Measurement of inorganic nitrogen (exchangeable ammonium, nitrite and nitrate) in soil is an accepted and straight forward procedure. The methods which have been adopted for determination of exchangeable ammonium, nitrite and nitrate in soils involve extraction of these forms of nitrogen and analysis of the extract by colorimetric or distillation techniques. Colorimetric methods have been extensively employed for determination of inorganic forms of nitrogen in soil extracts.

Various chemical salt solutions, such as potassium chloride, potassium sulphate, potassium acetate, sodium chloride, calcium chloride and Morgan's reagent (10% sodium acetate, 3% acetic acid pH 4.8) varying both in concentration and pH have been used for extracting inorganic forms of nitrogen (Bremner 1965). Jackson (1967) recommended the use of 10 % sodium chloride solution acidified to pH 2.5 for extracting ammonium-N in soils. Sahrawat and Prasad (1975) proposed the use of Morgan's reagent for simultaneous extraction of ammonium, nitrite and nitrate nitrogen from soil. Sahrawat (1979) compared potassium chloride, sodium chloride, sodium acetate and Morgan's reagent solutions for extracting ammonium nitrogen from rice soils. He reported the efficiency of

2.3 SELECTION OF A SUITABLE EXTRACTANT FOR THE EXTRACTION OF INORGANIC NITROGEN FROM COAL MINE SOIL

2.3.1 INTRODUCTION

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these chemicals in the following descending order by using the recovery of the known amount of ammonium-N applied to soils as a criterion.

KCl> NaCl> CH_3COONa> Morgan's reagent.

Extraction is one of the most important steps for the colorimetric determination of ammonium and other inorganic forms of nitrogen in the coal mine soil. In order to avoid any problem in the preparation of extracts for determination of ammonium, nitrite and nitrate-N in soils, it is necessary to obtain clear, colourless extracts which do not contain substances that interfere with the colorimetric method of determination of these forms of nitrogen. It has been reported by Bremner (1965) that procedures involving extraction with potassium chloride or sodium chloride solutions yielded turbid or coloured extracts, and chloride interfered with the methods generally favoured for colorimetric determination of nitrate. Moreover, it is also important to use an extractant that contains very low levels of inorganic nitrogen as impurities or that can be purified very easily. The aim of this experiment was to select a suitable extractant for coal mine soils amongst the various potassium salts commonly used for the extraction of inorganic nitrogen from soils.

2.3.2 MATERIALS AND METHODS

2.3.2.1. Reagents

Analar grade reagents and deionized water was used throughout.

1. Potassium sulphate solution (0.5 M)
2. Potassium chloride solution (1 M)
3. Potassium acetate solution (1 M)

The potassium sulphate and potassium chloride solutions were prepared from their respective salts using deionized water. The solutions were boiled and stirred at pH 11.0 for 15 minutes and then readjusted to pH 5.5-6.0 after cooling.

The potassium acetate solution was not boiled at pH 11, keeping in view the trace amount of ammonium contamination and buffering nature of the salt. Khan (1987) reported negligible amounts of ammonium (0.1-0.2 mg/kg salt) in the batch number of potassium acetate used in this experiment.

2.3.2.2. Soil samples

Eight soil samples collected from the following experimental plots at Baads were used as test samples.

1. Naturally vegetated area

No treatment had been applied to this area of the

bing and the spoil had become naturally vegetated mainly with trees and associated undergrowth. Two soil samples one each from organic and mineral horizons from this area were collected. The sample from the $_{A}$ organic horizon was further subdivided horizontally into three portions, indicated as natural organic 1, 2 and 3.

2. Chicken manure treated plot
3. NPK fertilizer treated plot
4. Birdsfoot trefoil species plot
5. White clover species plot

The above mentioned four plots had been specifically reclaimed and sown with a grass / clover mixture or as otherwise mentioned.

2.3.2.3. Procedure

An amount of fresh soil (partially air-dried to allow sieving through 4mm sieve) equivalent to 2.5 g on oven dry basis was shaken for 2 hours at 2 ^{o}C with 50 cm^{3} of extracting solution. The suspensions were filtered through previously washed Whatman filter paper No.40 and the filtrates obtained were analysed for ammonium, nitrite and nitrate-N with a Technicon AutoAnalyzer II (see section 2.1.5). All the analytical standards used contained equal concentrations of the corresponding extractant as the soil extracts.

2.3.3 RESULTS AND DISCUSSION

The criteria used for the selection of the extractant were (1) amount of inorganic nitrogen extracted from soil

samples (2) level of inorganic nitrogen in the extractant (3) interference with colorimetric method of nitrate-N determination.

Some of the results of the experiment, as shown in Table 2.3, indicate that significantly different amounts of ammonium nitrogen were extracted by the different extractants from half of the coal mine soil samples tested. There was, however, no trend whereby one extractant consistently removed more ammonium nitrogen than another.

Sample	Ammonium-N (mg/kg oven dry soil)			
	potassium sulphate	potassium chloride	potassium acetate	
Natural mineral	1.3	1.4	1.4	
Natural organic 1	19.4	18.7	20.2	
Natural organic 2	9.0	9.2	10.5	*
Natural organic 3	3.0	3.0	2.8	
Chicken manure plot	4.5	2.6	2.8	*
Fertilizer plot	3.3	3.0	2.6	
Birdsfoot trefoil plot	0.1	0.1	1.2	*
White clover plot	2.5	2.2	3.3	*

Table 2.3. Ammonium-N extracted from 8 soil samples using 0.5 M potassium sulphate , 1 M potassium chloride and 1 M potassium acetate.

* indicates a significant difference between extraction solutions ($p < 0.001$)

Sample	Nitrate-N (mg/kg oven dry soil)			
	potassium sulphate	potassium chloride	potassium acetate	
Natural mineral	0.0	0.3	0.0	*
Natural organic 1	0.0	0.3	0.3	*
Natural organic 2	0.0	1.9	0.2	*
Natural organic 3	0.0	1.8	0.0	*
Chicken manure plot	0.3	1.0	0.5	*
Fertilizer plot	0.0	0.7	0.1	*
Birdsfoot trefoil plot	0.0	0.9	0.5	*
White clover plot	0.0	0.5	0.2	*

Table 2.4. Nitrate-N extracted from 8 soil samples using 0.5 M potassium sulphate , 1 M potassium chloride and 1 M potassium acetate.

* indicates a significant difference between extraction solutions ($p < 0.001$)

The results of the experiment documented in Table 2.4 show that in spite of the very small amounts of nitrate nitrogen extracted by different reagents from various soil samples, potassium chloride (1 M) extracted significantly more nitrate nitrogen from all of the coal mine soil samples. The use of potassium chloride (1 M) cannot be recommended because it sometimes yielded coloured extracts and also due to the chloride ion interference in the colorimetric determination of the nitrate nitrogen. The comparatively higher levels of nitrate nitrogen extracted

by this salt solution may be due to this chloride ion interference.

As far as the level of contamination is concerned, Khan (1987) studied the nitrogen contamination in several batches of potassium sulphate, potassium chloride and potassium acetate. He reported:

1. Very low levels of nitrite in the various batches of potassium salts.
2. Higher and variable levels of ammonium in potassium sulphate compared with potassium chloride and potassium acetate salts.
3. Comparatively higher levels of nitrate in potassium acetate salts.

Keeping in view the higher and variable levels of nitrate-N contamination in potassium acetate salts, its use will be a bit risky because it cannot be purified easily as there is no simple method for the removal of nitrate-N.

Potassium sulphate solution does not interfere in the nitrate nitrogen analysis and contains low levels of nitrate nitrogen contamination. It does contain ammonium nitrogen contamination but it can be easily purified by boiling and stirring for fifteen minutes at pH 11.0 (Khan, 1987). Ammonium is converted into ammonia by increasing the pH of the solution to 11.0 with potassium hydroxide solution. Boiling and stirring for a period of 15 minutes will expel all the ammonia in the solution.

Coal mine soil extracted with potassium sulphate produced precipitation in some cases when the extract was

stored at 2 ^{o}C for 2 days but this drawback can be ignored as it does not affect the amount of nitrogen extracted from the soil. It was therefore, decided to use 0.5 M potassium sulphate as an extractant for the extraction of ammonium, nitrate and nitrite nitrogen from the coal mine soils.

2.3.4 METHOD OF INORGANIC-N EXTRACTION FROM COAL MINE SOIL

1. Preparation of 0.5 M potassium sulphate solution

87.125 g potassium sulphate was dissolved in about 800 cm^3 deionized water and made up to 1 litre. The pH of the solution was raised to 11.0 with potassium hydroxide solution. The solution was then boiled and stirred for 15 minutes. The pH of the solution was readjusted to normal with 0.5 M sulphuric acid after cooling. Deionized water was added for any loss of water during boiling due to evaporation.

2. Method of inorganic-N extraction

An amount of fresh coal mine soil (partially air-dried to allow sieving through a 4 mm sieve) equivalent to 2.5 g on an oven dry basis was shaken for 2 hours at 2 ^{o}C with 50 cm^3 of 0.5 M potassium sulphate. The suspensions were filtered through previously washed Whatman filter paper No. 40 or No. 2. (see section 2.2.4). The first 2 to 3 cm^3 of the filtrates including blanks were discarded. The filtrates obtained were analysed for ammonium, nitrite and nitrate nitrogen with a

Technicon AutoAnalyzer II by using methods described in section 2.1.5. All the analytical standards used contained equal concentrations of 0.5 M potassium sulphate solution as the soil extracts.

2.4 TESTING OF INCUBATION PROCEDURES

2.4.1 INTRODUCTION

Many methods for obtaining an index of the availability of soil nitrogen to plants have been proposed. It is generally accepted that the most satisfactory methods for the assessment of 'nitrogen supplying power' of a soil are those based on soil incubation. The basis of these techniques is the estimation of mineral nitrogen released when a soil is incubated under conditions that promote mineralization of soil organic nitrogen reserves (Bremner, 1965). Numerous incubation techniques have been used for estimation of mineralizable soil nitrogen, but methods involving determination of total inorganic nitrogen (ammonium, nitrite and nitrate) by incubation under aerobic conditions have been generally preferred. Many investigations have shown good correlation between the values produced by incubation methods and yields of vegetation subsequently produced in the field. As most of these incubation studies have been confined to normal agricultural soils, very little information is available on coal mine soils.

Incubation methods are probably the most meaningful in determining the plant availability of nitrogen in disturbed soil materials. Reeder and Berg (1977a) suggested that laboratory incubation tests could be useful in estimating the plant available nitrogen potentials of

certain drastically disturbed lands prior to extensive revegetation programme. They found a high correlation between mineral nitrogen content of laboratory incubation samples and total nitrogen uptake by barley grown under greenhouse conditions.

Research workers have used various incubation techniques differing with respect to quantity of soils, use of physical and/ or chemical amendments, temperatures, water level, incubation periods, type of incubation vessel, method of estimating mineral nitrogen production etc. Williams (1975) and Williams and Cooper (1976) incubated 10 g coal mine soil samples in the dark at 15% moisture and 27 ^{o}C, in loosely capped 250 cm^3 bottles, corrected the moisture lost after every two days and measured mineral nitrogen content after 0 and 40 days of incubation. Reeder and Berg (1977b) used an incubation procedure for studying mineralizable nitrogen, carbon dioxide evolution and nitrification by incubating 40 g coal mine soil wetted to field $_{\Lambda}^{\text{capacity}}$ at 31 ^{o}C for 3, 6, 12, 21, 42, 84 or 168 days. They used the entire 40 g sample for extractable nitrogen at the end of a specific incubation period. Jefferies et al. (1981b) used the aerobic incubation method of Keeney and Bremner (1967). The method involved determination of the inorganic nitrogen (ammonium, nitrite, and nitrate) in 2M KCl extracts of soil by a steam distillation method. 10 g of soil were mixed with 30 g of sand, treated with 6 cm^3 of water and incubated at 30 ^{o}C for 14 days.

Since an understanding of nitrogen cycling in coal

mine soils is limited, an incubation study was undertaken to determine mineralization rates of indigenous nitrogen, carbon turnover, and nitrification rates of added ammonium.

The main purpose of this work was to evaluate a simple and comparatively rapid method of aerobic incubation, which can be applicable and suitable for a survey study involving a large number (90) of samples.

2.4.2 MATERIALS AND METHODS

The coal mine soil samples collected from Baads colliery (described in section 2.3) were used as test samples for the evaluation of incubation procedures for studying mineralization of indigenous nitrogen and carbon, and nitrification of added ammonium (ammonium sulphate).

The soil samples were partially air-dried at room temperature to permit sieving (4 mm sieve) and for additions to be made without exceeding the desired moisture content for incubation.

2.4.2.1. Reagents

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

1. Sodium hydroxide (1M volumetric solution)
2. Ammonium solution (2,500 mg NH_4-N/l)

1.180 g dried ammonium sulphate was dissolved in water and made up the volume 100 cm^3.

2.4.2.2. Incubation procedure for studying N mineralization and CO_2 evolution

Two fresh coal mine soil samples, one from the chicken manure treated plot and the other from the birdsfoot trefoil species plot, were used as test samples for the evaluation of an incubation procedure for studying nitrogen mineralization and carbon dioxide evolution. Each soil sample was incubated in duplicate as described below.

An amount of fresh soil equivalent to 50 g on an oven dry basis was weighed into a plastic vessel 6 cm in diameter and 8 cm deep (made from a 250 cm^3 plastic bottle). The soil moisture was adjusted to -0.5 bar soil moisture potential by the addition of an appropriate weight of deionized water with the help of a Pasteur pipette. The soil container was then placed in a 1.5 litre glass Kilner jar together with 25 cm^3 M sodium hydroxide in an open 2 oz glass bottle. The Kilner jar also contained about 20 cm^3 deionized water in order to offset the drying effect of the alkali. Blank incubations, in which the jar contained water and sodium hydroxide but no soil, were also carried through to allow correction to be made for absorption of carbon dioxide from sources other than microbial respiration in the soil. The jars were large enough to ensure that there was enough oxygen for the period of incubation. The Kilner jar lid was then screwed on over a parafilm sealing ring to make it air-tight. Care was taken to avoid breathing near the open jar which may become an important source of error.

The soils were incubated at 20 ^{o}C for a period of 5 weeks. Carbon dioxide evolved by the soil and mineral nitrogen (ammonium, nitrite and nitrate-N) in the soil samples were measured at weekly intervals over 5 weeks. At the end of each week of incubation the bottle containing sodium hydroxide was removed and closed tightly. Fresh air was allowed to enter the Kilner jar for 10-15 minutes. Care was taken to re-adjust any loss in the weight of the soil before taking a sample for extractable-N, by adding an appropriate weight of deionized water.

Carbon dioxide absorbed by M sodium hydroxide was measured by the titration method of Jenkinson and Powlson (1976) as described in section 2.1.4. The mineral nitrogen (ammonium, nitrite and nitrate) was extracted and determined colorimetrically by Technicon AutoAnalyzer II using the methods described in sections 2.3.4 and 2.1.5.

2.4.2.3. Incubation procedure for measurement of nitrification of added ammonium.

Eight fresh coal mine soil samples collected from Baads colliery (see section 2.3.2.2) were used during this test. Each sample was incubated in duplicate by the method as described below.

A sample of fresh coal mine soil equivalent to 50 g (oven dry weight basis) was weighed into a 8 oz glass bottle which was left open to permit aeration. The sample was treated with 2 cm^3 of ammonium sulphate solution containing 2,500 mg NH_4-N/l. Following thorough mixing of the soil sample, the moisture content was adjusted to the

required level (-0.5 bar soil moisture potential) by the addition of an appropriate weight of deionized water with a Pasteur pipette. The bottle containing the sample was allowed to stand for 3 hours at 2 ^{o}C. After taking a sample for measuring extractable-N (ammonium, nitrite and nitrate) at 0 day incubation, the bottle was then placed in a 1.5 litre glass Kilner jar with a few cm^{3} water at the bottom in order to keep the air moist. The jar was large enough to ensure that there was sufficient oxygen for the incubation period. The Kilner jar lid was then screwed on over a parafilm sealing ring to make it air tight. The soil was incubated at 20 ^{o}C for 17 days. The changes in ammonium, nitrite and nitrate nitrogen were measured at 0, 3, 5, 7, 10, 12, and 17 days of incubation. The Kilner jar was allowed to stand open for 10-15 minutes to replenish the air at the end of each interval. Care was taken to readjust any loss in weight of the soil in the bottle with deionized water before taking a sample for measuring the extractable mineral nitrogen. The ammonium, nitrite and nitrate forms of nitrogen were measured colorimetrically by using a Technicon AutoAnalyzer II (see section 2.1.5).

Note:

Two coal mine soil samples, one from chicken manure treated plot and the other from birdsfoot trefoil species plot were incubated by using Kilner jar as an incubation chamber. Later the remaining 6 samples were incubated in a similar way, except that a 16 litre plastic bin lined with

soaked filter papers was used for a set of 10 bottles as an incubation chamber. In this case the mineral nitrogen (ammonium, nitrite and nitrate) was determined at 0, 4, 8 and 11 days of incubation.

2.4.3 RESULTS AND DISCUSSION

Fresh coal mine soils samples were used in testing incubation procedures and care was taken to ensure that the soil sample received the minimum amount of air drying so as to minimise any reduction in the nitrifying population.

Figs. 2.3 and 2.4 indicate the lack of mineralizable nitrogen in coal mine soils tested.

The cumulative amounts of carbon mineralized in the coal mine soils during 5 weeks of incubation as shown in Figs. 2.5 and 2.6 indicate that a large amount of carbon was mineralized. The N present in these coal mine soils may be in forms that are not readily available for microbial metabolism, therefore it was decided to measure the mineralizable nitrogen (ammonium, nitrite and nitrate) at 4 weeks interval instead of one week interval and the incubation period to be extended up to 8 weeks.

In contrast to other incubation procedures mentioned in the introduction, the aerobic incubation procedures tested were found more simple and suitable for use in a survey study. The method tested for mineralization study has the advantages that: (i) The mineralizable nitrogen and carbon turnover can be measured within the same procedure. (ii) It does not require the use of any

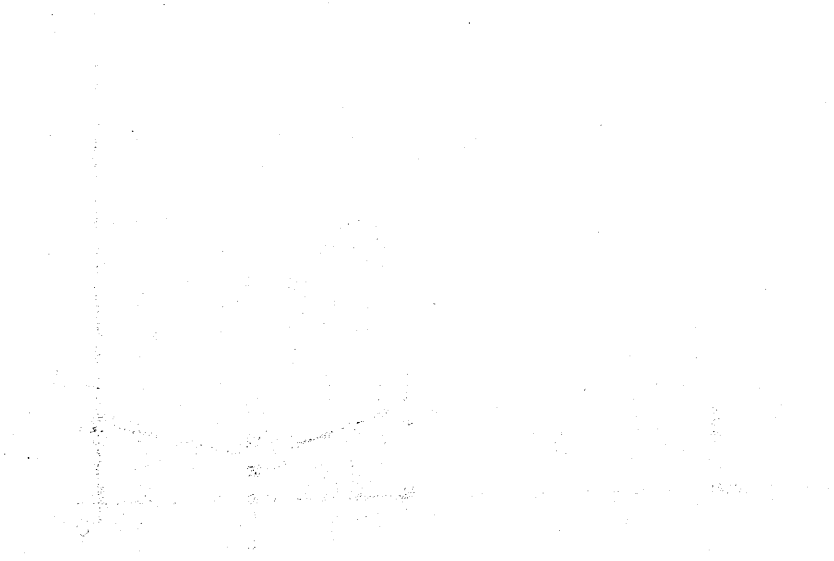
amendment like washed quartz sand. (iii) The aeration is simple and can be done along with the sodium hydroxide renewal once a week. (iv) The 0.5 bar moisture content of incubated soil, is an optimum moisture level for microbial growth, at which level the micro-organism metabolism would not be limited by water, but at this level they would have sufficient oxygen. (v) The automated colorimetric methods used to determine the nitrogen mineralized during incubation, determines the total mineral nitrogen (ammonium, nitrite and nitrate) and is rapid and precise.

The changes in ammonium, nitrite and nitrate as well as total mineral nitrogen during incubation period of the ammonium sulphate treated coal mine soils are shown in Figures 2.7 and 2.8. There was no measurable nitrification in most of the coal mine soil samples tested. An obvious decrease in the total mineral-N was observed which may be possibly due to ammonium fixation by clay or immobilization of inorganic nitrogen.

The sample from the chicken manure-treated plot showed a measurable nitrification rate, with a marked decrease in the total mineral-N. On the 17th day of incubation, the remaining coal mine soil in the bottle was treated again with 100 mg ammonium-N/kg soil and the changes in the ammonium, nitrite and nitrate nitrogen were determined at 19, 21, 24, 26 and 28 days of incubation. This time a measurable rate of nitrification of added ammonium sulphate was observed as shown in Fig 2.7. It shows that chicken manure may contain some nitrifying bacteria, or this plot might have received the responsible

organisms from the adjacent fields.

This incubation procedure showed that the coal mine soil which had some nitrifying bacteria exhibited measurable nitrification of added ammonium within 4 days of incubation, and almost 90-100 % of the added ammonium was changed to nitrate (Figure 2.7). It was, therefore, decided to determine the mineral-N (ammonium, nitrite and nitrate) at four days interval and incubation to be carried up to 16 days for survey samples. The use of a 16 litre plastic bin for a set of 10 bottles instead of individual Kilner jars for each bottle, was found to be a more rapid and easy method for incubation.



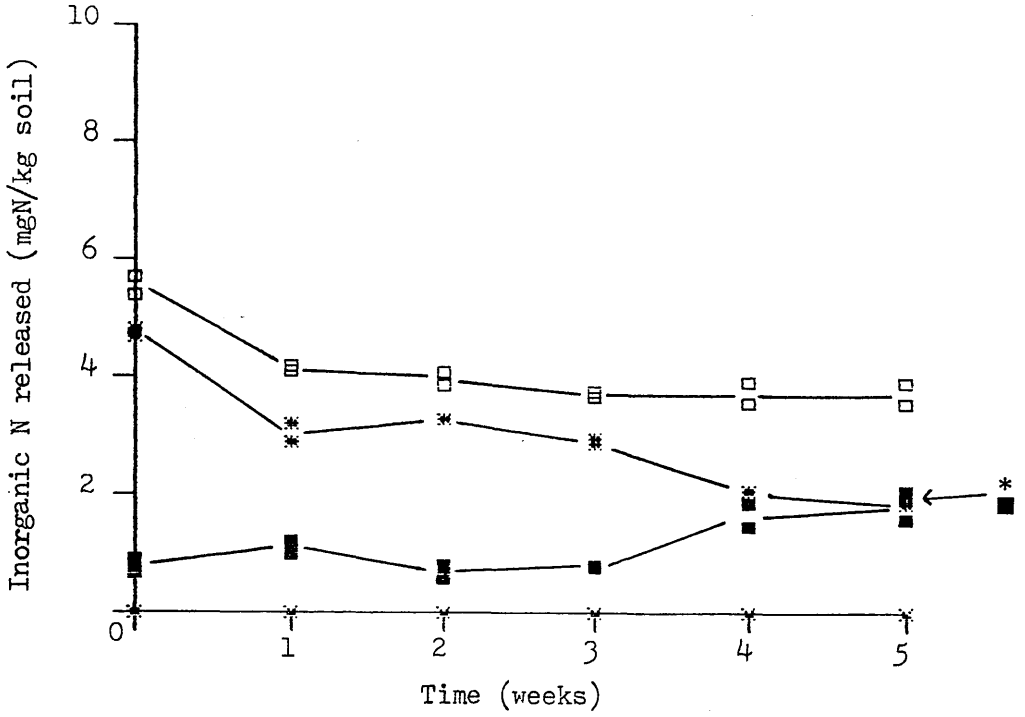


Fig. 2.3 N mineralized by soil from a chicken manure treated plot.
Total inorganic N (□), NH_4-N (*), NO_3-N (■), NO_2-N (×) Duplicate incubations

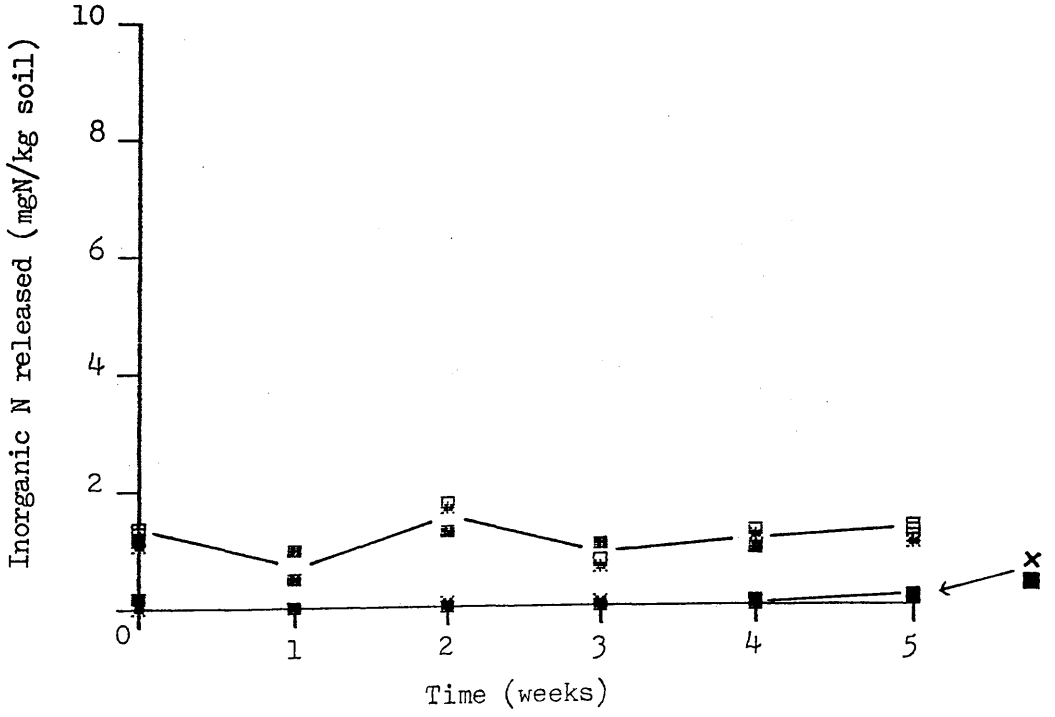


Fig. 2.4 N mineralized by soil from a birdsfoot trefoil plot.
Total inorganic N (□), NH_4-N (*), NO_3-N (■), NO_2-N (×) Duplicate incubations

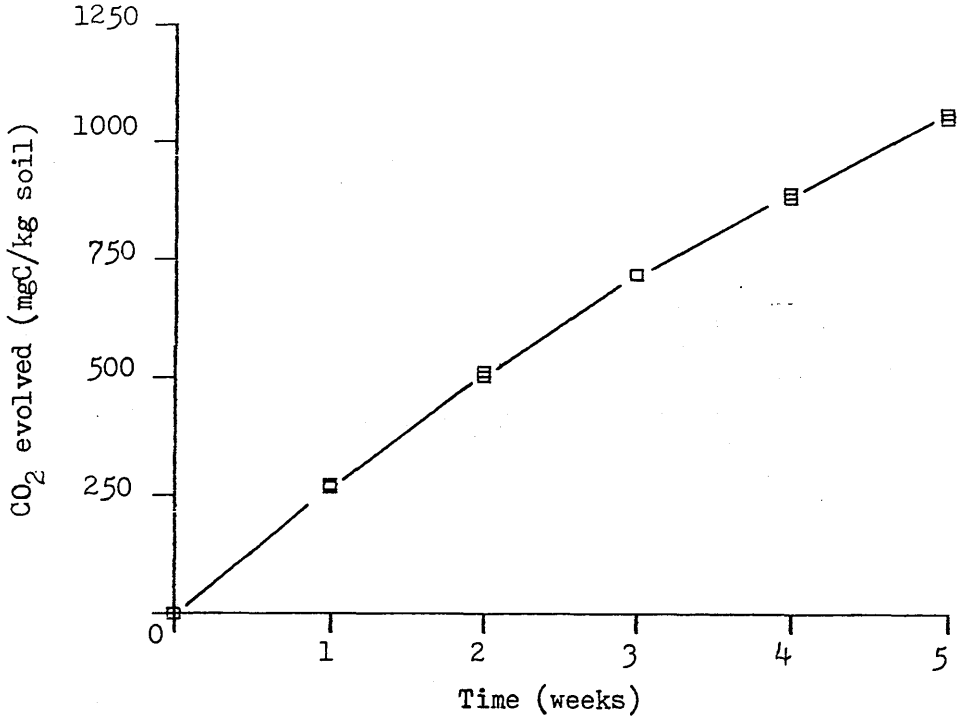


Fig. 2.5 CO_2-C evolved by soil from a chicken manure treated plot. Duplicate incubations

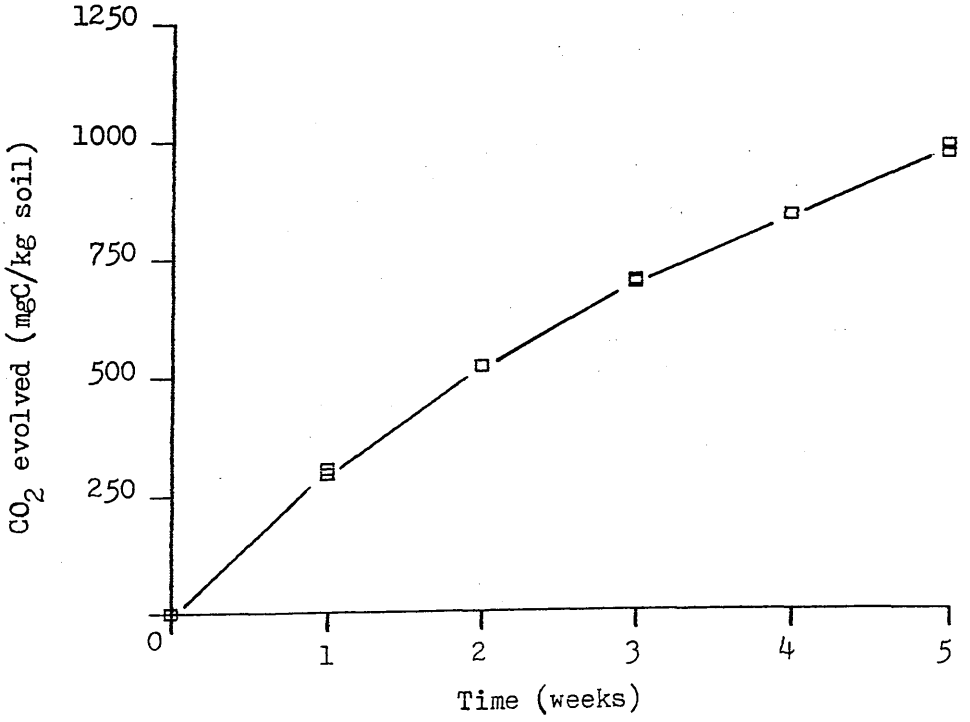


Fig. 2.6 CO_2-C evolved by soil from a birdsfoot trefoil plot. Duplicate incubations

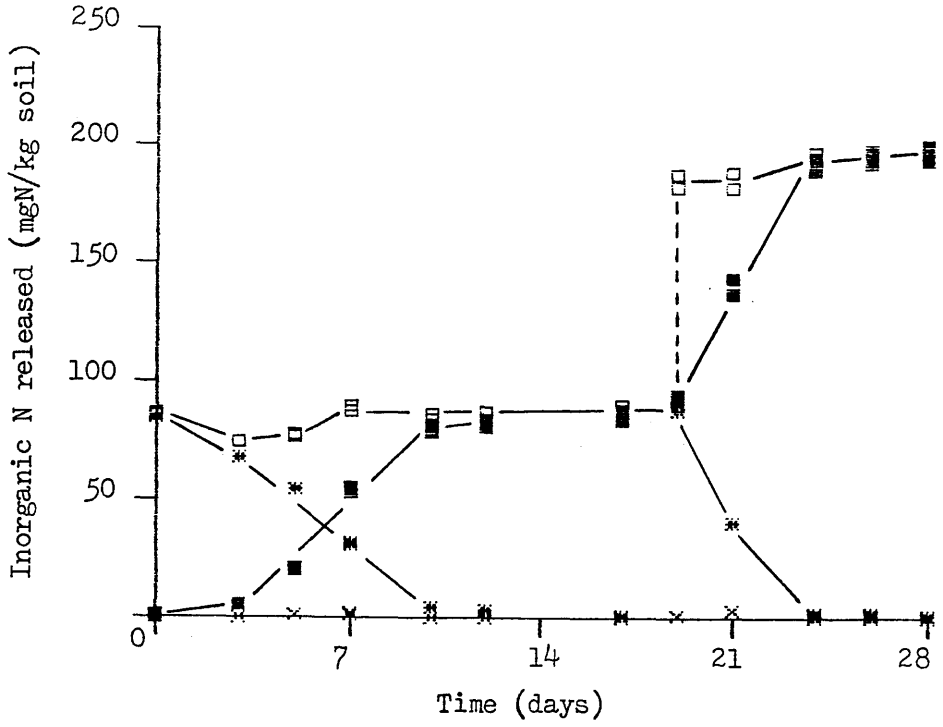


Fig. 2.7 Nitrification of added NH_4-N by soil from a chicken manure treated plot.
Total inorganic N (□), NH_4-N (*), NO_3-N (■), NO_2-N (×) Duplicate incubations

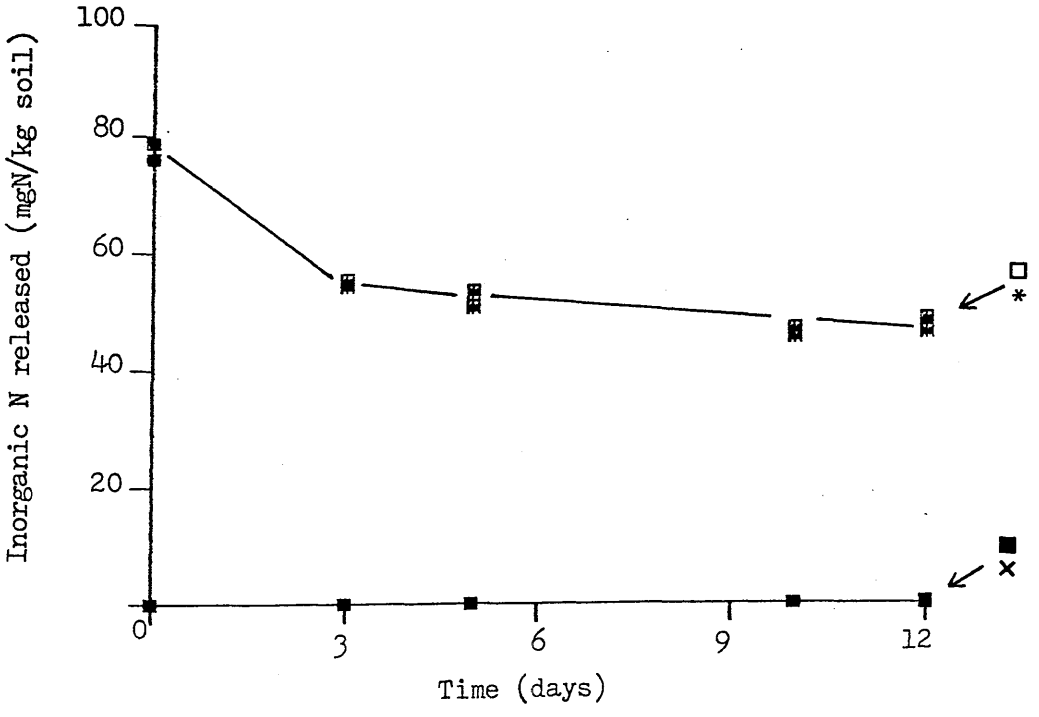


Fig. 2.8 Nitrification of added NH_4-N by soil from a birdsfoot trefoil plot.
Total inorganic N (□), NH_4-N (*), NO_3-N (■), NO_2-N (×) Duplicate incubations

2.5 TESTING OF AMMONIUM FIXATION TECHNIQUES

2.5.1 INTRODUCTION

From the incubation studies with addition of ammonium fertilizer (ammonium sulphate), it was observed that a certain amount of added ammonium was not recovered from the coal mine soil at zero day of incubation. Some ammonium ions added as ammonium salt are held in fixed forms by clays, as potassium is, and then are not accessible for quick exchange with other ions or for nitrification by bacteria. The amount of ammonium nitrogen fixed against extraction with 0.5 M potassium sulphate solution ranged from 13 mg/kg soil (chicken manure-treated plot) to 24mg/kg soil (birdsfoot trefoil species plot) in coal mine soil samples from Baads (Figs. 2.7 and 2.8). In some samples this loss of added ammonium was found to be less at zero time compared to after 16-days of incubation (Fig. 2.8) where only about 60 mg of the added 100 mg of ammonium-N/kg soil was recovered with no accumulation of nitrate.

Reeder and Berg (1977b) found a loss of added ammonium in coal mine spoil during an incubation experiment, and recovered 46 ppm ammonium-N of the added 60 ppm ammonium-N at zero time. Since the spoil sample they tested was calcareous, they attributed some of this initial loss to ammonia volatilization.

As no systematic study has been carried out on this aspect, the present work was undertaken to find a simple and rapid method for studying the loss of added ammonium

due to ammonium fixation in a wide range of coal mine soils collected from various colliery bings of Central Scotland.

In order to study the ability of coal mine soils to fix ammonium-N two methods were tested. The first method involved treatment of the soil with a known amount of ammonium sulphate solution (100 mg NH_4-N/kg soil) and after a definite time of contact, the extractable portion was removed with 0.5 M potassium sulphate extraction. The difference between the ammonium added and that recovered in the extract was considered as the ammonium fixed.

The second method, which is generally used for estimation of the capacity of soil to fix potassium was tested on 5 coal mine soil samples. As much of the basic information on ammonium fixation originates from the studies on fixation of potassium ion, the quantity-intensity curve method was tested for ammonium fixation study. In this experiment a quantity-intensity curve (Q/I) for ammonium was measured to examine the mechanism of holding exchangeable ammonium in the coal mine soil, where Q represents the change in the quantity of the exchangeable ammonium, held by the soil, and I the intensity of the ammonium ion activity relative to the dominant cations in the bathing solution, in this case calcium.

In addition, the reversibility of the isotherm was examined by measuring the desorption curve following an initial equilibrium with a high activity of ammonium in solution. A background solution of 0.01M calcium chloride

was used so that other cations can be ignored when calculating the total divalent ions present, by assuming the activity ratio to be $(NH_4^+) / (Ca^{2+})^{1/2}$.

2.5.2 MATERIALS AND METHODS

2.5.2.1. Reagents

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

1. Ammonium sulphate solution (250 mg NH_4-N/l)

1.180 g dried ammonium sulphate was dissolved in water and made the volume 1 litre.

2. Ammonium sulphate solution (125 mg NH_4-N/l)

0.590 g dried ammonium sulphate was dissolved in water and diluted to 1 litre.

3. Ammonium chloride solution (100 mM)

M ammonium chloride solution was prepared by dissolving 5.349 g ammonium chloride in water and diluted to 100 cm^3. 10 cm^3 of this solution was diluted to 100 cm^3 with water.

4. Calcium chloride solution (0.01M)

10 cm^3 M calcium chloride (BDH analytical volumetric solution) was diluted to 1 litre with water.

5. Solutions containing 0, 0.5, 1, 2, 3, 4 and 5 mM ammonium in 0.01 M calcium chloride were prepared by pipetting 5 cm^3 M calcium chloride into seven 500 cm^3 volumetric flasks, and then adding 0, 2.5, 5, 10, 15, 20 or 25 cm^3 100 mM ammonium chloride and making up the volume with water.

2.5.2.2. Single point method 1

Thirty coal mine soil samples with a wide range of pHs from Dykehead, Stane, Fauldhouse, Loganlee and North Addiewell colliery bings were used as test samples during this experiment. The details of the sites and sampling areas are given in chapter 3 section 3.2. The samples were partially air dried just sufficient to be sieved (4mm sieve).

Duplicate 2.5 g fresh samples of coal mine soil (oven dry weight basis) were weighed out into 100 cm^3 clean, dry plastic bottles. One sample was treated with 1 cm^3 ammonium sulphate solution containing 250 mg NH_4-N/l and the other was treated with 1 cm^3 water to allow quantification of ammonium-N already present in the coal mine soil. The bottles were shaken and allowed to stand for 3 hours at 2 ^oC. The inorganic-N (ammonium, nitrite and nitrate) in treated and untreated samples of the coal mine soil was extracted with 0.5 M potassium sulphate solution and measured colorimetrically by Technicon AutoAnalyzer II using the methods described in sections 2.3.4 and 2.1.5.

2.5.2.3. Single point method 2

Duplicate 2.5 g samples of coal mine soil (oven dry weight basis) were weighed out into 100 cm^3 clean, dry plastic bottles. One sample was treated with 2 cm^3 ammonium sulphate solution containing 125mg NH_4-N/l and the other was treated with 2 cm^3 deionized water to allow quantification of ammonium-N already present in the coal mine soil. The bottles were shaken and allowed to stand for 24 hours at 2 ^oC. The inorganic-N (ammonium, nitrite and nitrate) in treated and untreated samples of the coal mine soil was extracted with 0.5 M potassium sulphate solution and measured colorimetrically by Technicon AutoAnalyzer II using the methods described in sections 2.3.4 and 2.1.5.

2.5.2.4. Quantity/ intensity curve method

One 0.5 g sample and seven 5.0 g samples of the coal mine soil were weighed out into 4 oz glass bottles. 50 cm^3 0.01M calcium chloride solutions containing 0, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0 mM ammonium chloride were added to the bottles containing soil in the following order.

weight of soil (g)	solution (50 cm^3)
0.5	0.0 mM NH_4 in 0.01M $CaCl_2$
5.0	0.0 mM NH_4 in 0.01M $CaCl_2$
5.0	0.5 mM NH_4 in 0.01M $CaCl_2$
5.0	1.0 mM NH_4 in 0.01M $CaCl_2$
5.0	2.0 mM NH_4 in 0.01M $CaCl_2$
5.0	3.0 mM NH_4 in 0.01M $CaCl_2$
5.0	4.0 mM NH_4 in 0.01M $CaCl_2$
5.0	5.0 mM NH_4 in 0.01M $CaCl_2$

The soil suspensions were filtered through Whatman No 40 filter paper after shaking for 3 hours at 2 ^oC, and the filtrates were analysed for extractable inorganic-N colorimetrically by Technicon AutoAnalyzer II (see section 2.1.5).

For determination of ammonium-N in the filtrates, dilution was considered important because of the high concentration of ammonium in the filtrates. Therefore, a dilution step was incorporated into the ammonium manifold (Fig. 2.1) whereby the sample solution was diluted 1/14

(sample 0.16 ml/min, water 2.0 ml/min) or 1/3 (sample 0.6 ml/min, water 1.2 ml/min) using deionized water prior to being presented to the sample input tube.

Adsorption-desorption curve

Eight 1.0 g samples of coal mine soil were weighed out into 4 oz glass bottles, and to each bottle was added 10 cm^3 of 0.01 M calcium chloride solution containing 5mM ammonium. The suspensions were shaken for 3 hours at 2 ^oC and then additions of 0, 2, 5, 10, 15, 25, 50 and 100 cm^3 0.01M calcium chloride (with no ammonium) were made to separate bottles as shown below.

Soil weight (g)	Initial addition 5 mM_3NH_4 (cm^3)	after 3 hours 0 mM_3NH_4 (cm^3)
1.00	10.0	0.0
1.00	10.0	2.0
1.00	10.0	5.0
1.00	10.0	10.0
1.00	10.0	15.0
1.00	10.0	25.0
1.00	10.0	50.0
1.00	10.0	100.0

The soils were shaken for a further 3 hours at 2 ^oC and filtered through Whatman No. 40 filter paper. Ammonium, nitrite and nitrate in the filtrates were measured colorimetrically by Technicon AutoAnalyzer II.

In order to make sure that the method is applicable to ammonium fixation study, it was first tested on fresh and air dried coal mine soil samples, collected at Fauldhouse (FD 6) and North Addiewell (NAD 9). For further confirmation, the method was tested on 3 other fresh soil

samples. This time the soil samples were selected on the basis of results obtained in the previous experiment of ammonium fixation (single point method 2) and those samples were selected which showed some evidence of ammonium fixation without any nitrate formation from the added ammonium during 24 hours of standing the treated sample (100 mg NH_4-N/Kg) at 2 ^{o}C.

2.5.3 RESULTS AND DISCUSSION

The loss of ammonium-N was calculated by subtracting the amount of ammonium-N determined in the treated sample from that initially present in the sample plus added (100 mg N/kg). Nitrite-N was found to be negligible in treated and untreated samples. The nitrate-N contents were considered in the calculation, in order to allow for the correction of any ammonium nitrified. The loss of ammonium was corrected by including the loss or gain in the nitrate content of the sample.

The data in Table 2.5 show that there was no loss of ammonium during 3 hours at 2 ^{o}C, apart from soil samples Dykehead 1b and North Addiewell 3b which showed some measurable ammonium loss of 2.1 and 1.5 mg N/kg soil respectively. Most of the soils have shown gain in nitrogen content rather losing the added nitrogen due to ammonium fixation.

The data documented in Table 2.6 indicate that the amount of corrected ammonium loss during 24 hours at 2 ^{o}C ranged from 0.7 to 9.6 mg/kg soil. This method proved to be better than method 1. The 2 ^{o}C temperature worked well

and kept the nitrification of the added ammonium to a minimum ranging from 0.1 to 2.3 mg NO_3-N/kg soil.

The results obtained from the Q/I method, shown in Figs. 2.9-2.12, indicate that some ammonium was fixed by the soil samples tested. There was no difference in the amount of ammonium fixed by the air-dried and fresh samples of the same coal mine soil. Q/I curve method for potassium can be applied to the study of ammonium fixing ability of soil, but it was found time consuming and laborious. It was considered not suitable for a survey study with a large number of samples.

The single point method 2 was found very simple and rapid as compared to Q/I curve method and therefore, it was decided to use this method for studying the ammonium fixing ability of added ammonium of coal mine soils.

Sample	NH_4 loss	NO_3 gain	NH_4 loss (corrected)
	(mg N/kg soil)		
Dykehead 1	-2.5	-0.1	-2.6
	2.0	-0.1	2.1
	-3.3	0.0	-3.3
Dykehead 2	-2.8	0.5	-2.3
	-2.1	0.8	-1.3
	-1.1	-1.2	-2.3
Stane	-2.3	-3.5	-5.8
	-2.4	0.8	-1.6
	-0.4	0.5	0.1
Fauldhouse 1	-4.2	0.9	-3.3
	-3.4	-0.2	-3.6
	-4.1	-0.2	-4.3
Fauldhouse 2	-8.1	0.0	-8.1
	-3.9	0.0	-3.9
	-3.9	0.0	-3.9
Loganlee 1	-1.8	0.0	-1.8
	0.0	0.0	0.0
	0.9	0.0	0.9
Loganlee 2	-2.0	0.1	-1.9
	-2.2	0.0	-2.2
	-2.9	0.4	-2.5
N. Addiewell 1	-4.0	0.0	-4.0
	-2.5	-0.1	-2.6
	-3.9	0.1	-3.8
N. Addiewell 2	-0.3	0.0	-0.3
	-0.3	0.0	-0.3
	-1.7	0.0	-1.7
N. Addiewell 3	-1.2	0.1	-1.1
	1.4	0.1	1.5
	-2.3	0.9	-1.4

Table 2.5. Loss of ammonium during 3 hours at 2 ^{o}C.

Sample	NH_4 loss	NO_3 gain	NH_4 loss (corrected)
	(mg N/kg soil)		
Dykehead 1	4.0	0.0	4.0
	3.0	0.6	2.4
	2.1	0.3	1.8
Dykehead 2	1.9	0.7	1.2
	2.0	1.3	0.7
	1.6	0.6	1.0
Stane	3.4	0.1	3.3
	2.6	0.0	2.6
	4.4	2.2	2.2
Fauldhouse 1	1.2	2.3	-1.1
	1.1	1.4	-0.3
	1.2	1.8	-0.6
Fauldhouse 2	-1.3	0.0	-1.3
	2.3	0.0	2.3
	9.6	0.0	9.6
Loganlee 1	2.9	0.0	2.9
	2.3	0.0	2.3
	7.7	0.0	7.7
Loganlee 2	1.9	0.0	1.9
	4.0	-0.1	4.1
	3.2	0.0	3.2
N. Addiewell 1	2.9	0.0	2.9
	2.9	-3.2	6.1
	-0.1	0.8	-0.9
N. Adiewell 2	7.6	0.0	7.6
	6.1	0.0	6.1
	-6.8	0.0	-6.8
N. Addiewell 3	4.6	0.0	4.6
	5.1	1.1	4.0
	3.4	1.0	2.4

Table 2.6. Loss of ammonium during 24 hours at 2 ^{o}C.

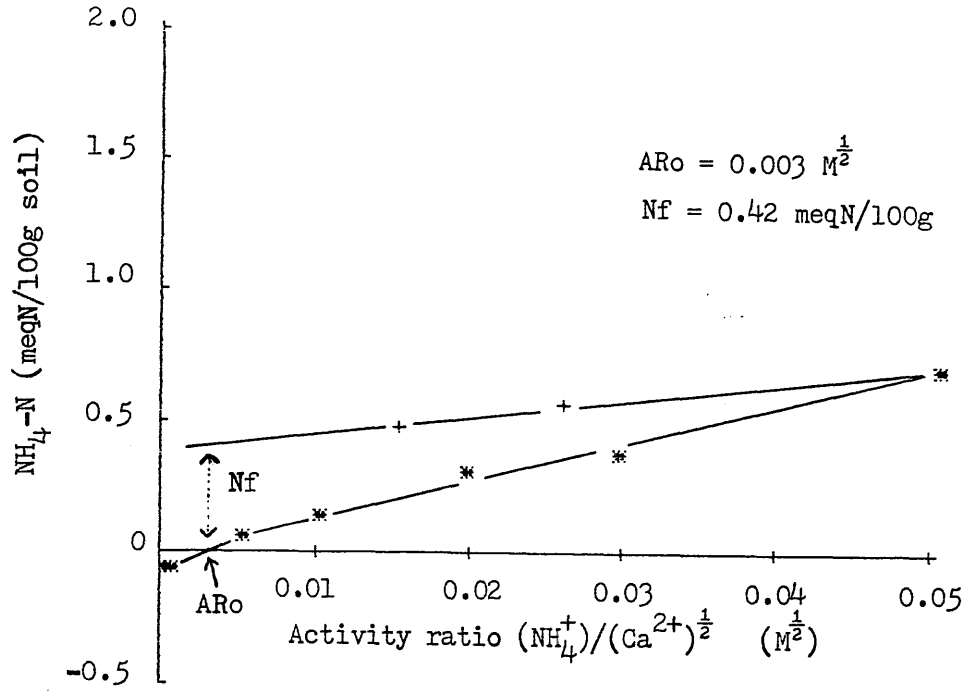


Fig. 2.9 Quantity Intensity curve for Ca-NH_4 exchange on Fauldhouse soil (fresh).
ARo Activity ratio of soil solution
Nf Ammonium-N fixed

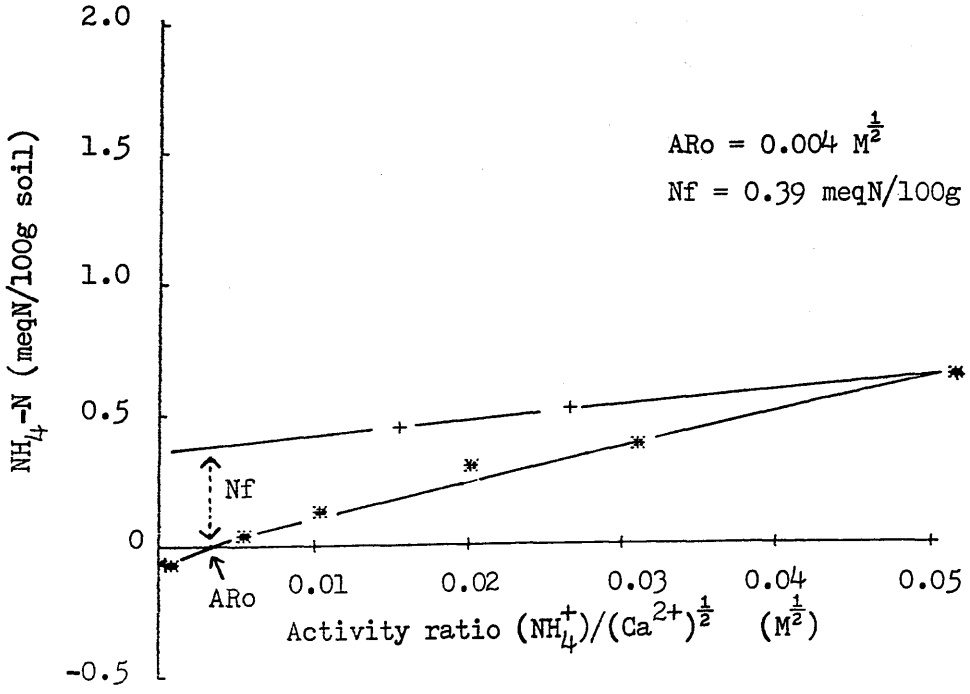


Fig. 2.10 Quantity Intensity curve for Ca-NH_4 exchange on Fauldhouse soil (air dry).
ARo Activity ratio of soil solution
Nf Ammonium-N fixed

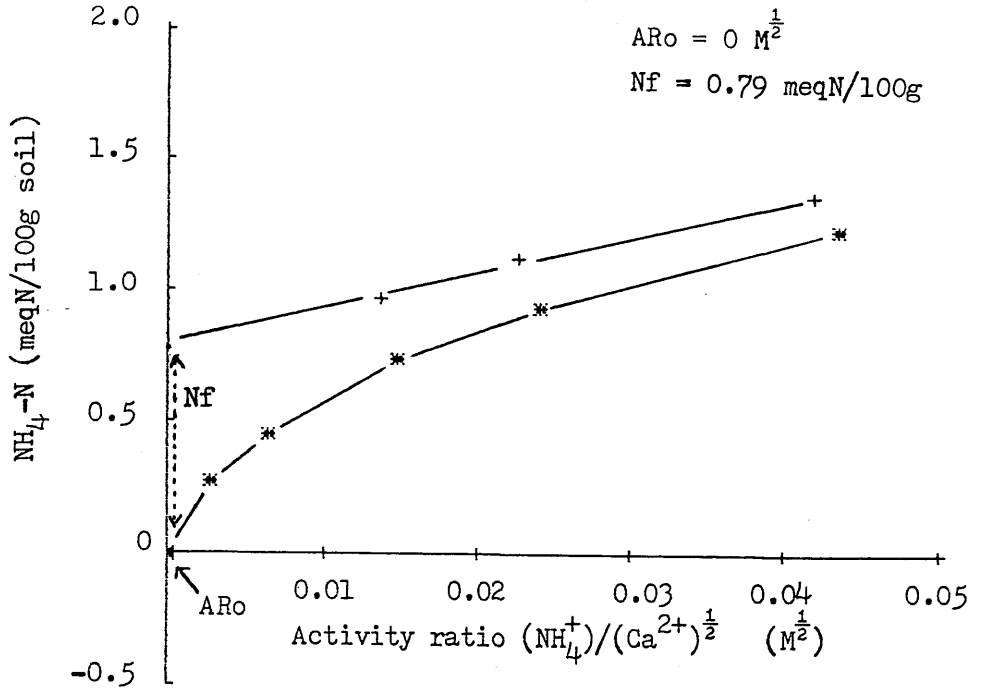


Fig. 2.11 Quantity Intensity curve for Ca-NH_4 exchange on North Addiewell soil (fresh).
ARo Activity ratio of soil solution
Nf Ammonium-N fixed

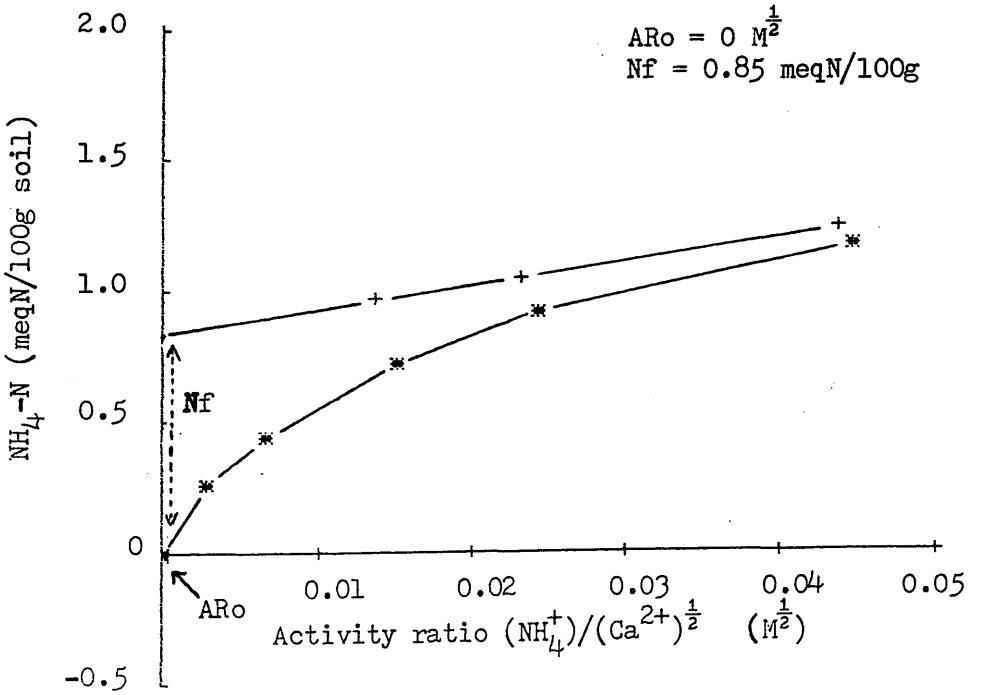


Fig. 2.12 Quantity Intensity curve for Ca-NH_4 exchange on North Addiewell soil (air dry).
ARo Activity ratio of soil solution
Nf Ammonium-N fixed

2.5.4 PROPOSED METHOD FOR AMMONIUM FIXATION STUDY

Duplicate 2.5 g samples of coal mine soil (oven dry weight basis) were weighed out into 100 cm^3 clean, dry plastic bottles. One sample was treated with 2 cm^3 ammonium sulphate solution containing 125 mg NH_4-N/l and the other was treated with 2 cm^3 deionized water to allow quantification of ammonium-N already present in the coal mine soil. The bottles were shaken and allowed to stand for 24 hours at 2 ^oC. The extractable-N (ammonium, nitrite and nitrate) in treated and untreated samples of the coal mine soil was extracted with 0.5 M potassium sulphate solution and measured colorimetrically by Technicon AutoAnalyzer II using the methods described in sections 2.3.4 and 2.1.5.

2.6 NITRATE MEASUREMENT: INTERFERENCE BY Mn AND pH INTERACTION

2.6.1 INTRODUCTION

During incubation experiments for studying native organic nitrogen and nitrification of added ammonium (ammonium sulphate), it was noted that some soil samples collected from Baads colliery were giving consistent negative values of nitrate nitrogen at different days of incubation. The estimated random error of the method for nitrate-N determination on the Technicon AutoAnalyzer II was from $\pm$ 0.1 to $\pm$ 0.2 ppm, but certain samples of coal mine soil were giving lower values than the estimated random error. One common interesting feature among these samples was their low pH (3.0-4.6) compared to other samples from the same site. The pH of the extracts of these samples with 0.5 M potassium sulphate solution was also measured, and was also found to be considerably lower than the extracts of the other soils. The erratic behaviour of these soil samples was thought to be due either to some chemical interference in the method of nitrate-N determination because of their low pH or some metals or a combination of both.

Due to the prevailing acid conditions in the coal mine spoil at Baads, certain metals are expected to dissolve, as indicated by iron oxide precipitation on shale particles and in streams. Kimber (1982) analysed

water samples from streams uncontaminated and contaminated by run-off from colliery spoil for various metals. He reported higher levels of Al, Mn, Co, Ni, Cu, Zn in the contaminated stream water compared to uncontaminated streams. Lister (1987) has measured the 0.5 M acetic acid extractable manganese of colliery spoil at Baads and reported a range from 18-118 mg Mn/kg of the spoil. Pulford et al. (1982) concluded from their findings that the mineral ankerite and the black shale in colliery spoil at Baads release manganese due to prevailing acid conditions.

Two combined soil extracts were made in large quantities by mixing the soil extracts of satisfactory soil samples into 'E1' and those of the suspected soil samples into 'E2'. The concentration of Ca, Cr, Mn, Fe, Ni, Cu, Zn, Pb, Mo, Mg, Cd and Co in the two combined extracts were determined by atomic absorption spectrophotometry.

The data in Table 2.7 indicate that the amounts of calcium, magnesium and iron found in the suspected extract 'E2' were much smaller than the amounts reported by Kamphake et al. (1967) and Markus et al. (1985) that have no effect on nitrate nitrogen determination. However, the suspected extract contained almost 24 times more Mn than the satisfactory combined extract. Moreover, 60 soil extracts with 0.5 M potassium sulphate solution for the study of mineralizable nitrogen in coal mine soils from Baads, were then tested for Mn content and a range of 0.5 to 2.5 mg Mn/l was found in extracts which were causing

the problem against 0.00 to 0.27 mg Mn/l of extracts which were giving satisfactory results. Therefore, some interference in either the colorimetric or reduction steps could be suspected due to comparatively higher Mn ion concentration of 'E2' suspected extract.

Metal	Concentration (mg/l)	
	E1	E2
Ca	61.00	84.00
Cr	0.00	0.00
Mn	0.10	2.40
Fe	0.20	0.10
Ni	0.08	0.08
Cu	0.05	0.10
Zn	0.05	0.15
Pb	0.00	0.00
Mo	0.00	0.00
Mg	3.18	2.95
Cd	0.06	0.06
Co	0.11	0.11

Table 2.7. Concentration of various cations in the two extracts.

A search of the literature revealed that Mn had been checked for possible interference with a colorimetric method of nitrate-N determination (Anon, 1981). In this case a small positive effect of Mn (10 mg/l) was reported on blanks but no effect on a solution containing 10 mg/l of nitrate-N was observed.

Some recovery tests were carried out, in order to investigate the effect of low pH and Mn content on the determinations of nitrite and nitrate nitrogen and to find a suitable solution to the problem.

2.6.2 MATERIALS AND METHODS

2.6.2.1. Reagents

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

1. 0.5 M potassium sulphate solution (see section 2.3.4).
2. Stock manganese solution (1000 mg Mn/l)

4.060 g manganous sulphate was dissolved and diluted to 1 litre with water. 100 mg Mn/l and 10 mg Mn/l in 0.5 M potassium sulphate solution were prepared from the stock solution.

3. Reagents for nitrate and nitrite nitrogen determination were prepared as described in section 2.1.5.2.

2.6.2.2. Experiment 1. Effect of Mn and pH on nitrite-N recovery.

Replicate solutions containing known concentrations of 0.0, 0.1, 1.0 and 5.0 mg manganese/l with 0.0, 1.0 and 4.0 mg nitrite-N/l in 0.5 M potassium sulphate solution (pH 5.8) were prepared and tested against 1 and 4 ppm analytical standards of nitrite-N for the recovery of added nitrite-N for investigating the interference effect of manganese in the nitrite-N analysis.

In order to study the effect of low pH on the recovery of nitrite-N, the pH of the above solutions was lowered to 3.4 by titrating with 0.5 M sulphuric acid. Nitrite-N was measured at 0, 2 and 24 hours after acidification.

2.6.2.3. Experiment 2. Effect of Mn and pH on nitrate-N recovery.

In order to investigate the effect of manganese on the nitrate-N recovery, another set of replicate solutions containing known concentrations 0.0, 0.1, 1.0 and 5.0 mg/l manganese with 0, 1.0 and 5.0 mg of nitrate-N/l were made in 0.5 M potassium sulphate solution at pH 5.8, and analysed for nitrate-N.

To study the effect of low pH (3.4) on the recovery of nitrate-N, the pH of the above solutions containing various known concentrations of manganese and nitrate-N was adjusted to 3.4 by titration with 0.5 M sulphuric acid and analysed for nitrate-N at 0, 2 and 24 hours after acidification.

2.6.2.4. Experiment 3. Effect of 5 and 10-fold dilutions on the recovery of nitrate-N.

In order to minimise the interfering effect of manganese in the various test solutions containing known concentrations of manganese and nitrate-N, it was decided to try 1:5 and 1:10 dilutions of the solutions. For this purpose a dilution step was incorporated into the nitrate manifold (Fig. 2.2) whereby the sample solution was

diluted 1/5 (sample 0.42 ml/min, water 1.6 ml/min) or 1/10 (sample 0.23 ml/min, water 2.0 ml/min) using deionized water prior to being presented to the sample input tube. The nitrate-N in the test solutions was remeasured at 5 and 10-fold dilutions.

2.6.2.5. Experiment 4. Effect of combined soil extract on the recovery of nitrite and nitrate-N.

Another recovery test of nitrite and nitrate nitrogen was conducted in order to confirm whether it was only manganese or some thing else which interfered in the nitrate-N determination of coal mine soils from Baads. Fresh extracts were prepared by shaking 3 soil samples collected from an experimental plot at Baads (the extracts of these samples were giving consistent negative values for nitrate-N) with 0.5 M potassium sulphate solution by using the method described in section 2.3.4. The three extracts were then mixed together. The pH of the solution was 4.9, while the manganese content of this solution was 1.7 mg/l. Nitrite-N was not detected, but 0.05 mg nitrate-N/l was found in this combined extract solution.

To study the effect of the combined extract on nitrite-N recovery, 1 and 4 cm^3 of solution containing 100 mg of nitrite-N as $NaNO_2$/l were pipetted into volumetric flasks and made up the volume with the soil extract. The solutions were prepared in triplicates and analysed for nitrite-N.

For the nitrate-N recovery test, 1 and 5 cm^3 of solution containing 100 mg of nitrate-N as KNO_3 /l were

pipetted into 100 cm^3 volumetric flasks and made up the volume with the combined extract. The solutions were made in triplicate and analysed for nitrate-N, undiluted as well as at 5 and 10-fold dilutions.

2.6.3 RESULTS AND DISCUSSION

All the analytical data reported represent the means of 3 replicate analyses. The % recovery was calculated by subtracting the blank from the amount recovered and dividing by the amount actually added.

2.6.3.1. Experiment 1. Effect of Mn and pH on nitrite-N recovery.

The results of the experiment 1, to examine the effect of manganese on nitrite-N recovery are presented in Table 2.8. The data indicate that concentration of Mn ions, up to 5 mg/l, produced no interference with nitrite-N determination, because almost 100 % of the added nitrite-N (1 or 4 mg NO_2-N/l) were recovered from the test solutions at pH 5.8. However, there was an obvious negative effect on the recovery of nitrite-N when the pH of the solutions was lowered to 3.4. The recovery of nitrite-N was at its lowest value after 24 hours of acidification. The failure to obtain completely quantitative recovery of nitrite-N added seems to be due to the instability of nitrite in the acidic media (VanCleemput and Baert, 1984).

$$3HNO_2 \text{ ---> } HNO_3 + 2NO + H_2O$$

$$2HNO_2 \text{ ---> } NO + NO_2 + H_2O$$

2.6.3.2. Experiment 2. Effect of Mn and pH on nitrate-N recovery.

The data presented in Table 2.9 show that manganese concentration, up to 0.1 mg/l, did not interfere with nitrate-N determination, but there was an obvious negative effect of 1 and 5 mg manganese/l on the recovery of added nitrate-N. The error ranged from 3-10 % and recovery of nitrate-N decreased with an increase in the manganese concentration from 1 to 5 mg/l. The data in Table 2.9 also indicate that the recovery of nitrate-N was not affected by acidification (pH 3.4) of the test solutions as compared to the negative effect of acidity (pH 3.4) on the nitrite-N recovery (Table 2.8).

2.6.3.3. Experiment 3. Effect of 5 and 10-fold dilutions on the recovery of nitrate-N.

The results of the experiment 3 documented in Table 2.10 indicate that 5-fold dilution yielded recoveries of added nitrate-N ranging from 94 to 99 %, and the recoveries were further improved (98-99%) with 10-fold dilution, by diluting the interfering cation (Mn) to a safe level.

Solutions (mg/l)	% Nitrite-N Recovery			
	pH 5.8	pH 3.4		
		0 hours	2 hours	24 hours
0 NO_2-N with 0.0 Mn	0.00	-	-	-
0 NO_2-N with 0.1 Mn	0.00	-	-	-
0 NO_2-N with 1.0 Mn	0.00	-	-	-
0 NO_2-N with 5.0 Mn	0.00	-	-	-
1 NO_2-N with 0.0 Mn	100	96	92	81
1 NO_2-N with 0.1 Mn	100	97	93	88
1 NO_2-N with 1.0 Mn	102	98	94	89
1 NO_2-N with 5.0 Mn	102	98	93	87
4 NO_2-N with 0.0 Mn	100	96	91	79
4 NO_2-N with 0.1 Mn	101	97	92	86
4 NO_2-N with 1.0 Mn	101	96	91	85
4 NO_2-N with 5.0 Mn	101	95	91	86

Table 2.8. Effect of different levels of manganese concentration and low pH on the recovery of nitrite-N.

Solutions (mg/l)	% Nitrate-N Recovery			
	pH 5.8	pH 3.4		
		0 hours	2 hours	24 hours
0 NO_3-N with 0.0 Mn	0.00	-	-	-
0 NO_3-N with 0.1 Mn	0.00	-	-	-
0 NO_3-N with 1.0 Mn	0.00	-	-	-
0 NO_3-N with 5.0 Mn	0.00	-	-	-
1 NO_3-N with 0.0 Mn	100	99	99	100
1 NO_3-N with 0.1 Mn	100	99	99	99
1 NO_3-N with 1.0 Mn	97	97	96	96
1 NO_3-N with 5.0 Mn	90	90	89	90
5 NO_3-N with 0.0 Mn	100	100	100	100
5 NO_3-N with 0.1 Mn	100	99	99	100
5 NO_3-N with 1.0 Mn	97	96	96	97
5 NO_3-N with 5.0 Mn	92	91	91	91

Table 2.9. Effect of different levels of manganese and low pH on the recovery of known amounts of nitrate-N added to 0.5 M potassium sulphate solution.

Solutions (mg/l)	% Nitrate-N Recovery			
	pH 5.8	pH 3.4		
		undil.	5-fold	10-fold
0 NO_3-N with 0.0 Mn	0.00	-	-	-
0 NO_3-N with 0.1 Mn	0.00	-	-	-
0 NO_3-N with 1.0 Mn	0.00	-	-	-
0 NO_3-N with 5.0 Mn	0.00	-	-	-
1 NO_3-N with 0.0 Mn	100	99	99	100
1 NO_3-N with 0.1 Mn	100	99	99	100
1 NO_3-N with 1.0 Mn	97	97	97	99
1 NO_3-N with 5.0 Mn	90	90	94	96
5 NO_3-N with 0.0 Mn	100	100	100	100
5 NO_3-N with 0.1 Mn	100	100	100	100
5 NO_3-N with 1.0 Mn	97	96	99	99
5 NO_3-N with 5.0 Mn	92	91	96	98

Table 2.10. Effect of 5 and 10-fold dilution on the recovery of nitrate-N added to 0.5 M potassium sulphate solution containing Mn at various concentration.

2.6.3.4. Experiment 4. Effect of combined soil extract on the recovery of nitrite and nitrate-N.

Some of the results of experiment 4, presented in Table 2.11, indicate that the added nitrite-N was completely (100%) recovered from the combined extract solution, and therefore, the combined extract showed no interference in the determination of nitrite-N. These results are similar to the experiment No.1 where manganese concentration (1-5 ppm) did not affect the recovery of the added nitrite-N from 0.5 M potassium sulphate solution.

Addition of NO_2-N (mg/l)	Recovery (%)
1	100
4	100

Table 2.11. Effect of combined extract on the recovery of nitrite-N.

The results of the effect of combined extract solution on the nitrate-N recovery, presented in Table 2.12, indicate that the amounts of added nitrate-N were not completely recovered from the combined extract. The recoveries ranged from 80 to 86 % as compared to 90-92 % recovery from the 0.5 M potassium sulphate solutions to which 5 mg of Mn/l was added (Table 2.9). The Mn content of the combined extract solution was much lower than 5 mg/l but the effect was found to be greater than expected, which suggests that Mn is not the complete answer. However, the results in Table 2.12 also reveal that

10-fold dilution improved the recovery (93-98%) of the added nitrate-N to the combined extract solution.

Addition of NO_3-N (mg/l)	Recovery (%)		
	undiluted	5 fold	10 fold
1	80	91	93
5	86	96	98

Table 2.12. Effect of dilution on the recovery of nitrate-N added to combined extract.

As no effect of manganese up to 5 mg/l was observed on the nitrite-N recovery (Table 2.8) the colorimetric step in the nitrate-N determination seemed to be not affected by manganese ions in the solution. Obviously, the reduction step in the nitrate nitrogen analysis seemed to be adversely affected by manganese ions.

A marked disagreement exists between the findings of Anon (1981) and those observed in this study concerning the interfering effect of Mn ions. A possible explanation of this divergence is that the method followed by Anon (1981) was that of Kamphake et al. (1967) in which the sample is treated with sodium hydroxide and reduction of nitrate to nitrite takes place at pH > 11 as compared to the method used here (Best, 1976) in which reduction was carried out at pH 9.6.

There were two possible methods for overcoming the problem of Mn interference in the nitrate-N determination. (i) The use of some chelating agent for retarding or preventing the interference of manganese. (ii) The use of

an appropriate dilution of the solutions for minimising the effect of manganese. The method of using chelating agent e.g citrate, tartrate, and ethylenediamine tetraacetate (EDTA), was not adopted because of possible chelating effect on the copper used as a catalyst in the nitrate-N determination (see section 2.1.5.2). Therefore, the dilution method was used for diluting the manganese ions concentration in the solutions.

2.6.4 CONCLUSIONS

During studies on the interference by manganese in the nitrate-N analysis it was concluded that:

1. Manganese definitely interfered with the nitrate-N determination causing an error ranging from 3-10 %. The recovery of the added nitrate-N to 0.5 M potassium sulphate solution decreased when the concentration of manganese was increased from 1 to 5 mg/l.

2. Manganese was not the only interfering component in the coal mine soils studied, because the recovery of the added nitrate-N to the soil extract containing 1.7 mg Mn/l was lower than the solution of 0.5 M potassium sulphate with a manganese content of 5 mg/l. Therefore, some further work is needed to find out the other interfering components of the coal mine soils.

3. Ten fold dilution effectively reduced the negative effect of the manganese and other interfering components

and yielded satisfactory recovery of added nitrate-N ranging from 98 to 99 % in standards containing 5mg Mn/l and 93-98% in soil extract.

4. The nitrate-N content of the coal mine soil is extremely low (mostly below 1 ppm), therefore, dilution to reduce the interference may affect the sensitivity with a risk of an increase in the expected random error of the method for nitrate-N determination, therefore only those samples of coal mine soil which were acidic (pH< 5) need to be checked with 10 fold dilution for nitrate-N determination.

2.7 UREASE AND AMIDASE ACTIVITIES IN COAL MINE SOILS

2.7.1 INTRODUCTION

Urea added to soil as a fertilizer or as animal urine is hydrolysed enzymatically to produce ammonia. Urease (urea amidohydrolase, EC 3.5.1.5) is the enzyme that catalyzes the hydrolysis of urea to carbon dioxide and ammonia.

$$NH_2\text{-}CO\text{-}NH_2 + H_2O \text{ ---> } 2NH_3 + CO_2$$

The method used for the assay of urease activity in coal mine soils was that of Tabatabai and Bremner (1972), except that released ammonium was measured by an automated colorimetric method. This method involved determination of ammonium released by urease activity when soil was incubated with buffered (pH 9.0) urea solution and toluene at 37 ^{o}C for 2 hours. Ammonium was extracted by shaking with 2.4 M potassium chloride containing silver sulphate (100ppm) as an urease inhibitor and measured colorimetrically by using a Technicon AutoAnalyzer II system.

The use of the automated colorimetric method for the determination of ammonium released by urease activity, was an attractive alternative to the method of steam distillation used by Tabatabai and Bremner (1972), because large numbers of samples can be analysed quickly and with a high degree of reproducibility.

McGarity and Myers (1967) used an automated colorimetric method for the determination of ammonium released by urease activity. They used a potassium

citrate-citric acid buffer (pH 6.7). Stroo and Jencks (1982) determined the urease activity in mine soils by the non-buffer method described by Zantua and Bremner (1975), except that evolved ammonia was measured colorimetrically by the indophenol blue method. None of the above research workers have mentioned the effects of the urease assay reagents on the colorimetric method of ammonium determination.

Before the use of the automated colorimetric method for measuring the ammonium released by urease activity, it was considered necessary to investigate whether the various reagents, THAM buffer (tris (hydroxymethyl) -aminomethane), urea and silver sulphate, used in the assay of urease activity had any interfering effect on the method of analysis. Therefore, some recovery experiments were carried out to check on the suitability of the automated colorimetric method of ammonium for studying the enzyme activities in coal mine soils.

Amidase (acylamide amidohydrolase, EC 3.5.1.4) is the enzyme that catalyzes the hydrolysis of amides and produces the corresponding carboxylic acid and ammonia.

$$R.CONH_2 + H_2O \text{ ---> } NH_3 + R.COOH$$

The method of Frankenberger and Tabatabai (1980a) with some modifications was used for assay of amidase activity in coal mine soils. Silver sulphate was used as an amidase inhibitor instead of uranyl acetate and the released ammonium was measured colorimetrically by Technicon AutoAnalyzer II.

Frankenberger and Tabatabai (1980a) have shown that

hydrolysis of formamide by soil amidase during extraction of soils with 2 M KCl could be inhibited completely by use of 2 M KCl-uranyl acetate (0.005 M) solution, and the ammonium released could be quantitatively determined by the steam distillation method. But in the present work it was found during a recovery test that a yellow precipitate immediately appeared when THAM buffer solution and 2.4 M KCl-uranyl acetate solutions were mixed together. Therefore, the use of uranyl acetate as amidase inhibitor proposed by Frankenberger and Tabatabai (1980a) was found unsuitable for the automated colorimetric method of ammonium determination. It was decided to use silver sulphate as an amidase inhibitor. Frankenberger and Tabatabai (1981b) reported an average inhibition of 55 % of amidase activity by using 5 μmol of Ag per g of soil. Although silver sulphate is not as good an inhibitor of amidase activity as uranyl acetate, it was hoped that silver sulphate along with low temperature (2 ^{o}C) shaking for 1 hour would be enough to inactivate the soil amidase during extraction.

Before the introduction of this modified method for amidase activity in coal mine soils, it was important to carry out some recovery experiments, in order to investigate any interference effect of the reagents involved in the method and to test the inhibition of amidase by silver sulphate.

2.7.2 ASSAY OF UREASE ACTIVITY IN COAL MINE SOILS

2.7.2.1. Reagents

Analar grade reagents and deionized water were used, unless otherwise specified.

1. Toluene
2. THAM buffer, pH 9.0 (0.05 M)

6.1 g reagent grade tris (hydroxymethyl)-aminomethane was dissolved in about 700 cm^3 water, the pH of the solution was lowered to 9.0 by the addition of 0.2 M sulphuric acid and then the volume made to 1 litre with water.

3. Urea solution (0.2 M)

1.2 g reagent grade urea was dissolved in about 80 cm^3 THAM buffer and diluted to 100 cm^3 with THAM buffer. The solution was stored in a refrigerator until required.

4. Potassium chloride (2 M)

149 g potassium chloride was dissolved in about 700 cm^3 water and diluted to 1 litre.

5. Potassium chloride (2.4 M)-silver sulphate (100 ppm)

100 mg reagent grade silver sulphate was dissolved in about 700 cm^3 water. 179 g potassium chloride was dissolved in this solution and diluted to 1 litre with water. The potassium chloride-silver sulphate solution was prepared by addition of potassium chloride to silver sulphate solution because the latter would not dissolve in potassium chloride solution.

6. Reagents for determination of ammonium by colorimetric method with Technicon AutoAnalyzer II (see section 2.1.5.1).

Standard solutions containing 0, 1, 5, 10 and 50 mg of NH_4-N /l were prepared in triplicate both in 2 M potassium chloride solution and in the complete mixture of reagents to be used in the determination of urease activity. For the preparation of standards in the complete mixture 2 cm^3 0.2 M urea solution, 15 cm^3 THAM buffer and the appropriate volume of NH_4-N stock solution were pipetted into 100 cm^3 volumetric flasks and made up to volume with 2.4 M KCl-Ag_2SO_4solution. The amounts of the reagents were calculated in accordance with their corresponding concentration in the soil extracts for urease activity.

The combined mixed solution of 0.2 M urea, THAM buffer (pH 9) and 2.4 M KCl-Ag_2SO_4 solution is hereafter refered to as complete mixture.

2.7.2.2. Effect of urea, THAM buffer and silver sulphate on the recovery of added ammonium-N.

For studying the combined effect of urea, THAM buffer and silver sulphate on the NH_4-N analysis, the mixture solutions spiked with 0 and 1 mg NH_4-N/ 1 were run through the Technicon AutoAnalyzer II for NH_4-N analysis against 0 and 1 ppm standards of NH_4-N in 2 M KCl solution. The system was run at varying concentrations of sodium hypochlorite solution ranging from 5 to 50 cm^3 / litre.

2.7.2.3. Effect of 14 and 21-fold dilutions on the recovery of added ammonium-N from mixture solution.

For minimizing the interfering effect of the reagents a dilution step was incorporated into the Technicon ammonium manifold (Fig. 2.1) whereby the sample solution was diluted 1/14 (sample 0.16 ml/min, water 2.0 ml/min) or 1/21 (sample 0.1 ml/min, water 2.0 ml/min) using deionized water prior to being presented to the sample input tube.

All the test solutions spiked with known concentrations of ammonium-N were analysed for ammonium against 0 and 10 ppm as well as 0 and 50 ppm ammonium-N standards in 2 M potassium chloride solution.

2.7.2.4. Testing of calibration curve

After getting 87 % recovery of added ammonium-N from the mixture solution, it was important to check the linearity of the calibration curve of ammonium-N standards in the mixture solution. For this purpose a set of triplicate standard solutions containing 0, 2, 4, 6, 8 and 10 mg of ammonium-N / 1 were prepared in the mixture solution. 2 cm^3 0.2 M urea solution, 15 cm^3 THAM buffer solution and an appropriate volume of ammonium-N stock solution were pipetted into 100 cm^3 volumetric flasks and made up to volume with 2.4 M KCl-Ag_2SO_4 solution. Then this set of standards was fed to the Technicon AutoAnalyzer II for ammonium determination, using 0 and 10 ppm standards in the mixture. Both 14 and 21 times dilution of the sample solutions were tried by building a dilution step into the Technicon ammonium manifold.

2.7.2.5. Extraction of added ammonium from coal mine soils in the presence of urease reagents.

In order to check the validity of the automated colorimetric method for measuring ammonium in enzyme assay of coal mine soils, a spike test was carried out. The coal mine soils selected for this test were surface (0-15 cm) samples collected from 3 amended plots at Baads to obtain a wide range in pH (4.4-7.2).

The following solutions were prepared for the test.

1. Urea solution A (0.02 M).

0.12 g urea was dissolved in about 70 cm^3 THAM buffer solution and made up the volume 100 cm^3 with THAM buffer solution.

2. Urea solution B (0.02 M)-ammonium-N (25 ppm).

0.12 g urea was dissolved in about 70 cm^3 THAM buffer solution. Then 2.5 cm^3 ammonium stock solution containing 1000 mg NH_4-N/l was added to that solution and the solution diluted to 100 cm^3 with THAM buffer solution.

6 g fresh coal mine soil was weighed into four 4 oz, screw cap, clear glass bottles. 0.2 cm^3 toluene was added to the soil in each bottle and mixed by swirling the bottles for a few seconds. To two bottles 10 cm^3 0.02 M urea solution B containing 25 ppm ammonium-N was added and the contents were mixed by swirling. To two bottles (controls) 10 cm^3 0f 0.02 M urea solution A (with no added ammonium) was added and mixed by swirling the bottles for

a few seconds. 50 cm^3 2.4 M potassium chloride-silver sulphate solution was immediately dispensed into each of the four bottles. The suspensions were shaken for one hour at 2 ^oC and filtered through a Whatman filter paper No. 40.

A blank extraction was also carried out, for which 0.2 cm^3 toluene and 10 cm^3 of urea solution B were pipetted into 100 cm^3 plastic bottle. 50 cm^3 2.4 M $KCl-Ag_2SO_4$ was added to the bottle, thoroughly mixed by swirling the bottle and filtered through a Whatman filter paper No 40.

The filtrates obtained from ammonium treated and control samples of coal mine soil were analysed against standards of 0 and 10 ppm ammonium-N in the complete mixture by using 21-fold dilution.

2.7.3 RESULTS AND DISCUSSION (UREASE)

The results of the effect of urea, THAM buffer (pH 9) and silver sulphate in the mixture solution on the recovery of added ammonium-N, indicate that the standard method of ammonium determination did not work. In the presence of THAM buffer there was no colour formation due to ammonium. The negative interference was probably due to the buffering effect of the THAM buffer (pH 9). A possible explanation would be a decrease in the pH below the optimum for the indophenol reaction.

Dilution was considered as a method of reducing the buffering effect of the THAM buffer in the ammonium system. Using 14-fold or 21-fold dilution the system

responded to ammonium but there was a high background colour in the presence of THAM buffer. However, it was found that by decreasing the hypochlorite concentration the background colour was reduced.

Since reducing the hypochlorite concentration also reduced the sensitivity of the method to ammonium a compromise was necessary. The hypochlorite concentration was reduced to one quarter of the original concentration. The method was not optimised and the concentrations of other reagents were not changed. This gave an acceptable background (complete mixture produced a background equivalent to 1.9 mg ammonium-N/litre) and sensitivity (permitting measurement in the range of 0 to 10 mg/litre ammonium-N).

There are conflicting reports in the literature on interferences from organic nitrogen with the colorimetric method of ammonium. Fenton (1962) found that positive interference by a number of organic nitrogen compounds was a result of ammonium contamination in these reagents. In some cases, this reaction has been found to be specific for ammonium-N (Krom, 1980) whereas in other cases amino acids and urea have interfered (White and Gosz, 1981). This is probably due to the different combination of reagents and reaction conditions available for this method.

The results presented in Table 2.13 show that 14-fold dilution yielded recoveries ranging from 75 to 80 % with a mean recovery of 78 %. The recovery of added ammonium-N was further improved by trying 21-fold dilution of the test solutions. Recoveries thus obtained were between 83 to 89 % with a mean recovery of 87 %.

NH_4-N added (^{4}mg/l)	NH_4-N recovered (%)			
	10ppm standard		50ppm standard	
	14-fold	21-fold	14-fold	21-fold
5	80	88	76	88
10	79	83	75	86
50	-	-	77	89

Table 2.13. Effect of 14- and 21-fold dilutions on the recovery of added ammonium-N from the mixture solution.

Figs. 2.13 and 2.14 show the calibration curves with 14- and 21-fold dilutions. For all standard solutions containing 0, 2, 4, 6, 8 and 10 mg of NH_4-N/ l of mixture solution linear graphs were obtained.

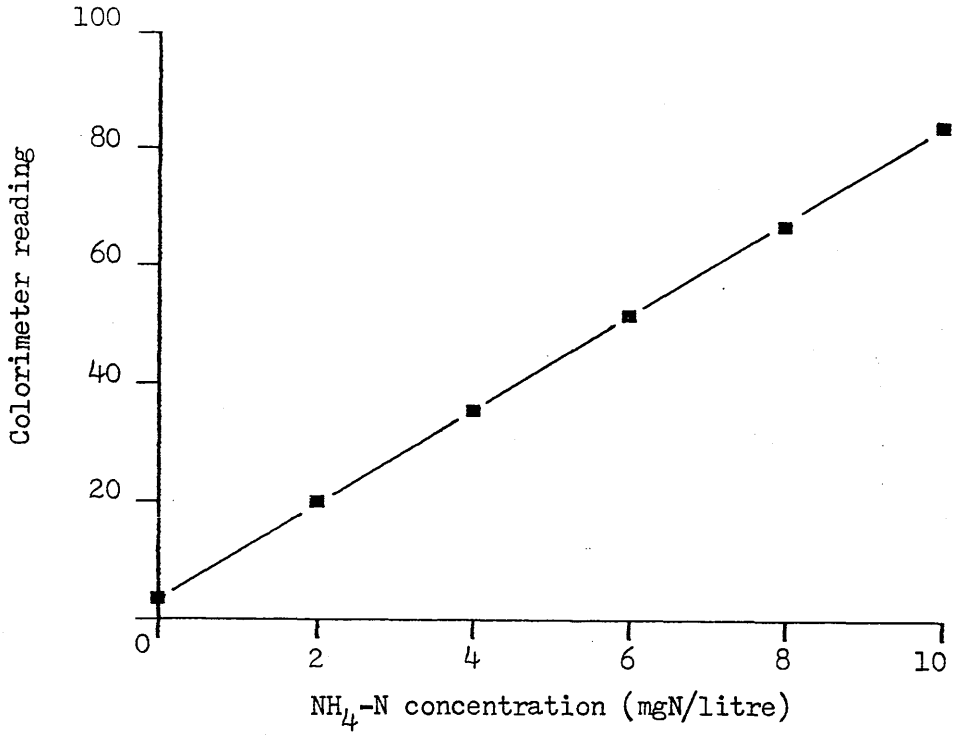


Fig. 2.13 Calibration curve for the solution containing known concentrations of NH_4-N and all the reagents used in the assay of urease activity (14 fold dilution).

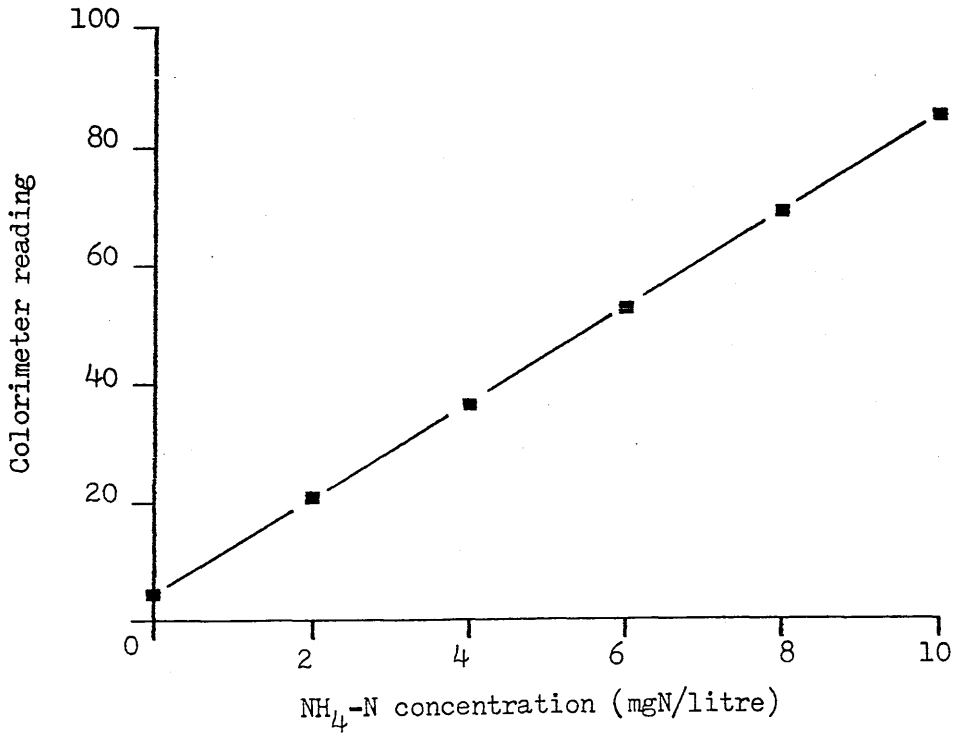


Fig. 2.14 Calibration curve for the solution containing known concentrations of NH_4-N and all the reagents used in the assay of urease activity (21 fold dilution).

The data from the spike test presented in Table 2.14 show that there was no significant difference in the amounts of ammonium-N found in the spoil samples spiked with a known amount of ammonium-N and the theoretically expected value. The amounts of ammonium-N recovered from the treated samples spiked with 25 ppm ammonium-N in the 0.02 M urea solution B were compared with the expected theoretical amounts. The theoretical values were calculated by summing the amount of ammonium-N found in control treatment and that detected in the Blank solution B.

Soil sample	pH	Ammonium-N (mg/kg soil)	
		Calculated	Found
S14	6.0	4.4	4.5
S20	4.4	6.5	6.9
S30	7.2	4.4	4.3

Table 2.14. Ammonium-N theoretically expected and found in spoil samples spiked with 4.1 mg of ammonium N per kg of coal mine soil.

It is concluded from the results of recovery tests, linear calibration curve of standards in the complete mixture and extraction of added ammonium from soil in the presence of urease reagents that:

i. Estimation of ammonium-N in the soil extracts for urease activity would be quantitative if measured against

ammonium standards prepared in the complete mixture, with a 21-fold dilution step built into the Technicon ammonium manifold.

ii. The automated colorimetric method could be successfully employed for the measurement of ammonium released by urease activity in coal mine soils, if the concentration of sodium hypochlorite is reduced to 12.5 cm^3/litre.

2.7.4 UREASE ASSAY PROCEDURE

The reagents were prepared as described in section 2.7.2.1, except that the sodium hypochlorite solution was prepared containing 12.5 cm^3/litre sodium hypochlorite (12% W/V available chlorine).

Duplicate urea treated samples of coal mine soil were prepared as follows.

5.0 g (oven dry basis) fresh coal mine soil was weighed into a 4 oz glass screw cap bottle. 0.2 cm^3 toluene and 9 cm^3 THAM buffer were also added to the bottle and swirled for a few seconds to mix the contents. Then 1 cm^3 0.2 M urea solution was added and the bottle again swirled for a few seconds. The bottle was closed and incubated at 37 ^oC for 2 hours. After incubation 50 cm^3 2.4 M potassium chloride containing silver sulphate was added and the bottle shaken for 1 hour at 2 ^oC. The suspension was filtered through a Whatman filter paper No. 40.

Duplicate controls were also carried through for each coal mine soil sample. The influence of coloured extracts, and any ammonium initially present on the exchange complex and extracted from the soil, was accounted for in the measurement of the control. In the case of the controls, addition of substrate (1 cm^3 0.2 M urea solution) was made after the addition of 50 cm^3 of potassium chloride-silver sulphate solution.

The ammonium was determined on the filtrate by an automated colorimetric method, using 0 and 10 ppm ammonium

standards in the complete mixture, with 21-fold dilution. The analytical standards were prepared by pipetting 2 cm^3 urea solution, 15 cm^3 THAM buffer, corresponding amount of ammonium stock solution and diluting to 100 cm^3 with 2.4 M potassium chloride containing 100 ppm silver sulphate.

The ammonium released by urease from the coal mine soil was calculated by subtracting the mean control value from that determined in the sample incubated with buffered urea solution.

2.7.5 ASSAY OF AMIDASE ACTIVITY IN COAL MINE SOILS

2.7.5.1. Reagents

Analar grade reagents and deionized water were used unless otherwise specified.

1. Toluene

2. THAM buffer, pH 8.5 (0.1 M)

12.2 g reagent grade tris (hydroxymethyl)-aminomethane was dissolved in about 800 cm^3 water. The pH was adjusted to 8.5 by titration with 0.2M sulphuric acid, and the solution diluted with water to 1 litre.

3. Formamide solution (0.5M)

2 cm^3 reagent grade formamide were pipetted into a 100 cm^3 volumetric flask and made up to volume with THAM buffer, the contents were thoroughly mixed. (The solution must be stored in a refrigerator).

4. Potassium chloride (2 M)

149 g potassium chloride was dissolved in about 700 cm^3 water and diluted to 1 litre.

5. Potassium chloride (2.4 M)-silver sulphate (100 ppm)

This solution was prepared as described in section 2.7.2.1.

7. Reagents for determination of ammonium were prepared as describedin section 2.1.5.1, except that the sodium hypochlorite solution was prepared containing 12.5 cm^3 sodium hypochlorite (12% W/V available chlorine) solution.

2.7.5.2. Effect of amidase reagents on the determination of ammonium

In order to study the effect of various reagents used in the assay of amidase activity, i.e. formamide, THAM buffer, and silver sulphate individually as well as in different combinations on the automated colorimetric method of ammonium analysis, some recovery experiments were carried out for the recovery of added ammonium from various solutions containing these reagents in different combinations.

Four sets of ammonium standards, containing 0, 10 and 20 mg of ammonium-N/ litre were prepared in triplicate in the following solutions. Care was taken to maintain equal concentration of various reagents in all test solutions. The values were calculated on the basis of the concentration of each reagent in the extracts after shaking the incubated soil with buffered formamide solution and toluene at 37 ^{o}C.

(1). 2 M KCl + Formamide

2 cm^3 0.5 M formamide solution and an appropriate volume of ammonium stock solution were added to 100 cm^3 volumetric flasks and the volume made up with 2 M KCl solution.

(2). 2.4 M KCl + THAM buffer

17 cm^3 0.1 M THAM buffer (pH 8.5) and an appropriate volume of ammonium stock solution were pipetted into 100 cm^3 volumetric flasks and the solutions diluted to the

mark with 2.4 M KCl.

(3). 2.4 M KCl + THAM buffer + Formamide

15 cm^3 0.1 M THAM buffer (pH 8.5), 2 cm^3 0.5 M formamide solution and an appropriate volume of ammonium stock solution were pipetted into 100 cm^3 volumetric flasks. The solutions were made up to 100 cm^3 with 2.4 M KCl solution.

(4). 2.4 M KCl-Ag_2SO_4 + THAM buffer + Formamide

15 cm^3 0.1 M THAM buffer (pH 8.5), 2 cm^3 0.5 M formamide solution and an appropriate volume of ammonium stock solution were pipetted into 100 cm^3 volumetric flasks and the volume made up with 2.4 M KCl-Ag_2SO_4 (100 ppm) solution.

A dilution step was incorporated into the Technicon ammonium manifold (Fig. 2.1) whereby the sample solution was diluted 1/21 as described in section 2.7.2.2.

All the test solutions spiked with known concentration of ammonium-N were fed to the Technicon AutoAnalyzer with analytical standards of 0 and 20 ppm ammonium-N made in 2 M KCl solution.

The % recovery was calculated by subtracting the blank from the amount recovered and dividing by the amount actually added.

2.7.5.3. Inhibition of amidase activity during extraction

In order to investigate whether silver sulphate along with 1 hour shaking (extraction time) at 2^{o}C for ammonium extraction can stop the amidase activity, the following experiment was carried out.

Six coal mine soil samples with a range of pH (4.2-6.6) from various experimental plots at Baads were selected for this test. The ammonium released from formamide by amidase in each coal mine soil (duplicate determinations) was determined after incubation of the sample with buffered (0.1 M THAM buffer, pH 8.5) formamide solution and toluene at 37 ^{o}C for 2 hours. The incubated samples were shaken for one and three hours with 50 cm^{3} of 2.4 M KCl containing Ag_2SO_4 (100 ppm) as an amidase inhibitor.

Duplicate controls were also carried through for 1 and 3 hours extraction of each coal mine soil, to allow for ammonium-N not derived from formamide through amidase activity.

2.7.6 RESULTS AND DISCUSSION (AMIDASE)

The data presented in Table 2.15 show the effect of various reagents used in the assay of amidase activity on the recovery of added ammonium-N. The levels of ammonium-N from the various mixed solutions to which no ammonium-N was added clearly indicated that formamide and THAM buffer solutions were the main sources of background ammonium contamination. So far as the interference effect of these

reagents with the automated colorimetric method of ammonium are concerned, formamide itself showed a very small effect because of 97-98 % recovery of added ammonium from 2 M KCl containing formamide only. However, an obvious negative interference effect of the buffer on the automated colorimetric method of ammonium was found because of consistent low recoveries from 6 spiked solutions containing THAM buffer. The recoveries ranged from 82-85 % (with one value at 74 % which seemed to be an experimental error), the average value being 82%.

The chemical reason for this interference was not known. However, one possible reason could be the buffering effect on the pH of the indophenol reaction of ammonium. Background ammonium in blanks may be due to the contamination of ammonia in the reagents used.

Treatment	NH_4-N added	NH_4-N Recovered	
	(mg/l)	(mg/l)	(%)
KCl+ Formamide	0	1.3	-
	10	11.0	97
	20	20.9	98
KCl+ Buffer	0	2.7	-
	10	11.1	84
	20	19.3	83
KCl+ buffer+ Formamide	0	3.8	-
	10	11.2	74
	20	20.7	85
KCl+ Ag_2SO_4 + Formamide+ Buffer	0	4.2	-
	10	12.5	83
	20	20.5	82

Table 2.15. Effect of formamide, THAM buffer and silver sulphate alone and in different combinations on the measurement of ammonium-N.

Results in Table 2.16 show the ammonium released from the formamide through amidase activity of the coal mine soil samples after 1 and 3 hours extraction. Replicate analyses are presented along with the mean values for comparing the expected (random) experimental errors.

Sample	pH	Amidase activity (mg of NH_4-N released/kg/hour)			
		1 hour		3 hour	
		replicates	mean	replicates	mean
1	4.2	10.9		11.0	
		8.5	9.7	9.3	10.2
2	4.2	8.5		8.1	
		8.3	8.4	7.6	7.9
3	4.6	15.8		17.0	
		17.5	16.7	15.7	16.4
14	6.0	24.6		26.2	
		24.6	24.6	27.4	26.8
19	4.5	14.6		14.7	
		12.9	13.8	13.1	13.9
22	6.6	17.4		19.9	
		16.2	16.8	18.7	19.3

Table 2.16. Effect of extraction time with 2.4 M potassium chloride containing silver sulphate (100 ppm) as an amidase inhibitor on amidase activity of coal mine soils.

There seemed to be no significant difference in the mean amidase activity of the coal mine soil measured after 1 and 3 hours shaking with 2.4 M potassium chloride

containing silver sulphate as an amidase inhibitor. However, some difference was found for samples No. 14 and 22 between the two extraction times, but it was comparatively small, if the variability among the replicates of the same sample was taken into account. This difference of amidase activity determined after 1 or 3 hours extraction seemed to be not more than random experimental error.

It can be concluded from this test that satisfactory inhibition of amidase in coal mine soils is possible with 2.4 M $KCl-Ag_2SO_4$ (100 ppm) extraction for 1 hour at 2^oC and that the automated colorimetric method of ammonium can be applied satisfactorily to extracts obtained with this 2.4 M $KCl-Ag_2SO_4$ solution, provided the sodium hypochlorite concentration is reduced to 12.5 cm^3/litre.

2.7.7 AMIDASE ASSAY PROCEDURE

The reagents were prepared as described in section 2.7.5.1. Duplicate formamide treated samples of coal mine soil were prepared as follows.

An amount of fresh coal mine soil equivalent to 5 g on oven dry basis was weighed into 4 oz screw cap clear glass bottle. 0.2 cm^3 of toluene and 9 cm^3 of THAM bufer were added to the bottle and swirled for a few seconds for mixing the contents. Then 1 cm^3 of 0.5 M formamide solution was added and the bottle swirled again for a few seconds. The bottle was closed tightly and incubated at 37 ^oC for 2 hours. After incubation 50 cm^3 of 2.4 M potassium chloride containing silver sulphate was added to the bottle. The resulting suspension after 1 hour shaking at 2 ^oC was filtered using Whatman filter paper No. 40.

Duplicate controls were also carried through for each sample of coal mine soil to allow for NH_4-N not derived from formamide through amidase activity. In the case of controls the same procedure as described above was followed, except that the addition of substrate, 1 cm^3 of 0.5 M formamide solution, was made after the addition of KCl-Ag_2SO_4 solution.

The filtrates were analysed for ammonium-N by an automated colorimetric method, against 0 and 10 ppm ammonium standards prepared in 2.4 M KCl containing all amidase assay reagents, with 21-fold dilution.

The analytical standards were prepared by pipetting 2cm^3 of 0.5 M formamide, 15 cm^3 of THAM buffer,

corresponding amount of ammonium stock solution and diluting to 100 cm^3 with 2.4 M $KCl-Ag_2SO_4$ (100 ppm) solution.

The ammonium released by amidase activity of the coal mine soil was calculated by subtracting the mean control value from the mean of that determined in the sample incubated with buffered formamide solution.

2.8 METHOD OF ACID DIGESTION OF HERBAGE SAMPLES

Approximately 0.2000 g of finely ground herbage sample was carefully weighed using a subtraction method into a Kjeldhal digestion flask. 2 g sodium sulphate and 1 tablet of Kjeldahl catalyst (BDH) containing 1 g sodium sulphate and the equivalent of 0.05 g selenium were added to the flask, to give a sodium sulphate to selenium ratio of 60:1. This catalyst mixture was chosen to allow N, P and K measurements in the same digest. After the addition of 5 cm^3 concentrated sulphuric acid (AR) the flask was first heated gently on a gas heater to prevent frothing and then more strongly until the solution became clear. The flask was then left at gentle heating with the acid condensing one third of the way up the flask neck for about 2 hours. After cooling, 20 cm^3 deionized water was added and swirled 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 water and the aliquots added to the volumetric flask through the filter paper. The digest was allowed to cool before making up to volume.

The digest was analysed for its nitrogen, phosphorus and potassium contents. Potassium was measured by flame photometry method using calibration standards in the range of 0 to 50 ppm. Phosphate-P was determined as a vanadomolybdate yellow complex at 420 nm based on a Technicon AutoAnalyzer method using 0-50 ppm standards (Technicon corporation, 1972). Total nitrogen as

ammonium-N was measured using the method given in section 2.1.5.1. Care was taken that all the standards contained equal concentrations of the reagents used in the digest material.

CHAPTER 3 SURVEY OF NITROGEN STATUS IN COAL MINE SOILS

3.1 INTRODUCTION

It is generally accepted that coal mine soils cannot adequately provide the nitrogen requirements of a growing plant, although the total nitrogen content can be comparable to that of undisturbed soils. Since an understanding of nitrogen cycling is needed for successful reclamation and proper maintenance of plant growth on such disturbed materials, a detailed study was undertaken to measure mineral nitrogen status, mineralization rates of carbon and nitrogen, nitrification rate and fixation of added ammonium. These measurements were made on 90 samples of coal mine soils collected from various bings throughout Central Scotland. Some of these survey samples collected from plots of established experiments at Baads colliery bing were also assayed for urease and amidase activities. The selection of sites was based on the extensive background work carried out in the Agricultural Chemistry Department of Glasgow University.

3.2 MATERIALS AND METHODS

3.2.1 DESCRIPTION OF SITES

Three individual samples were collected at random from the surface spoil (0-15 cm) from each sampling area. Areas on a bing of uniform appearance, or as experimental plots, were considered as a representative sampling area. A visual assessment was made for selecting an area of uniform appearance over the bing, keeping in view the colour of the spoil, topography, steepness of the slope, absence or presence of vegetation, density of vegetation and any treatment in the past on each bing under study.

1. Dykehead (DK) Grid reference NS 867608

This site is situated on the outskirts of the town of Shotts. It is approximately six miles north-east of Wishaw. Coals from both the productive Coal Measures and the Limestone Coal Group were mined in this area.

The bing is in the form of a single steep-sided conical mound and very poor natural vegetation has established. Three soil samples were collected from each of the following sampling areas.

DK 1. Flat shoulder with poor vegetation.

DK 2. Steep slope with no vegetation.

2. Stane (ST) Grid reference NS 884587

The site had been regraded and a layer of top soil (6-12 cm) applied to the surface of the coal mine spoil. The area had received 2.5 t/ha of lime, 500 kg/ha of a 12:24:12 NPK compound fertilizer, 250 kg/ha of triple superphosphate, and been seeded to a mixture of grasses and clovers during 1976. The surface soil was well structured sandy loam with loose and friable underlying spoil. The site was under good vegetative cover. One sampling area was selected on this site. Three coal mine soil samples were taken from the exposed side of a drainage ditch.

3. Fauldhouse (FH) Grid reference NS 939618

Two sampling areas were selected on this bing, one was a reclaimed area which was sown with grass while the other was not reclaimed.

FH 1. Reclaimed, flat with poor vegetation.

FH 2. Unreclaimed, steep slope and naturally vegetated.

4. Loganlee (LL) Grid reference NS 976622.

This site is situated in close proximity to the villages of Loganlee and Stoneyburn, alongside the Breich Water. The soil materials of the area are derived from the Limestone Coal Group. The site was reclaimed in 1983. Two sampling areas were selected on the bing.

LL 1. Poor grass (sown species with infestation by weed grasses)

LL 2. Thick grass and very wet (poorly drained).

5. North Addiewell (NA) Grid reference NT 002630

The material on the site was derived from oil shale wastes and was under a good cover of mixed natural vegetation. Three sampling area were identified for sampling.

NA 1. Naturally vegetated, under trees.

NA 2. Steep slope, naturally vegetated mainly heather.

NA 3. Flat, under clover.

6. Baads (BD) Grid reference NT 003609

This bing is situated near the town of West Calder. Some parts of the bing are highly pyritic and acid patches are common. Reclamation of the site was carried out in 1980. A part of the site received 50 t/ha of lime and 300 kg/ha of a 15:10:10 NPK compound fertilizer before being seeded with a grass-white clover mixture (Lister, 1987).

Two field experiments were established during the year 1980 by the Agricultural Chemistry Department of Glasgow University. On one area various organic amendment treatments; chicken manure, sewage sludge, peat and a seaweed-based soil conditioner are being compared for their ability to supply organic matter and nutrients to the spoil. On the other area a range of grass and legume species are being tested for their ability to produce a self-sustaining vegetation cover with particular interest in the nitrogen fixing abilities of the legumes.

Ten sampling areas each representing an individual experimental plot were selected on this bing keeping in

view the residual effect of various organic manures and leguminous species on the nitrogen status of coal mine soils. At the time of soil sampling actual plot areas were no longer very distinguishable in the leguminous species trial. However, red clover and white clover were the dominant species.

BD 1. Limed (25t/ha), no organic amendment, and sparsely vegetated.

BD 2. Limed (25t/ha), soil conditioner (11t/ha) treated and well vegetated.

BD 3. Limed (25t/ha), peat (20t/ha) treated and well vegetated.

BD 4. Limed (25t/ha), sewage sludge (20t/ha) treated and well vegetated.

BD 5. Limed (25t/ha), chicken manure (4t/ha) treated and well vegetated.

BD 6. Control, no lime no manure and unvegetated.

BD 7. Unreclaimed area of the bing, naturally vegetated.

BD 8. Red clover and grass mixture

BD 9. Red clover and grass mixture

BD 10. Grass mixture

7. Pennyvenie (PV) Grid reference NS 486068

This bing is situated on the B 741 north east of the village of Dalmellington. The site occupies an area of about 16 hectares. The soils of the area are mainly derived from sandstones and shales of the limestone groups and productive Coal Measures. Vegetation is well developed on most parts of the site with a wide variety of species.

21 samples were collected from the bing, 3 from each of the following seven recognisable sampling areas.

PV 1. Plateau-shaped mound, well weathered shales and vegetated.

PV 2. Conical in shape, steep slope and not vegetated. The samples were collected going down the slope.

PV 3. Conical in shape, steep slope and vegetated. Three samples were collected going up the slope.

PV 4. The eastern end of the bing, having well weathered shale and good vegetation. The samples were taken going down a gentle slope.

PV 5. Slope and unvegetated.

PV 6. Flat top and vegetated.

PV 7. Large flat area, compacted and sparsely vegetated.

8. Minnivey (MV) Grid reference NS 476071

This bing is situated immediately north of Burnton, a district on the north west outskirts of Dalmellington. The site comprises an area area of 6-7 hectares.

The bing is flat-topped and roughly oval in plan. The slopes along the southern and western flanks are very steep and comprise a series of gullies due to erosion. The bing itself occupies 20-25% of the area, the remainder comprising a flat area of compact hard stand material. The shale at Minnivey is sparsely vegetated, particularly on the bing itself where only an occasional plant is evident. At the eastern extremity of the site, vegetation is much more luxuriant. The samples were collected from the following two sampling areas.

MV 1. Steep slope, no vegetation.

MV 2. Flat-topped, sparsely vegetated.

9. Laight (LT) Grid reference NS 458073

Laight is situated just south of Laight Farm which lies north-east of the A 713 midway between Waterside and Dalmellington. The site, which is roughly triangular in shape, occupies an area of approximately 9 hectares. Vegetation on the Laight shale is extremely sparse. Three samples were collected from a flat, sparsely vegetated sampling area.

3.2.2 LABORATORY PRETREATMENTS AND METHODS

The freshly collected samples were brought to the laboratory as soon as possible and spread out on clean plastic sheets. The samples were kept in a cold room (10^{o}C) if it was not possible to sieve on the day of collection. Any vegetation, roots, stones etc were discarded. The samples were partially air-dried just sufficient to permit sieving through a 4 mm sieve with the minimum of disturbance. Care was taken to minimise any chemical and biological changes in the samples during the preparation process e.g drying of the samples at low temperatures (room temperature) and in a ammonia free atmosphere. The sieved samples were thoroughly mixed and refrigerated at 2 ^{o}C in tied plastic bags until they were to be used.

1. pH determination

The pH of coal mine soil samples was determined in water by the method described in section 2.1.1.

2. Extractable mineral nitrogen

Mineral nitrogen (ammonium-N, nitrite-N and nitrate-N) in coal mine soil samples was extracted by the method described in section 2.3.4 and the extracts were analysed by automated colorimetric methods (section 2.1.5).

3. Mineralization of carbon and nitrogen

The incubation technique described in section 2.4.2.2 was used for the measurements of nitrogen and carbon mineralization rates.

Carbon dioxide evolved was determined weekly by the method described in section 2.1.4 as a linear rate which was expressed in mg/kg/week. Best-fit lines were fitted by linear regression in order to calculate the mineralization rate constant.

The nitrogen mineralized was determined at 0, 4 and 8 weeks of incubation. The mineralization rate expressed as mg N/kg/week was estimated by subtracting the initial inorganic nitrogen extracted from the sample at time zero from the total inorganic nitrogen mineralized after 8 weeks of incubation.

The C:N ratio was calculated from the ratio of rates of mineralization of carbon and nitrogen.

4. Nitrification rate

The nitrification rate of ammonium-treated coal mine soil was measured at 4 day intervals up to 16 days, by an aerobic incubation method, as described in section 2.4.2.3. It was calculated as a linear rate expressed as mg N/kg/day. Best-fit lines were fitted by linear regression in order to calculate the nitrification rate constant.

5. Loss of nitrogen during incubation

The loss of inorganic nitrogen during incubation was calculated by subtracting the total inorganic-N determined after 12 days of incubation from that measured at day zero of the incubation of the ammonium-treated coal mine soil sample. The extractable inorganic-N was at its lowest after 12 days incubation in most of the coal mine soils studied.

6. Loss of nitrogen due to ammonium fixation

The loss of nitrogen due to ammonium fixation was determined by the method given in section 2.5.2.3. This loss was calculated by subtracting the ammonium-N recovered from a treated sample from that initially present in the sample plus the added amount (100 mg ammonium-N/kg soil). The loss of ammonium-N was corrected for any nitrification during the 24 hours incubation at 2 ^{o}C.

7. Assay of soil enzymes

Urease and amidase activities in coal mine soils from Baads were assayed by using the methods described in section 2.7.4 and 2.7.7 respectively.

3.3 RESULTS

In order to show the variability and range of values of the various parameters of the nitrogen cycle between thirty different sampling areas and within three samples from each sampling area, the data for all 90 samples are presented as such in Tables 3.1 to 3.4.

Table 3.1 presents the results of extractable mineral nitrogen in the form of ammonium, nitrate and total inorganic-N.

Table 3.2 shows the mineralization rates of carbon and nitrogen measured during an incubation study. The C:N ratio reported was calculated from the rates of mineralization of carbon and nitrogen. The C:N ratios are not given where the nitrogen mineralized was found to be zero.

Table 3.3 indicates the nitrification rates of added ammonium to the coal mine soil during an incubation study. The loss of nitrogen given was calculated by subtracting the total inorganic-N recovered after 12 days of incubation from that determined at zero days of incubation. The missing values represent a gain in extractable inorganic nitrogen. Table 3.3 also shows the amounts of ammonium fixed by the clay fraction of coal mine soils.

Table 3.4 shows the urease and amidase activities determined in thirty samples collected from spoil at Baads colliery.

Sample	pH	Extractable Inorganic-N (mg/kg soil)		
		Ammonium-N	Nitrate-N	Total-N
DK 1A*	6.0	0.6	0.0	0.7
DK 1B	5.5	1.4	0.0	1.5
DK 1C	5.5	0.4	0.0	0.4
DK 2A	6.5	0.8	1.3	2.2
DK 2B	6.5	1.1	1.2	2.4
DK 2C	6.2	0.4	0.9	1.4
ST A	6.5	3.0	4.0	7.0
ST B	6.4	2.5	1.4	4.0
ST C	6.1	2.5	4.2	6.8
FH 1A	5.3	1.8	4.1	5.9
FH 1B	4.1	25.9	5.5	31.4
FH 1C	3.9	16.4	0.8	17.3
FH 2A	4.4	2.2	0.0	2.2
FH 2B	4.1	0.2	0.0	0.2
FH 2C	3.8	0.1	0.0	0.1
LL 1A	4.2	0.8	0.0	0.8
LL 1B	3.5	0.7	0.0	0.7
LL 1C	5.1	4.8	0.1	5.0
LL 2A	4.8	0.4	0.0	0.4
LL 2B	5.2	0.4	0.0	0.4
LL 2C	4.9	0.1	0.0	0.1
NA 1A	4.8	1.0	0.0	1.0
NA 1B	4.5	6.8	0.7	7.5
NA 1C	4.6	2.4	0.0	2.4
NA 2A	4.1	0.7	0.0	0.7
NA 2B	3.8	0.8	0.0	0.8
NA 2C	4.1	5.1	0.0	5.1
NA 3A	5.1	1.3	0.0	1.3
NA 3B	7.8	1.4	0.3	1.7
NA 3C	6.2	1.5	5.0	6.5

Table 3.1.(i). Extractable inorganic-N in coal mine soil samples.

* site codes, see section 3.2.1.

Table 3.1 continued on next page

Sample	pH	Extractable Inorganic-N (mg/kg soil)		
		Ammonium-N	Nitrate-N	Total-N
BD 1A	4.2	2.0	0.0	2.0
BD 1B	4.2	3.4	0.0	3.4
BD 1C	4.6	1.6	0.0	1.6
BD 2A	4.6	2.2	0.0	2.2
BD 2B	7.2	1.7	0.3	2.2
BD 2C	6.0	2.2	0.4	2.8
BD 3A	6.6	4.0	0.4	4.6
BD 3B	5.2	2.1	0.1	2.2
BD 3C	5.6	1.5	0.5	2.2
BD 4A	6.6	2.1	0.4	2.8
BD 4B	4.8	4.9	0.1	5.1
BD 4C	6.6	3.3	0.4	4.0
BD 5A	5.4	3.2	0.3	3.6
BD 5B	6.0	4.6	0.8	5.5
BD 5C	4.4	4.9	0.2	5.2
BD 6A	3.0	13.6	0.7	14.8
BD 6B	3.6	3.3	0.0	3.4
BD 6C	3.4	3.3	0.1	3.8
BD 7A	4.5	0.5	0.0	0.5
BD 7B	4.4	10.5	0.6	11.2
BD 7C	4.5	7.6	0.5	8.1
BD 8A	6.6	2.0	0.2	2.3
BD 8B	5.8	1.4	0.3	1.8
BD 8C	6.4	1.0	0.2	1.4
BD 9A	6.6	1.7	0.3	2.3
BD 9B	6.6	2.4	0.3	3.0
BD 9C	7.0	0.4	0.4	1.0
BD 10A	6.2	1.5	0.1	1.7
BD 10B	6.8	0.3	0.3	0.8
BD 10C	7.2	0.3	0.3	0.8

Table 3.1.(ii). Extractable inorganic-N in coal mine soil samples.

Table 3.1 continued on next page

Sample	pH	Extractable Inorganic-N (mg/kg soil)		
		Ammonium-N	Nitrate-N	Total-N
PV 1A	5.6	0.8	0.0	0.8
PV 1B	5.6	1.1	0.0	1.1
PV 1C	5.8	3.3	0.0	3.3
PV 2A	5.5	6.5	9.0	15.5
PV 2B	6.7	0.8	6.2	7.0
PV 2C	6.0	3.0	2.8	5.8
PV 3A	7.1	2.0	1.4	3.4
PV 3B	5.7	1.3	0.3	1.6
PV 3C	5.8	1.5	2.6	4.1
PV 4A	4.8	5.3	0.0	5.3
PV 4B	4.5	3.8	2.7	6.5
PV 4C	4.7	2.5	0.0	2.5
PV 5A	8.4	0.6	0.7	1.3
PV 5B	8.4	0.1	2.3	2.4
PV 5C	8.2	0.1	1.4	1.5
PV 6A	6.2	2.0	0.0	2.0
PV 6B	6.1	2.1	0.0	2.1
PV 6C	5.2	1.9	0.0	1.9
PV 7A	7.5	0.6	0.1	0.7
PV 7B	8.1	1.2	0.2	1.4
PV 7C	8.0	0.9	0.3	1.2
MV 1A	8.5	0.6	1.0	1.6
MV 1B	8.5	0.4	1.0	1.4
MV 1C	8.5	0.5	1.0	1.5
MV 2A	8.0	0.8	0.8	1.6
MV 2B	8.1	0.9	1.0	1.9
MV 2C	8.0	0.3	1.6	1.9
LT A	8.6	0.4	0.2	0.6
LT B	8.0	1.1	1.2	2.3
LT C	8.8	0.4	1.1	1.5

Table 3.1.(iii). Extractable inorganic-N in coal mine soil samples.

Sample	pH	Rate of mineralization (mg/kg/week)		C/N ratio
		Carbon	Nitrogen	
DK 1A*	6.0	43	0.0	-
DK 1B	5.5	51	0.2	255
DK 1C	5.5	43	0.3	143
DK 2A	6.5	27	0.1	270
DK 2B	6.5	35	0.2	175
DK 2C	6.2	28	0.1	280
ST A	6.5	139	2.7	51
ST B	6.4	138	2.1	66
ST C	6.1	151	2.5	60
FH 1A	5.3	43	1.7	25
FH 1B	4.1	37	1.3	28
FH 1C	3.9	47	0.8	59
FH 2A	4.4	ND	0.6	ND
FH 2B	4.1	ND	0.1	ND
FH 2C	3.8	117	0.0	-
LL 1A	4.2	34	0.1	340
LL 1B	3.5	48	0.2	240
LL 1C	5.1	100	0.4	250
LL 2A	4.8	27	0.2	135
LL 2B	5.2	46	0.1	460
LL 2C	4.9	35	0.1	350
NA 1A	4.8	75	0.5	150
NA 1B	4.5	57	2.0	29
NA 1C	4.6	102	3.0	34
NA 2A	4.1	89	0.1	890
NA 2B	3.8	74	0.2	370
NA 2C	4.1	119	0.2	595
NA 3A	5.1	46	0.1	460
NA 3B	7.8	63	0.9	70
NA 3C	6.2	46	3.1	15

Table 3.2.(i). Mineralization rate of organic carbon and nitrogen in coal mine soils.

ND not determined.
- not applicable.
* site codes, see section 3.2.1.

Table 3.2 continued on next page

Sample	pH	Rate of mineralization (mg/kg/week)		C/N ratio
		Carbon	Nitrogen	
BD 1A	4.2	28	0.6	47
BD 1B	4.2	49	1.2	41
BD 1C	4.6	53	0.8	66
BD 2A	4.6	26	0.7	37
BD 2B	7.2	142	0.4	355
BD 2C	6.0	91	0.6	152
BD 3A	6.6	181	0.9	201
BD 3B	5.2	66	1.0	66
BD 3C	5.6	102	0.3	340
BD 4A	6.6	146	0.2	730
BD 4B	4.8	45	1.1	41
BD 4C	6.6	135	0.3	450
BD 5A	5.4	66	1.3	51
BD 5B	6.0	100	2.7	37
BD 5C	4.4	31	0.8	39
BD 6A	3.0	20	0.4	50
BD 6B	3.6	74	2.5	30
BD 6C	3.4	27	1.1	25
BD 7A	4.5	59	0.3	197
BD 7B	4.4	43	3.2	13
BD 7C	4.5	68	3.1	22
BD 8A	6.6	112	0.3	373
BD 8B	5.8	45	1.2	38
BD 8C	6.4	103	0.3	343
BD 9A	6.6	102	0.3	340
BD 9B	6.6	97	0.5	194
BD 9C	7.0	109	0.2	545
BD 10A	6.2	64	0.6	107
BD 10B	6.8	79	0.0	-
BD 10C	7.2	92	0.0	-

Table 3.2.(ii). Mineralization rate of organic carbon and nitrogen in coal mine soils.

- not applicable.

Table 3.2 continued on next page

Sample	pH	Rate of mineralization (mg/kg/week)		C/N ratio
		Carbon	Nitrogen	
PV 1A	5.6	56	0.4	140
PV 1B	5.6	38	0.2	190
PV 1C	5.8	90	0.0	-
PV 2A	5.5	21	1.3	16
PV 2B	6.7	20	1.3	15
PV 2C	6.0	25	0.6	42
PV 3A	7.1	43	2.1	20
PV 3B	5.7	35	2.0	18
PV 3C	5.8	50	2.3	22
PV 4A	4.8	47	1.6	29
PV 4B	4.5	24	0.2	120
PV 4C	4.7	36	0.9	40
PV 5A	8.4	34	0.1	340
PV 5B	8.4	22	0.4	55
PV 5C	8.2	26	0.2	130
PV 6A	6.2	88	0.1	880
PV 6B	6.1	89	1.1	81
PV 6C	5.2	98	2.3	43
PV 7A	7.5	45	0.3	150
PV 7B	8.1	40	0.2	200
PV 7C	8.0	44	0.6	73
MV 1A	8.5	33	0.1	330
MV 1B	8.5	22	0.1	220
MV 1C	8.5	27	0.1	270
MV 2A	8.0	43	0.5	86
MV 2B	8.1	48	0.7	69
MV 2C	8.0	45	0.5	90
LT A	8.6	45	0.5	90
LT B	8.0	42	1.3	32
LT C	8.8	25	0.1	250

Table 3.2.(iii). Mineralization rate of organic carbon and nitrogen in coal mine soils.

- not applicable.

Sample	pH	Nitrification (mg/kg/day)	Loss of Nitrogen (mg/kg)	
			Ammonium Fixation	Incubation
DK 1A*	6.0	1.6	4.0	15.8
DK 1B	5.5	1.8	2.5	17.0
DK 1C	5.5	1.7	1.8	18.1
DK 2A	6.5	2.1	1.3	7.2
DK 2B	6.5	2.0	0.7	1.9
DK 2C	6.2	2.0	1.0	-
ST A	6.5	7.0	3.4	10.8
ST B	6.4	6.5	2.6	15.7
ST C	6.1	6.6	2.2	17.5
FH 1A	5.3	0.0	-1.1	-
FH 1B	4.1	0.0	-0.3	4.0
FH 1C	3.9	0.0	-0.6	-
FH 2A	4.4	0.0	-1.3	-
FH 2B	4.1	0.0	2.3	4.2
FH 2C	3.8	0.0	9.6	2.2
LL 1A	4.2	0.0	2.9	20.1
LL 1B	3.5	0.0	2.3	15.5
LL 1C	5.1	0.0	7.7	14.7
LL 2A	4.8	3.2	1.9	29.2
LL 2B	5.2	3.2	4.1	5.8
LL 2C	4.9	3.5	3.2	23.1
NA 1A	4.8	0.4	2.9	18.3
NA 1B	4.5	0.4	6.0	19.4
NA 1C	4.6	0.3	-0.9	13.9
NA 2A	4.1	0.0	7.6	5.2
NA 2B	3.8	0.0	6.1	16.8
NA 2C	4.1	0.0	-6.8	14.4
NA 3A	5.1	7.2	4.6	-
NA 3B	7.8	7.0	4.0	15.5
NA 3C	6.2	7.1	2.6	6.2

Table 3.3.(i). Nitrification rate of added ammonium, ammonium fixation, and inorganic-N loss after 12 days of incubation of ammonium treated coal mine soils (100 mg N/kg soil).

- gain in nitrogen
* site codes, see section 3.2.1.

Table 3.3 continued on next page

Sample	pH	Nitrification (mg/kg/day)	Loss of Nitrogen (mg/kg)	
			Ammonium Fixation	Incubation
BD 1A	4.2	0.0	6.7	7.3
BD 1B	4.2	0.0	2.5	9.5
BD 1C	4.6	0.0	7.1	-
BD 2A	4.6	0.0	5.2	22.4
BD 2B	7.2	10.2	12.5	9.6
BD 2C	6.0	2.5	10.3	19.3
BD 3A	6.6	8.0	13.7	9.7
BD 3B	5.2	0.0	5.4	8.7
BD 3C	5.6	3.0	7.9	22.5
BD 4A	6.6	7.3	22.9	45.2
BD 4B	4.8	0.0	6.1	11.5
BD 4C	6.6	8.1	17.2	31.8
BD 5A	5.4	1.5	7.2	5.5
BD 5B	6.0	6.6	8.3	2.6
BD 5C	4.4	0.0	5.7	7.4
BD 6A	3.0	0.0	-0.9	30.1
BD 6B	3.6	0.0	0.4	31.2
BD 6C	3.4	0.0	-0.9	14.9
BD 7A	4.5	0.0	2.0	24.5
BD 7B	4.4	0.0	-0.6	23.9
BD 7C	4.5	0.0	-0.9	8.5
BD 8A	6.6	0.0	14.9	61.8
BD 8B	5.8	0.0	10.0	19.1
BD 8C	6.4	1.8	13.1	20.8
BD 9A	6.6	2.1	17.7	14.3
BD 9B	6.6	4.2	16.1	8.8
BD 9C	7.0	2.5	21.6	22.3
BD 10A	6.2	0.0	12.0	31.8
BD 10B	6.8	1.9	20.0	60.8
BD 10C	7.2	0.0	19.5	39.1

Table 3.3.(ii). Nitrification rate of added ammonium, ammonium fixation, and inorganic-N loss after 12 days of incubation of ammonium treated coal mine soils (100 mg N/kg soil).

- gain in nitrogen

Table 3.3 continued on next page

Sample	pH	Nitrification (mg/kg/day)	Loss of Nitrogen (mg/kg)	
			Ammonium Fixation	Incubation
PV 1A	5.6	0.0	1.0	10.4
PV 1B	5.6	0.0	-0.5	1.9
PV 1C	5.8	0.0	-1.8	11.7
PV 2A	5.5	1.4	0.1	9.7
PV 2B	6.7	5.0	2.3	-
PV 2C	6.0	0.3	1.3	9.4
PV 3A	7.1	3.1	10.7	18.9
PV 3B	5.7	0.0	5.6	9.0
PV 3C	5.8	1.2	4.1	4.7
PV 4A	4.8	0.0	-0.5	0.2
PV 4B	4.5	0.0	-0.2	2.5
PV 4C	4.7	0.0	0.9	2.5
PV 5A	8.4	0.0	12.7	43.4
PV 5B	8.4	0.0	11.4	34.9
PV 5C	8.2	3.4	4.6	20.6
PV 6A	6.2	0.0	3.4	23.3
PV 6B	6.1	0.0	4.3	13.9
PV 6C	5.2	0.0	0.5	5.3
PV 7A	7.5	4.8	3.1	11.6
PV 7B	8.1	7.4	7.4	29.5
PV 7C	8.0	7.0	6.0	8.6
MV 1A	8.5	0.0	12.3	29.7
MV 1B	8.5	0.0	14.3	25.3
MV 1C	8.5	0.0	13.1	24.5
MV 2A	8.0	0.8	11.5	10.6
MV 2B	8.1	1.2	10.8	22.5
MV 2C	8.0	1.0	9.6	24.6
LT A	8.6	0.7	4.9	14.5
LT B	8.0	2.5	3.0	1.8
LT C	8.8	0.8	9.5	29.9

Table 3.3.(iii). Nitrification rate of added ammonium, ammonium fixation, and inorganic-N loss after 12 days of incubation of ammonium treated coal mine soils (100 mg N/kg soil).

- gain in nitrogen

Sample	pH	Soil Enzyme Activity (NH_4-N released mg/kg/h)	
		Urease	Amidase
BD 1A*	4.2	8.3	9.6
BD 1B	4.2	3.9	7.9
BD 1C	4.6	11.7	11.7
BD 2A	4.6	8.1	12.7
BD 2B	7.2	20.5	26.5
BD 2C	6.0	22.6	17.8
BD 3A	6.6	33.8	41.4
BD 3B	5.2	12.5	12.5
BD 3C	5.6	32.6	28.3
BD 4A	6.6	19.8	22.8
BD 4B	4.8	5.1	8.7
BD 4C	6.6	28.0	27.9
BD 5A	5.4	20.2	14.8
BD 5B	6.0	27.3	26.7
BD 5C	4.4	5.8	4.5
BD 6A	3.0	4.5	3.0
BD 6B	3.6	13.5	29.4
BD 6C	3.4	5.6	10.5
BD 7A	4.5	24.0	13.2
BD 7B	4.4	10.4	10.8
BD 7C	4.5	12.4	19.3
BD 8A	6.6	21.3	16.7
BD 8B	5.8	16.8	10.8
BD 8C	6.4	24.7	10.5
BD 9A	6.6	15.2	10.8
BD 9B	6.6	21.4	17.9
BD 9C	7.0	14.7	6.6
BD 10A	6.2	12.7	8.4
BD 10B	6.8	19.4	8.5
BD 10C	7.2	26.0	9.7

Table 3.4. Urease and amidase activity in coal mine soils from Baads.

* site codes, see section 3.2.1.

3.4 DISCUSSION

The data presented in Tables 3.1 to 3.4 are summarised in Table 3.5 for the general assessment of nitrogen status in a wide range of coal mine soil samples from sites throughout Central Scotland. The large ranges of values encountered illustrate the large variation in the type of materials found.

3.4.1 EXTRACTABLE INORGANIC NITROGEN

The levels of ammonium-N, nitrate-N and total inorganic-N in 90 samples of coalmine soils presented in Table 3.1 indicate a great variation between sampling areas as well as within three samples from the same sampling area. Nitrite levels were always very low (< 1 mg N/kg soil) and were therefore not reported. The levels of ammonium-N ranged from 0.1 to 25.9 mg N/kg soil with a mean value of 2.5 mg N/kg. Most of the inorganic-N was found in the ammonium form, especially in acid spoils. For example, comparatively higher values of ammonium-N (>10 mg/kg soil) were generally found in samples having pH values below 4.5 (FH 1B, FH 1C, BD 6A and BD 7B). These results agree with those reported by Williams and Cooper (1976) who observed that mineral nitrogen concentrations were greater in acid coal mine soils (pH<5) than in neutral coal mine soils, and that in acid coal mine soils the predominant form was ammonium-N.

Property	Maximum	Minimum	Mean	Median	SD
pH (in water)	8.8	3.0	5.9	5.8	1.5
Extractable Ammonium (mg N/kg)	25.9	0.1	2.5	1.5	3.7
Extractable Nitrate (mg N/kg)*	9.0	0.0	1.3	0.7	1.8
Total Extractable Inorganic-N (mg N/kg)	31.4	0.1	3.4	2.2	4.4
Carbon dioxide evolved (mg C/kg/week)	181.4	19.5	62.6	46.8	36.8
Nitrogen Mineralization (mg N/kg/week)	3.2	0.0	0.8	0.5	0.9
Mineralizable C:N ratio	890	13	178	90	191
Nitrification rate (mg N/kg/day)**	10.2	0.0	3.6	2.5	2.7
Ammonium fixation (mg N/kg)	22.9	0.0	6.0	4.2	5.7
Nitrogen loss in incubation (mg N/kg)	61.8	0.0	15.7	14.3	12.6
Urease activity (mg NH_4-N/kg/h)	33.8	3.9	16.8	16.0	8.5
Amidase activity (mg NH_4-N/kg/h)	41.4	3.0	15.3	12.1	8.9

Table 3.5. Survey of Nitrogen status of coal mine soils.

* The mean, median and standard deviation of the mean (SD) are calculated on the basis of 59 samples, which had some detectable nitrate-N.

** The mean, median and SD are calculated on the basis of 46 samples which showed detectable nitrification.

The data in Table 3.1 also indicate that nitrate-N was either undetectable or present at low levels ranging from 0 to 9.0 mg N/kg soil with a mean of 0.9 mg N/kg. Although there was no observed relationship of pH with the extractable nitrate-N, in some sampling areas like FH 2 and NA 2 having pH in the range of 3.8-4.4 no nitrate-N was detected, which can be expected due to inhibition of nitrification at low pH (Table 3.3).

The extractable inorganic-N in coal mine soils under study ranged from 0.1 to 31.4 mg N/kg soil with a mean of 3.4 mg N/kg. This seems to be very low when compared with 9.0 to 162.6 mg N /kg soil, found in 9 agricultural soils extracted under similar conditions (Khan, 1987).

3.4.2 MINERALIZATION OF CARBON AND NITROGEN

Carbon and nitrogen mineralization rates presented in Table 3.2 show that large amounts of carbon, but little nitrogen were turned over. The rate of carbon turnover was found to be in the range of 19.5 to 181.4 mg C/kg soil/week with a mean of 62.6 mg C/kg/week. The mineralizable nitrogen rate values ranged from 0 to 3.2 mg N/kg soil/week with a mean value of 0.8 mg N/kg/week. The overall ratio of C/N mineralized was very high, varying from 13 to 890 which indicates a wide range of substrates in the coal mine soils under study.

Comparison of the results found during the present study with those reported by previous workers (Reeder and Berg, 1977b; Palmer and Chadwick, 1985; and Fyles and McGill, 1987) is difficult, because of different methods

used. For example they used air-dried coal mine soils, whereas in this study fresh samples of coal mine soil were used. Similarly there are a number of incubation systems which have been used. Despite these variations however it has usually been the case that far more carbon than nitrogen is released in this type of incubation. Similarly the C/N ratios reported by previous research workers (Down, 1975b; Reeder and Berg, 1977b; Schafer et al., 1980 and Stroo and Jencks, 1982) cannot be compared with that found during the present study. They calculated C/N ratio on the basis of total or organic carbon (different methods) and total nitrogen (Kjeldhal method) of air dried spoil, whereas in this study mineralized carbon and nitrogen of fresh spoil investigated during incubation study were compared.

Some of the results found in the present study are in agreement with those reported by Pulford et al. (1988) who incubated fresh samples of coal mine soil from Baads colliery spoil at 22 ^{o}C. They reported carbon mineralization rates from 16.5 to 146.4 mg C/kg soil/week and nitrogen mineralization rates from 0.68 to 3.80 mg N/kg soil/week, with the C/N ratios in the range of 24 to 58. Similarly, Rimmer and Gildon (1986) who carried out an incubation study at 23 ^{o}C of coal mine soils adjusted to 70 % of the water holding capacity, reported C mineralization rates ranging from 14 to 18 mg C/kg soil/day (98 to 126 mg C/kg soil/week). They found higher microbial activity in topsoiled and sewage sludge treated spoils than untreated spoils.

Different rates of carbon and nitrogen mineralization of agricultural soils have been reported by various researchers, which may partly be due to different temperature of incubation they used. For example, Khan (1987) used 9 different agricultural soils in the fresh condition for his incubation study at 10 ^{o}C, and reported carbon mineralization rates from 8.63 to 50.63 mg C/kg soil/week and nitrogen mineralization rates from 0.87 to 1.93 mg N/kg soil/week, with the ratio C/N mineralized in the range of 8 to 44. Jenkinson and Powlson (1976) studied carbon mineralization in 9 fresh soils incubated at 25 ^{o}C. They reported carbon mineralization rates from 4.5 to 27.5 mg C/100g soil/10 days (31.5 to 192.5 mg C /kg soil/week). Tabatabai and Alkhafaji (1980) studied nitrogen mineralization in 12 fresh soil samples at 20 ^{o}C. They reported nitrogen mineralization rates from 1.7 to 4.2 mg N/kg soil/week with a mean value of 2.9 mg N/kg soil/week. Addiscott (1983) reported values of 0.09 to 0.234 mg N/kg soil/day (0.63 to 1.64 mg N/kg/week) for nitrogen mineralization rates in fresh soils incubated at 20 ^{o}C. Similarly, Flowers and Arnold (1983) used 2 different soils in their incubation experiment at 15 ^{o}C, and reported nitrogen mineralization rates of 0.188 and 0.378 mg N/kg soil/day (1.316 to 2.646 mg N/kg/week).

The carbon mineralization rates of coal mine soils at 20 ^{o}C found in the present study were comparable to those reported by Jenkinson and Powlson (1976) at 25 ^{o}C, but were much higher than those found by Khan (1987) at 10 ^{o}C in agricultural soils.

The nitrogen mineralization rate constants for coal mine soils measured in this study clearly indicate that most of the rates were very low compared to those reported in agricultural soils. Half of the samples mineralized small amounts of nitrogen (0 to 0.5 mg N/kg soil/week) which resulted in high ratios of C/N mineralized (Table 3.2).

The rates of respiration indicate that coal mine soils have a microbial population which can break down organic matter at a rate comparable to agricultural soils. Microbial respiration was enhanced in the vegetated compared to non-vegetated coal mine soils. In the soils from Baads, for example, carbon turnover rate was highest in the vegetated (BD 3A) coal mine soil (181 mg C/kg soil/week) and lowest in the barren (BD 6A) coal mine soil (20 mg C/kg soil/week). The samples from a non-vegetated sampling area (DK 2) showed lower respiration rates of 27 to 35 mg C/kg soil/week compared with 43 to 51 mg C kg soil/week in the vegetated area (DK 2) of the same site. Similarly on Pennyvenie bing the vegetated sampling area PV 6 had a higher carbon turnover rate than the non-vegetated sampling area PV 5. The increased CO_2-C evolution from vegetated coal mine soil suggested an increase in microbial activity which would result from the addition of plant residues and root exudates to the coal mine soil. Microbial activities have been shown to be higher in vegetated amended than barren coal mine soil (Stroo and Jencks, 1982).

The coal mine soils have some fossil nitrogen in the

carbonaceous materials contained within them, but this does not seem to be mineralized. The low levels of nitrogen mineralized in most of the samples may also indicate that either ammonification was not occurring or that the heterotrophic microbial population was immobilising all ammonium that was produced via ammonification or the released ammonium being fixed into the clay fraction of the waste material.

In spite of the fact that the levels of nitrogen mineralized in coal mine soils are very low, they may represent the most important pool of N available to plants. Reeder and Berg (1977a) and Palmer and Chadwick (1985) found a high correlation between mineralizable nitrogen and N uptake by the plants grown in coal mine soils.

3.4.3 NITRIFICATION OF ADDED AMMONIUM

The data pertaining to the nitrification rates of coal mine soil samples documented in Table 3.3 clearly indicate the lack of nitrification on about half of the sites. Nitrification rates of added ammonium (100 mg N/kg soil) varied among the remaining sites which exhibited nitrification. This process of microbial transformation was highly pH dependent, being inhibited below pH 4.8. However, even on some sites above this pH there was no measurable nitrification. Those soil samples which showed some transformation of ammonium-N had nitrification rates in the range of 0.3 to 10.2 mg N/kg soil/day with a mean of 3.6 mg N/kg/day.

Nitrification rates of added ammonium in most of the coal mine soils under study were low compared to that reported in agricultural soils, although some samples showed comparable rates of this process. Addiscott (1983) studied nitrification of added ammonium as ammonium chloride (1.2 mg ammonium-N/15 g soil) during an incubation study at 15 ^{o}C. He reported nitrification rates from 5.32 to 12.73 mg N /kg soil/day. Similarly Flowers and O'Callaghan (1983) reported the nitrification of added ammonium sulphate (100 mg ammonium-N/kg soil) from 2.5 to 5.6 mg N/kg soil/day in 4 fresh agricultural soils during an incubation experiment at 15 ^{o}C.

One possible reason for the apparent lack of nitrification in coal mine soil could be the low levels of available nutrients in maintaining the population of nitrifying bacteria in soils. The coal mine soils studied contained very low levels of substrate material i.e. ammonium-N (Table 3.1). This, coupled with its low levels of available phosphorus reported by various research workers (Fitter and Bradshaw, 1974; Pulford, 1976; Palmer, 1978; and Lister, 1987) probably explain the paucity of nitrifying bacteria.

Williams (1975) found similarity between the nitrification processes in acid soil and spoils and neutral soil and spoils, and suggested that there was no fundamental differences between the soils and spoils in this process. He reported that incubating the acid spoil with calcium carbonate not only resulted in increased nitrification but also increased the quantity of

mineralizable nitrogen.

Reeder and Berg (1977b) quoted that the lack of nitrification in the coal mine soils could be attributed to toxic substances inhibiting the growth of the nitrifying bacteria. However, the apparent lack of this microbial process in some samples during the present study having pH suitable for this process suggests that the introduction of nitrogen transformation organisms, particularly nitrifying microorganisms, into coal mine soil was also an important factor. This can be possible through the application of organic amendments (Fresquez and Lindemann, 1982).

3.4.4 AMMONIUM FIXATION AND INCUBATION LOSS

The data presented in Table 3.3 also indicate that some nitrogen was lost by ammonium fixation, but greater amounts of nitrogen loss were found during incubation of ammonium sulphate treated samples of coal mine soil. A range of 0 to 61.8 mg/kg soil of total inorganic-N was lost during the 16-day incubation. The high CO_2-C evolved: N mineralized ratio of coal mine soils (Table 3.2) indicated that the heterotrophic microbial population may have assimilated the added ammonium into their cells.

The loss of nitrogen due to ammonium fixation by the clay fraction of coalmine soil was very high in some samples. It ranged from 0 to 22.9 mg ammonium-N/kg soil with a mean of 6.0 mg ammonium-N/kg. This clearly indicates that a considerable amount of nitrogen applied in the form of ammonium salts is likely to get fixed in

coalmine soils or assimilated by the heterotrophic microorganisms.

Reeder and Berg (1977b) observed losses of added ammonium-N during an incubation study of coal mine soil at zero time as well as after 168 days incubation. Since the spoil they used was calcareous, they attributed some of the initial loss of ammonium-N to ammonia volatilization. They further stated that the possibility of ammonium fixation by the soil colloidal material cannot be ignored.

Some samples of coal mine soils showed an increase in extractable inorganic-N, represented by the negative values of ammonium fixation. In view of the incubation time (24 hours) and temperature (2 ^{o}C), such gains are most likely to arise through experimental error or contamination. But they are generally very small and can be regarded as negligible. Similarly the missing values in the incubation loss were for those samples which showed a gain of total inorganic-N during incubation at 20 ^{o}C. This type of gain may be due to nitrogen mineralization.

3.4.5 UREASE AND AMIDASE ACTIVITIES

The summary of the data presented in Table 3.5 indicates that the urease activity in coal mine waste ranged from 3.9 to 33.8 mg ammonium-N released/kg soil/hour with a mean value of 16.8 mg of ammonium-N released /kg/hour.

The values for urease activity found in this survey are similar to, or somewhat greater than, values of 6.8 to 23.6 mg urea hydrolysed/kg soil/hour (3.2-11.0 mg of

ammonium-N released/kg soil/hour) reported by Stroo and Jencks (1982) for coal mine soils. The comparatively higher values of urease activity than those reported by Stroo and Jencks (1982) may partly be due to the non-buffer method of urease activity they used. Higher values of urease activity determined by the buffer method than by the non-buffer method of the same soils have been reported by Zantua and Bremner (1975). However, enzymatic activities in coal mine soils were found to be lower than those reported in agricultural soils by various researchers. Zantua and Bremner (1975) studied urease activity in 16 different soils, and reported 6.4 to 233.8 mg urea hydrolysed/kg soil/hour (3.0 to 109.1 mg ammonium-N released/kg soil/hour). Tabatabai (1977) reported urease activity for agricultural soils ranging from 9.0 to 131.5 mg ammonium-N released/kg soil/hour.

The amidase activity measured ranged from 3.0 to 41.4 mg ammonium-N released/kg soil/hour with a mean value of 15.3 mg of ammonium released /kg/hour. A search of the literature indicates that amidase activity has not been studied previously in coal mine soils. Frankenberger and Tabatabai (1981b) reported the mean values of this enzyme in 3 agricultural soils from 97.5 to 167.5 mg ammonium-N released/kg soil/hour. Frankenberger and Tabatabai (1982) studied urease and amidase activities in 5 agricultural soils. Their reported values of urease activity ranged from 16.5 to 110 mg ammonium-N released/kg soil/hour and amidase activity using formamide as substrate ranged from 52.5 to 224.5 ammonium-N released/kg soil/hour.

3.4.6 GENERAL DISCUSSION

The coal mine soils represent a wide range of heterogeneous parent materials, which leads one to question the use of composite samples. It should be recognized that even what are taken as individual samples are in reality composites of individual particles, because the material tipped may have had different origins. Keeping in view the heterogeneity of material, instead of taking a composite sample from each site, it was decided to take 3 individual samples at random from each sampling area. Care was taken in the selection and distribution of sampling areas on a bing. Each sampling area represented a piece of land, roughly equal to 20-25 square meters, with uniform appearance in respect of vegetation, topography, colour of the soil or any treatment in the past.

Fresh samples of coal mine soils were used in the present study, because it is well established that drying of soil has an immediate and major effect on many of its biological activities (McGarity and Myers, 1967). Air drying of soil samples can lead to small but significant increases in exchangeable ammonium-N and even nitrate-N (Nelson and Bremner, 1972). Moreover, the actual field conditions can be best represented by fresh soil samples and not by air dried.

The pH, which is considered to be a major determining factor in nitrogen transformations, of the coal mine soil ranged from 3.0 to 8.8, and showed a considerable variation between different sites on the same bing as well as within 3 samples from the same sampling area.

To assess the relationships between various properties studied, correlation coefficients for all sites (90 samples) and for the Baads site (30 samples) only were computed. Table 3.6 presents the correlation coefficients of all samples, while Table 3.7 shows the correlation coefficients of samples collected from Baads. The correlation coefficients of nitrification rates with other properties are presented separately in Table 3.8. Because about 50% of coal mine soils did not exhibit nitrification of added ammonium, these calculations were made on the basis of those samples which showed detectable nitrification rates.

In general, correlation coefficients tended to be higher when considering only the data from Baads compared to that from all 90 sites. This may be because all the Baads samples were taken from the same bing, thereby reducing the variability of the spoil.

Some graphs of correlation of Baads samples are presented in Figures 3.1 to 3.6.

Property		Inorganic-N			Mineralization				
	pH	NH_4	NO_3	Total	C	N	C/N	Nitr. rate	NH_4 fixed
Ammonium-N	-0.41								
Nitrate-N	0.13	0.28							
Total-N	-0.29	0.94	0.59						
C Mineralization rate	-0.01	-0.04	-0.15	-0.08					
N mineralization rate	-0.19	0.31	0.34	0.37	0.14				
C/N ratio	0.07	-0.24	-0.30	-0.30	0.35	-0.57			
Nitrification rate	0.37	-0.14	0.18	-0.05	0.43	0.11	0.13		
Ammonium Fixation	0.52	-0.29	-0.19	-0.30	0.38	-0.32	0.40	0.23	
Incubation N loss	0.32	-0.16	-0.18	-0.19	0.14	-0.25	0.30	-0.02	0.57

Table 3.6. Correlation coefficients between various components of N status of 90 coal mine soil samples collected from various sites throughout Central Scotland.

Significance of correlation coefficients at 88 degrees of freedom (N=90)

values > 0.21 significant at 5%
values > 0.27 significant at 1%
values > 0.34 significant at 0.1%

Property		Inorganic-N			Mineralization						
	pH	NH_4-N	NO_3-N	Total-N	C	N	C/N	Nitr. rate	NH_4 Fixed	N Loss	Urease
Ammonium-N	-0.56										
Nitrate-N	0.24	0.51									
Total-N	-0.51	1.00	0.58								
C mineralization rate	0.79	-0.33	0.33	-0.28							
N mineralization rate	-0.45	0.53	0.25	0.51	-0.25						
C/N ratio	0.71	-0.40	0.19	-0.35	0.74	-0.58					
Nitrification rate	0.59	-0.13	0.43	-0.07	0.82	-0.16	0.59				
Ammonium fixation	0.91	-0.56	0.15	-0.51	0.69	-0.62	0.83	0.51			
Incubation N loss	0.30	-0.14	0.06	-0.12	0.20	-0.36	0.51	-0.05	0.43		
Urease activity	0.68	-0.39	0.37	-0.34	0.79	-0.23	0.50	0.61	0.52	0.20	
Amidase activity	0.30	-0.09	0.27	-0.06	0.73	0.19	0.28	0.72	0.14	-0.05	0.69

Table 3.7. Correlation coefficients between various components of N status of 30 coal mine soil samples collected at Baads.

Significance of correlation coefficients at 28 degrees of freedom (N=30)
values > 0.36 significant at 5%
values > 0.46 significant at 1%
values > 0.57 significant at 0.1%

PROPERTY	NITRIFICATION RATE	
	All sites (46 samples)	Baads site (13 samples)
PH	0.14	0.42
Ammonium-N	0.13	0.49
Nitrate-N	0.05	0.28
Total inorganic-N	0.11	0.50
C mineralization rate	0.51***	0.80***
N mineralization rate	0.15	0.17
C/N ratio	0.04	-0.18
Ammonium fixation	0.18	0.08
Incubation N loss	-0.04	-0.12
Urease activity	ND	0.36
Amidase activity	ND	0.76**

Table 3.8. Correlation coefficients of nitrification rate with other properties of coal mine soils which showed detectable nitrification.

ND not determined

Significance of correlation coefficient

significance at 5% *
significance at 1% **
significance at 0.1% ***

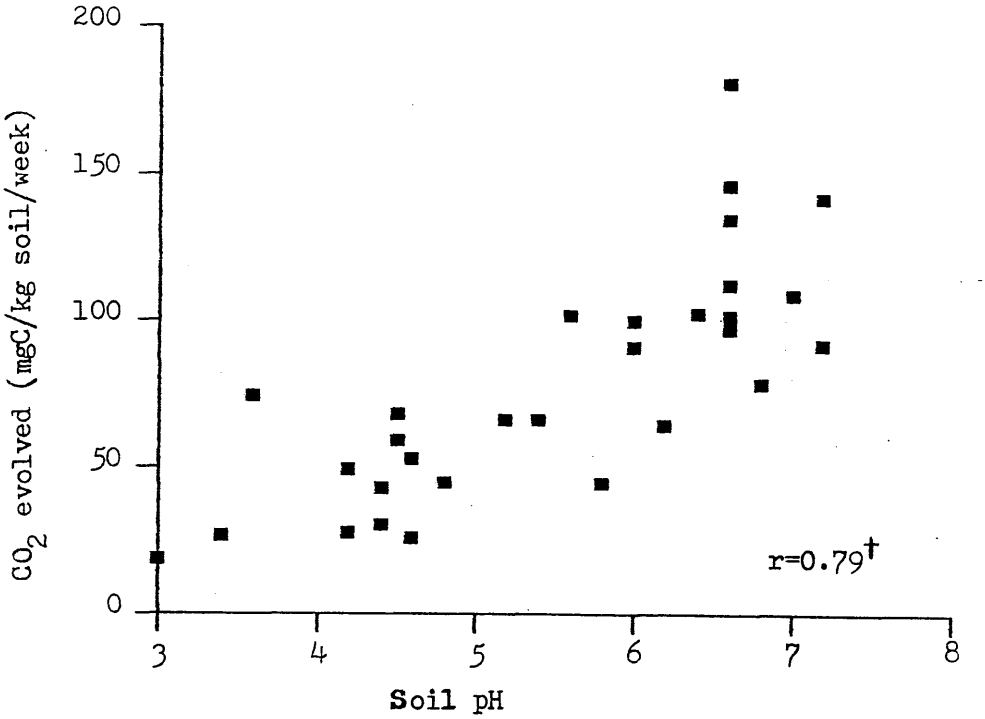


Fig. 3.1 Relationship between pH and CO_2 evolved by the soils.
† r is the Pearson product-moment correlation coefficient

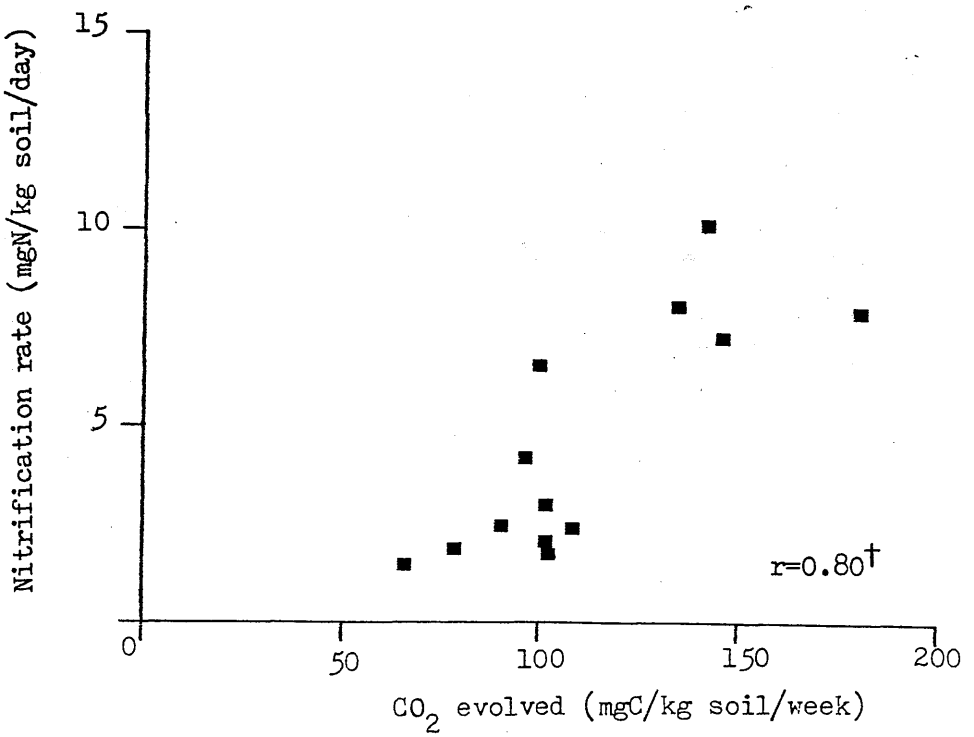


Fig. 3.2 Relationship between nitrification rate constants and CO_2 evolved by the soils.
† r is the Pearson product-moment correlation coefficient

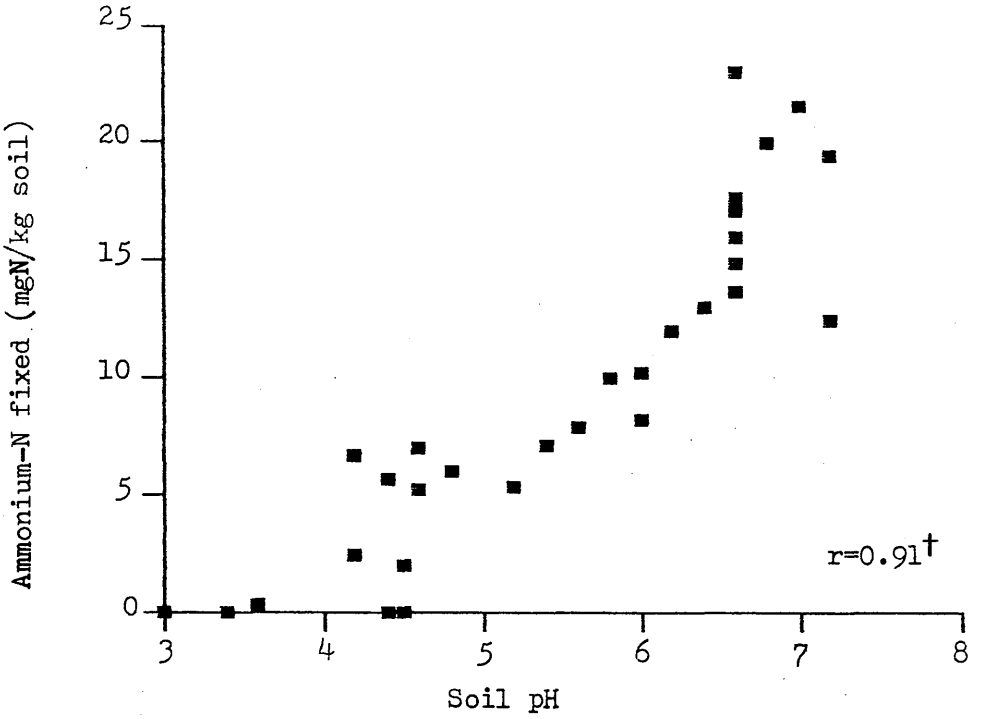


Fig. 3.3 Relationship between pH and ammonium-N fixed by the soils.

† r is the Pearson product-moment correlation coefficient

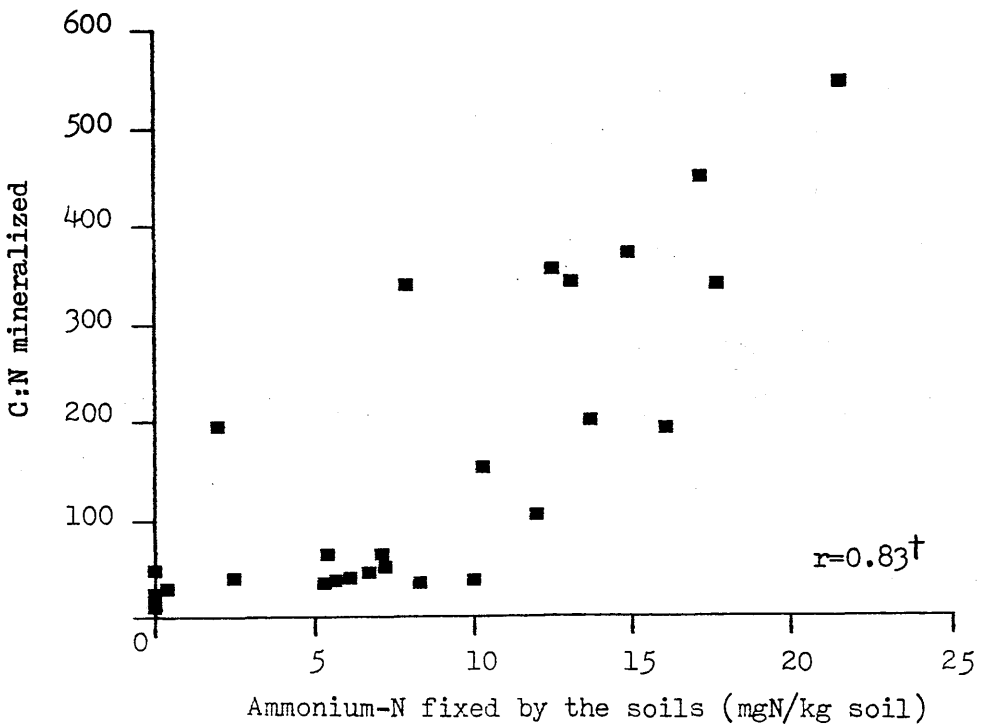


Fig. 3.4 Relationship between C:N mineralized and ammonium-N fixed by the soils.

† r is the Pearson product-moment correlation coefficient

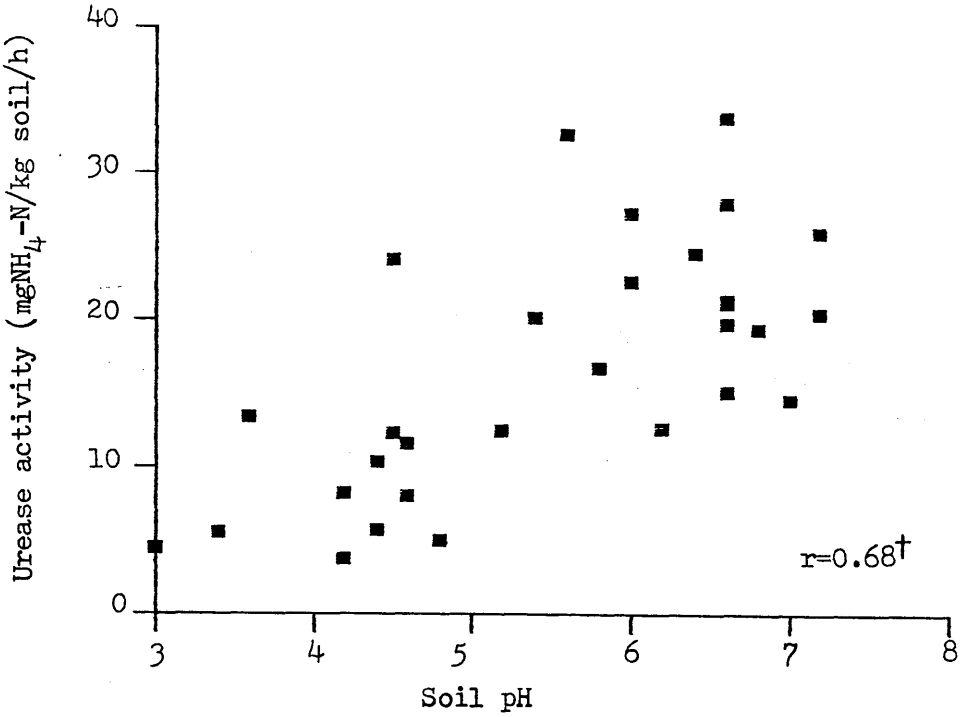


Fig. 3.5 Relationship between urease activity and soil pH.

† r is the Pearson product-moment correlation coefficient

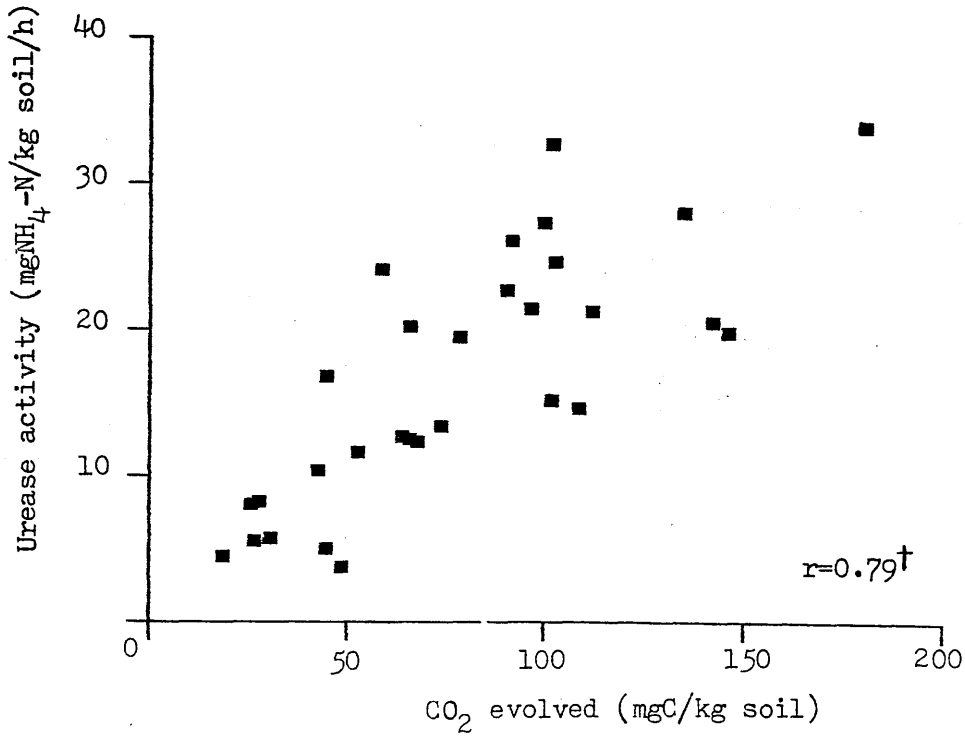


Fig. 3.6 Relationship between urease activity and CO_2 evolved by the soils.

† r is the Pearson product-moment correlation coefficient

Figs. 3.1 and 3.2 show that carbon mineralization was significantly correlated to pH (r=0.79) and nitrification rate (r=0.80).

Figs. 3.3 and 3.4 indicate a strong relationship of fixation of added ammonium to both the pH (r=0.91) and to the ratio of mineralizable carbon and nitrogen (r=0.83) of coal mine soils. The data in Table 3.7 also shows a highly significant relationship (r=0.69) between carbon mineralization and ammonium fixation. Burge and Broadbent (1961) reported a linear relationship between the amount of ammonium fixed and the carbon content of agricultural soil. They suggested that hydroxyl groups present in the organic matter may be the site of the reaction with the added ammonium. The significant negative correlation observed between ammonium fixation and nitrogen mineralization rates (r=-0.62) indicates that the ammonium produced through mineralization was being fixed into the clay minerals of the coal mine soils. These correlations were still significant when all sites were considered (Table 3.6).

However, unlike the significant relationship of pH to carbon and nitrogen mineralization rates, and C/N ratios between Baads samples (Table 3.7), there were no significant correlations between pH and these properties when all sites were considered (Table 3.6). A possible explanation for this divergent behaviour would be that factors other than pH e.g. parent material, age of the bing, type of vegetation, and reclamation etc were also affecting the various properties of coal mine soils.

The significant correlation of urease activity with pH (r=0.68) and carbon mineralization (r= 0.79) are shown in Figs. 3.5 and 3.6. McGarity and Myers (1967) reported that the urease activity in 100 Australian soils was highly correlated with organic carbon and weakly correlated with pH. The significant correlation between carbon mineralization and amidase activity (r=0.73) supports the findings of Frankenberger and Tabatabai (1981a), who found significant correlation between total organic carbon and amidase activity.

Unlike the relationship between pH and urease activity, there is no significant relationship between amidase activity and soil pH. There is, however, a highly significant correlation (r= 0.69) between amidase activity and urease activity in the coal mine soils studied. The significant correlation between amidase activity and urease activity is not unusual. Frankenberger and Tabatabai (1981a) also found significant correlation and reported that amidase and urease in soils are believed to be of microbial origin, possibly involving the same group of microorganisms. These two enzymes are related in that both are amidohydrolases and have similar kinetic properties (Tabatabai, 1973; Frankenberger and Tabatabai, 1980b).

Carbon mineralization rate, nitrification rate and amidase activity were found significantly correlated with each other. These results reflect the importance of organic matter for both microbial activity and extracellular soil enzyme stabilization.

The significant negative correlation of extractable ammonium-N with the pH (r=-0.56) and the positive correlation of pH with the ammonium fixation (r=0.91) and with mineralizable C/N ratio (r=0.71) explain the fate of ammonium-N at a high pH. The highly significant linear relationship between extractable ammonium-N with total inorganic-N (r=1.00) was expected, due to lack of nitrification in most of the coal mine soils studied.

A favourable effect of organic amendments and legumes was generally noticed on the microbial activities of coal mine soils from Baads. For example, lower rates of carbon turnover, lack of nitrification and comparatively lower values of urease and amidase activities were found in the coal mine soils collected from sampling area BD 6 (no lime, no manure) and BD 1 (only limed). The amended plots were also under good vegetation which emphasizes the importance of vegetation to coal mine soil microbial activity.

The presence of enzymatic activities suggests that enzyme inhibition would not be a problem on these coal mine soils, although such problems may occur in mine soils due to higher acidities or heavy metal concentrations (Cundell,1977).

3.5 CONCLUSIONS

It was concluded from the survey regarding the study of various aspects of nitrogen cycle in 90 coal mine soil samples from sites throughout Central Scotland that:

i. The inorganic nitrogen status of the waste material was very low.

ii. The rate of carbon dioxide evolved was very high compared to the low rate of nitrogen mineralized.

iii. Nitrification of added ammonium fertilizer was measurable on only half of the samples studied. Coal mine soil samples with pH < 4.8 showed no nitrification of added ammonium fertilizer. However on some sites above this pH no net nitrification was measured, which clearly indicated the absence of nitrifying bacteria.

iv. A considerable amount of applied ammonium nitrogen could be lost due to ammonium fixation by clay minerals of these waste materials, or assimilated by the heterotrophic population present due to wide mineralizable C:N ratio.

v. Significant correlations of carbon turnover, urease activity, amidase activity and nitrification rate with each other suggest the importance of organic matter for both microbial activity and extracellular enzyme stabilization.

vi. Reasonable values of the enzyme activities measured suggest that the use of urea or amide-N fertilizers may be possible on these coal mine soils.

CHAPTER 4 NITROGEN TRANSFORMATIONS DURING INCUBATION OF COAL MINE SOILS

4.1 INTRODUCTION

From the results of the survey of nitrogen status in coal mine soils of Central Scotland (chapter 3), it was found that mineralization of soil organic nitrogen and nitrification of ammonium-N added as ammonium sulphate were generally very low. Further study was undertaken to investigate these features in greater depth.

Until recently, most laboratory incubation studies of coal mine soils used to estimate nitrogen transformation in coal mine soils have utilized inorganic nitrogen fertilizer (Williams and Cooper, 1976; Reeder and Berg, 1977b; and Fyles and McGill, 1987). Information regarding the fate of organic fertilizers and organic wastes are limited. Voos and Sabey (1987) studied nitrogen mineralization in sewage sludge amended coal mine spoils and topsoils during a 16-week incubation at 25 ^{o}C. They reported that the total amount of inorganic-N that accumulated increased significantly with increasing rates of sewage sludge. They observed that more organic nitrogen was turned over in topsoil than in the coal mine spoil.

Urea is an organic fertilizer and has to undergo hydrolysis for transformation into an inorganic form of nitrogen. Upon application to soil, urea is acted on by

the enzyme urease. The ammonia released during this process may cause a temporary increase in the soil pH (Tisdale et al., 1985). These changes in soil properties may influence nitrogen transformations and crop development. The enzymatic conversion of organic nitrogen substances yielding ammonium ions can also be considered as a rate limiting step in nitrogen mineralization.

Chicken manure has been used on reclaimed spoils mainly because of its nutrient content (Bradshaw et al., 1973). The chemical composition and fertilizer value of this manure has been evaluated by Bradshaw and Chadwick (1980b) and Keefer et al. (1983). Its application may improve the physical condition of coal mine soil due to its high organic matter content. Keefer et al. (1983) reported that chicken manure addition not only increased the yield of grass, but also improved the concentration of nutrients in the soil and plant. It also lowered the concentrations of potentially toxic Al and Mn ions, and reduced the acid producing potential of coal mine soils. Pulford et al. (1984) compared the effects of various organic amendments on the yield of vegetation in coal mine soils. They reported that addition of chicken manure to a limed colliery spoil resulted in an increased yield of vegetation and more nitrogen retained in the system compared to other treatments. Apart from supplying nutrients, chicken manure could also be used as an inhibitor for the oxidation of pyrite in acid coal mine soils (Backes et al., 1987).

Efficient management of chicken manure as a nitrogen source requires a knowledge of its transformation in coal mine soils. Nitrogen mineralization in the organic manure treated soil should be dependent on the composition of the organic waste and the chemical and physical makeup of the soil receiving the organic waste (Chae and Tabatabai, 1986). Information on the fate of inorganic fertilizers (ammonium sulphate) in the coal mine soil may not therefore be applicable to the nitrogen in urea or in chicken manure.

Since information regarding the fate of nitrogen added as urea or chicken manure is lacking, a 16-week laboratory incubation was conducted to determine and compare the rates of nitrogen mineralization, nitrification and immobilization in coal mine soils treated with 100 mg N/kg soil as ammonium sulphate, urea or chicken manure.

4.2 MATERIALS AND METHODS

4.2.1 COAL MINE SOIL SAMPLES

Five coal mine soil samples (0-15 cm) were collected from colliery spoil at Baads (BD 11 to BD 15) to represent a wide range of pH and other properties. The details of the site are given in section 3.2. The first two samples were selected from the plots of an established organic manure trial. Soil sample BD 11 was collected from a plot which received 25 t/ha of lime and 4t/ha of chicken manure, while sample BD 12 was from a plot which received 50t/ha of lime and 20t/ha of sewage sludge during 1980. The remaining three samples, BD 13, BD 14 and BD 15 were collected from the area of the nitrogen response trial (chapter 5) which received 50 t/ha of lime and 300 kg/ha of a 15:10:10 NPK compound fertilizer during 1980. The samples were air-dried only just enough to permit sieving (4 mm) and for subsequent addition of amendment solution to be made without exceeding the desired moisture content (-0.5 bar soil moisture potential) for incubation. After sieving, the field moist samples (fresh) were stored in tied plastic bags at 2 ^{o}C until used.

The samples were tested for various properties; pH, extractable mineral-N (ammonium-N, nitrite-N and nitrate-N), fixation of added ammonium-N (100 mg N/kg soil as ammonium sulphate) and urease activity. These properties were measured by using the methods described in chapter 2. The relevant characteristics of the coal mine soils are given in Table 4.1.

Properties	Coal mine soils				
	BD 11	BD 12	BD 13	BD 14	BD 15
pH (in water)	6.2	7.2	7.0	7.3	3.8
0.5 bar moisture (%)	27.3	27.6	24.0	29.1	20.1
Ammonium-N (mg/kg soil)	8.8	4.2	0.7	2.7	3.9
Nitrate-N (mg/kg soil)	0.0	0.2	0.1	0.2	0.0
Inorganic-N (mg/kg soil)	8.8	4.4	0.8	2.9	3.9
NH_4-N fixed (mg/kg soil)	24.8	17.1	18.9	23.2	2.1
Urease activity *	21.9	26.8	14.6	25.9	8.2

Table 4.1. Characteristics of coal mine soils used in the incubation study.

* Ammonium-N released mg/kg soil/hour (37^{o}C).

4.2.2 CHICKEN MANURE

A sample of chicken manure mixed with bedding material (wood shavings) was obtained from an uncovered manure heap at Baads. In order to get a more homogeneous mixture of the material, the manure was thoroughly mixed and passed through a 4 mm sieve. It was stored in a tied plastic bag at 2 ^{o}C until used.

Inorganic-N was extracted by the method of Hoyle and Mattingly (1954). 1.0 g moist material was shaken with 100 cm^3 of 0.1 M HCl for 30 minutes. The contents were filtered through Whatman filter paper No. 40, discarding the first few cm^3 of the filtrate. The filtrate was then analysed for ammonium-N and nitrate-N by automated

colorimetric methods described in section 2.1.5.

The ammonium-N content of chicken manure was found to be 1,343 mg N/g fresh material. Nitrate-N was not detected. The pH was determined with a manure: deionized water ratio of 1:2.5 (w/v) by the method described in section 2.1.1 and was found to be pH 8.8. The moisture content (61.5 % fresh weight basis) was determined by the method described in section 2.1.3.

4.2.3 EXPERIMENTAL PROCEDURE

Twelve 50 g samples (oven dry equivalent) in fresh condition of each coal mine soil were weighed into wide mouth 8 oz glass bottles. The following treatments were applied to triplicate samples of each soil, and an appropriate amount of deionized water was added to each bottle to bring the soil to -0.5 bar moisture potential.

TREATMENTS

1. Control: no addition of nitrogen
2. 100 mg N/kg soil as ammonium sulphate.
3. 100 mg N/kg soil as urea.
4. 100 mg N/kg soil as chicken manure.

In control treatments the required moisture content of the coal mine soils in glass bottles was adjusted with deionized water and the contents mixed thoroughly.

In the case of the ammonium sulphate or urea treatments, 2 cm^3 of a solution containing

2,500 mg N/litre as ammonium sulphate or urea was added to the soil in the bottle. The contents in the bottles were thoroughly mixed after the addition of an appropriate amount of deionized water for the desired moisture content.

Chicken manure treated coal mine soils were prepared by mixing 3.72 g fresh chicken manure with 50 g soil, and the correct amount of deionized water (allowing for the moisture already present in the manure) was added for the desired moisture content. The contents in the bottles were thoroughly mixed.

A 16 litre plastic bin lined with moist filter paper was used as an incubation chamber for a set of ten bottles. The bottles containing the soils were left open in the bin to permit aeration. The bins were closed and kept in a room at 25 ^{o}C for incubation. The bottles with samples therein were weighed before each sampling period, deionized water was added to compensate for moisture loss and thoroughly mixed in (see section 2.4.2.3). With long intervals between sampling dates, the plastic bins were opened each week and the samples allowed to aerate for about 10 minutes, weighed and brought to the required moisture content if needed before closing.

The pH of the incubated soil samples was determined at 0, 1, 2, 7, 15, 28, 56 and 112 days of incubation. The extractable mineral nitrogen (ammonium-N, nitrite-N and nitrate-N) was measured after 5, 9, 15, 21, 28, 56, 84, and 112 days of incubation.

4.2.4 ANALYTICAL METHODS

Measurements of pH were made with a glass electrode in soil suspensions shaken for half an hour. The soil suspensions were prepared at a soil: deionized water ratio of 1:2.5 (section 2.1.1).

The inorganic nitrogen (ammonium-N, nitrite-N and nitrate-N) was extracted (section 2.3.2.3) by shaking 2.5 g (on oven dry basis) of moist soil with 50 cm^3 0.5 M potassium sulphate solution for 2 hours at 2 ^{o}C. The suspensions were filtered using acid washed Whatman filter paper No. 2. The filtrates were analysed for ammonium-N, nitrite-N and nitrate-N by colorimetric methods using a Technicon AutoAnalyzer II (section 2.1.5).

4.3 RESULTS

The transformations of soil nitrogen and that added in the form of ammonium sulphate, urea or chicken manure during incubation at 25 ^{o}C are shown in Figures 4.1 to 4.20. The changes in pH of the coal mine soils during incubation are given in Table 4.2.

4.3.1 UNTREATED COAL MINE SOILS

Changes in the soil inorganic nitrogen of untreated coal mine soils are shown in Figures 4.1, 4.5, 4.9, 4.13 and 4.17. Two soils BD 11 and BD 12 showed some measurable mineralization of native organic nitrogen. There was a lag period which lasted up to 21 days in the case of BD 11 (Fig. 4.1) after which the increase in total inorganic nitrogen was linear with time of incubation. There was an accumulation of about 50 mg N /kg soil in this soil after 112 days of incubation. Fig. 4.5 shows that the lag period in case of soil BD 12 was continued up to 42 days, followed by a linear increase in soil total inorganic nitrogen. This resulted in accumulation of about 29 mg N/kg soil at the end of 112 days of incubation.

In soil BD 12 the inorganic nitrogen measured after 112 days incubation was nearly all in the nitrate form, whereas in soil BD 11 about 20 % of total inorganic-N was present as ammonium-N. However, the inorganic nitrogen accumulated was mainly in the nitrate form indicating that the ammonium produced via ammonification was being nitrified in soils BD 11 and BD 12.

There was no net mineralization of organic nitrogen in coal mine soils BD 13 and BD 14 at any stage of incubation (Figs. 4.9 and 4.13). The acid coal mine soil BD 15 showed a slow release of mineralizable nitrogen, which resulted in an accumulation of about 18 mg ammonium-N/kg soil after 112 days of incubation. Nitrate-N was not found at any stage of incubation (Fig. 4.17).

4.3.2 TREATED COAL MINE SOILS

So far as nitrogen transformations in treated coal mine soils were concerned, ammonium sulphate or urea behaved similarly in each sample. All coal mine soils showed an initial decline in total inorganic nitrogen after the addition of the 3 different sources of nitrogen, with the exception of acid soil BD 15 which showed a very small decrease in the total inorganic nitrogen following the addition of ammonium sulphate or urea (Figs. 4.18 and 4.19). After the initial decline in the total inorganic-N there was generally a lag period which was comparatively shorter, and of the same length in the ammonium sulphate and urea treatments. The length of this phase in the chicken manure treatments of all soils was generally greater than that observed in other amended treatments.

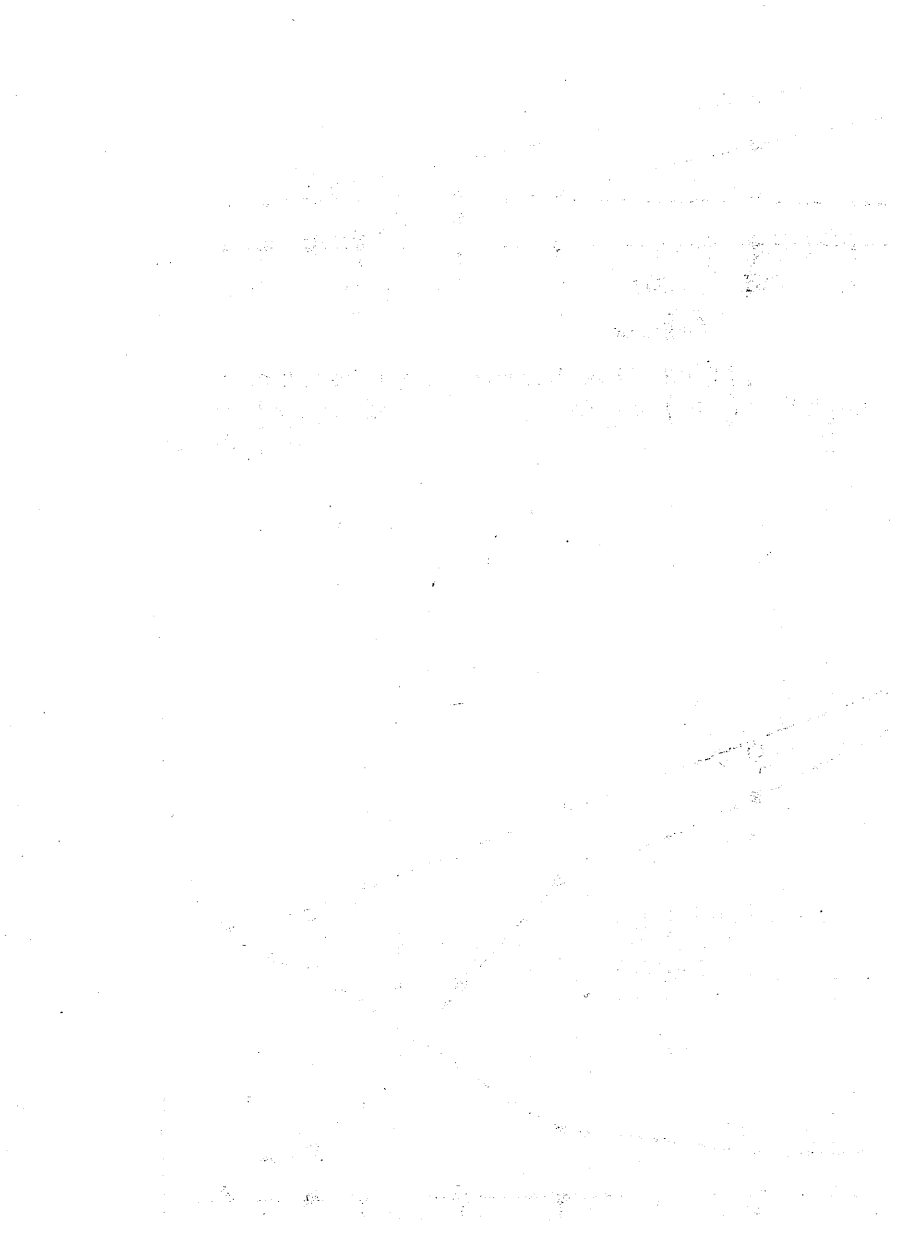
After the initial decline and a lag phase, the increase in total inorganic nitrogen was linear with time of incubation in soils which exhibited mineralization. Ammonium sulphate and urea treatments of soils BD 11 and BD 12 showed mineralization of soil nitrogen (Figs. 4.2,

4.3, 4.6 and 4.7). The same treatments of soils BD 13, BD 14 and BD 15 did not show any net mineralization at any stage of incubation (Figs. 4.10, 4.11, 4.14, 4.15, 4.18 and 4.19). The chicken manure treatment of coal mine soil BD 11 accumulated the greatest amount of inorganic nitrogen after 112 days of incubation, with an average amount of 161 mg N/kg soil (Fig. 4.4).

In the acid sample BD 15, which showed a slow release of mineralizable nitrogen (ammonification) in the control treatment, addition of ammonium sulphate or urea decreased the mineralization of soil nitrogen (Figs. 4.18-4.19). However in the later stages of the incubation there was a clear contribution to mineralization from chicken manure (Fig. 4.20). There was an obvious favourable effect of manure on this process in all soils except in soil BD 14 (Fig. 4.16).

In all coal mine soils the added nitrogen, whether in the form of ammonium sulphate, urea or chicken manure was converted to nitrate nitrogen, except the acidic one BD 15, where only the manure treatment showed nitrification. Ammonium sulphate and urea treatments of the acid soil did not exhibit nitrification (Figs. 4.18 and 4.19). The graphs of nitrogen transformation in the chicken manure treatments of all soils showed a somewhat different pattern compared to other treatments. Most of the ammonium-N added as chicken manure was nitrified in the first 9 days of incubation, followed by a lag period up to 21 days of incubation. Thereafter nitrification became slower, being governed by the rate at which the

soil mineralized nitrogen. After the initial flush of nitrification there was a sharp fall in the nitrate nitrogen in some soils especially soil BD 13 and BD 14 due to immobilization (Figs. 4.12 and 4.16).



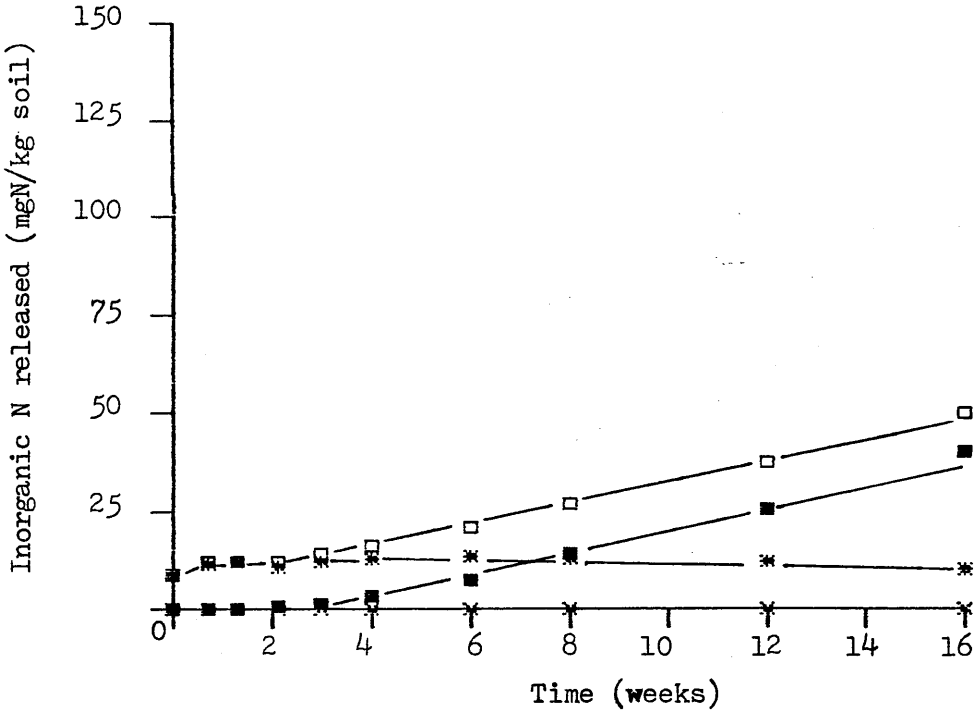


Fig. 4.1 N mineralized by untreated soil BD 11.
Total inorganic N (□), NH_4-N (*), NO_3-N (■), NO_2-N (×)

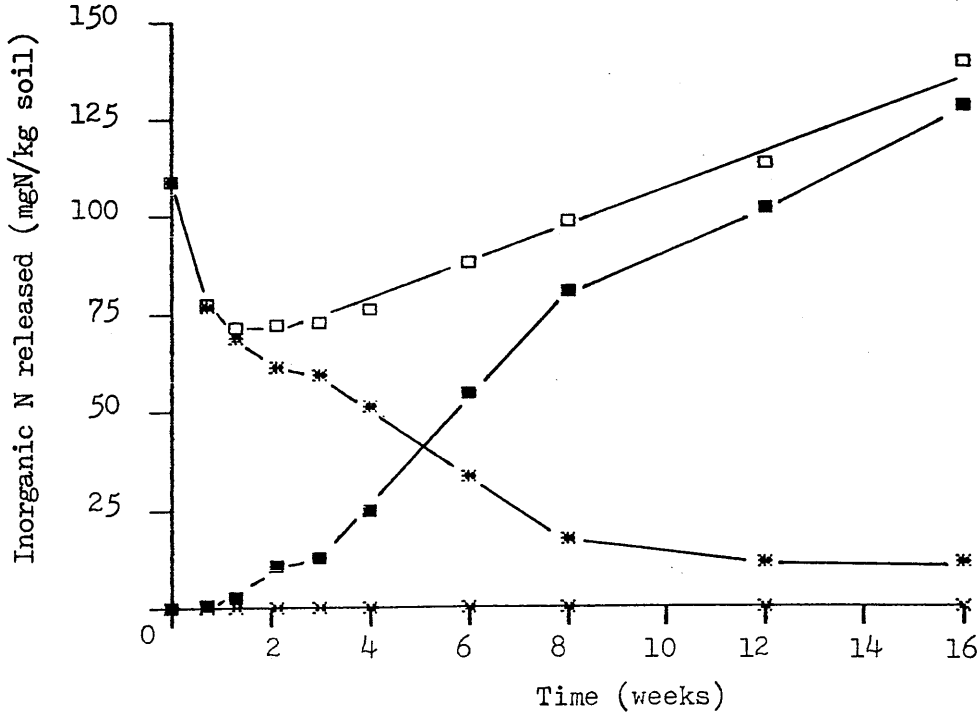


Fig. 4.2 N mineralized by ammonium sulphate treated soil BD 11.
Total inorganic N (□), NH_4-N (*), NO_3-N (■), NO_2-N (×)

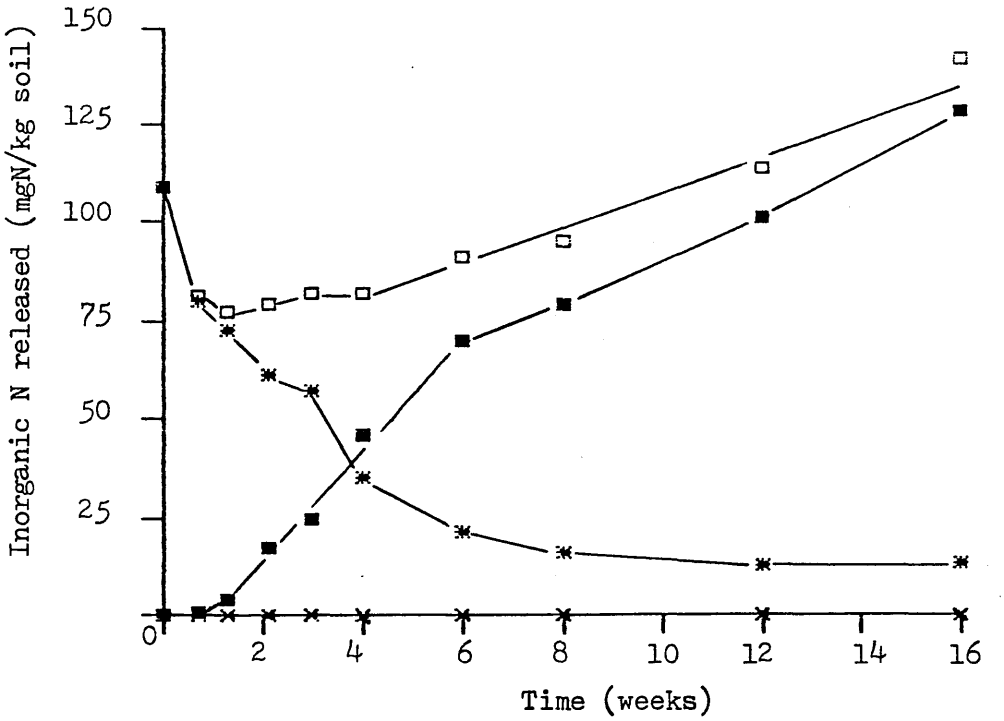


Fig. 4.3 N mineralized by urea treated soil BD 11.
Total inorganic N (□), NH_4-N (*), NO_3-N (■),
NO_2-N (×)

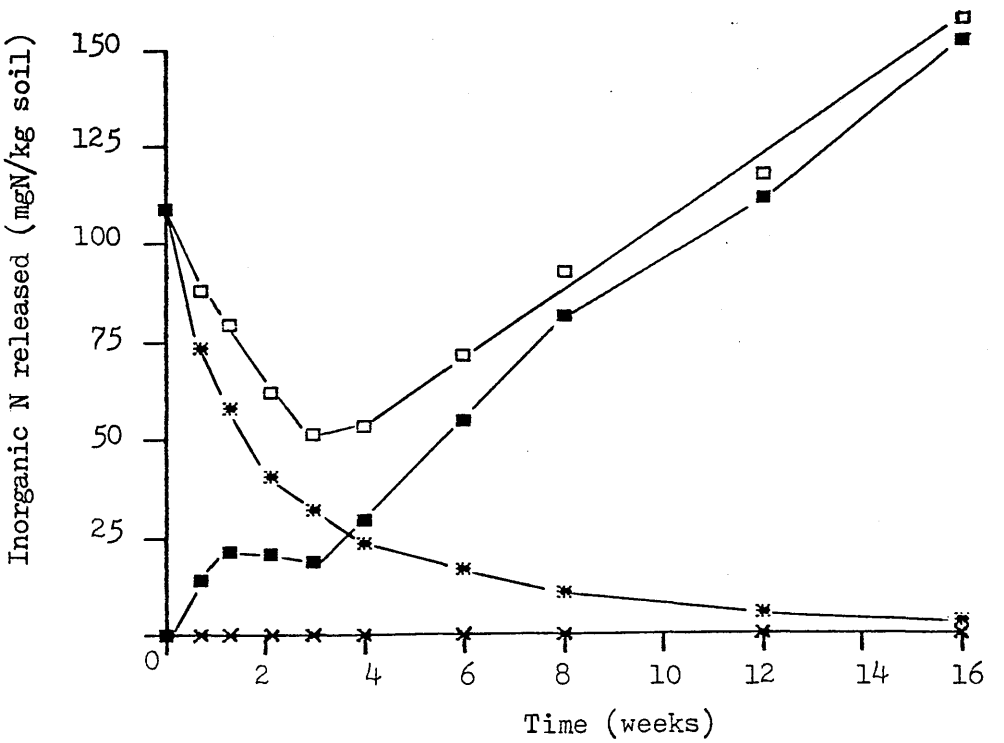


Fig. 4.4 N mineralized by chicken manure treated soil BD 11.
Total inorganic N (□), NH_4-N (*), NO_3-N (■),
NO_2-N (×)

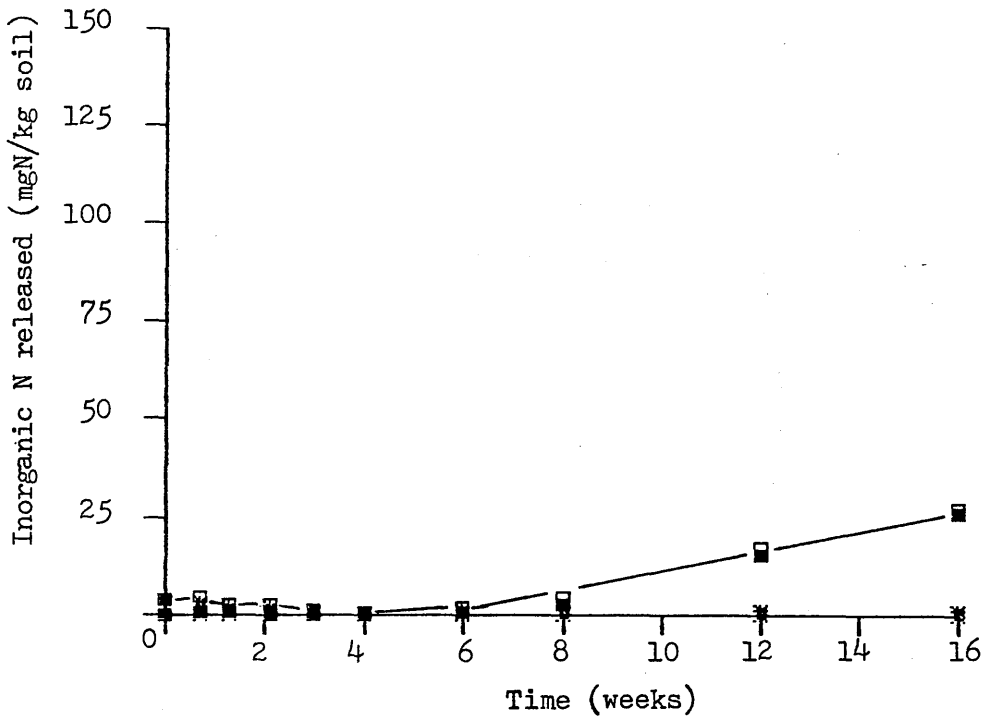


Fig. 4.5 N mineralized by untreated soil BD 12.
Total inorganic N (□), NH_4-N (*), NO_3-N (■), NO_2-N (×)

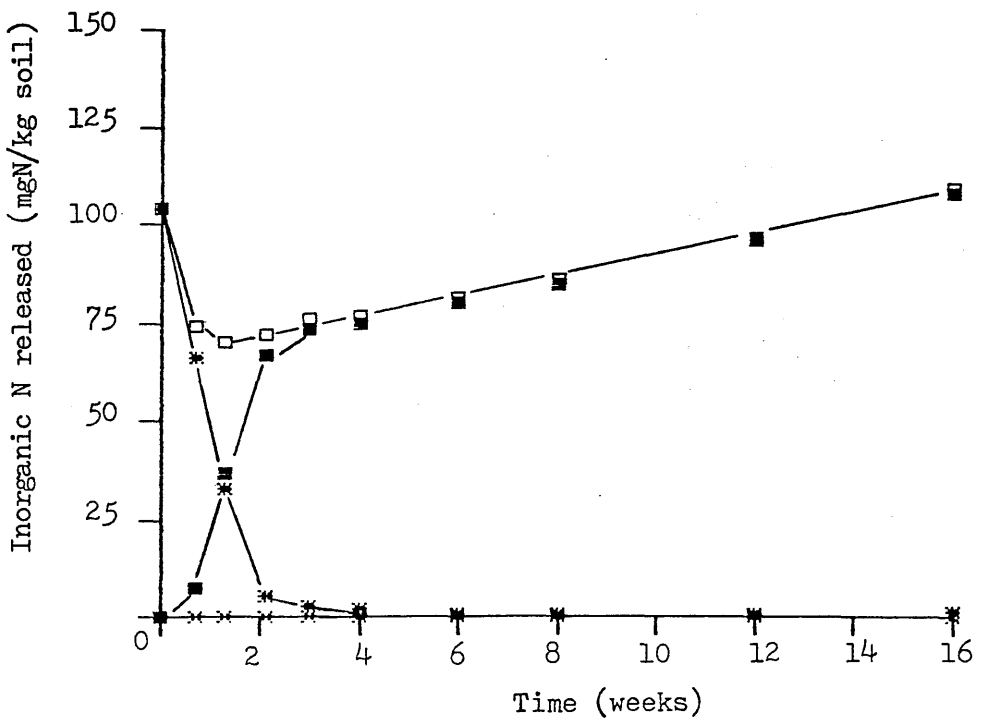


Fig. 4.6 N mineralized by ammonium sulphate treated soil BD 12.
Total inorganic N (□), NH_4-N (*), NO_3-N (■), NO_2-N (×)

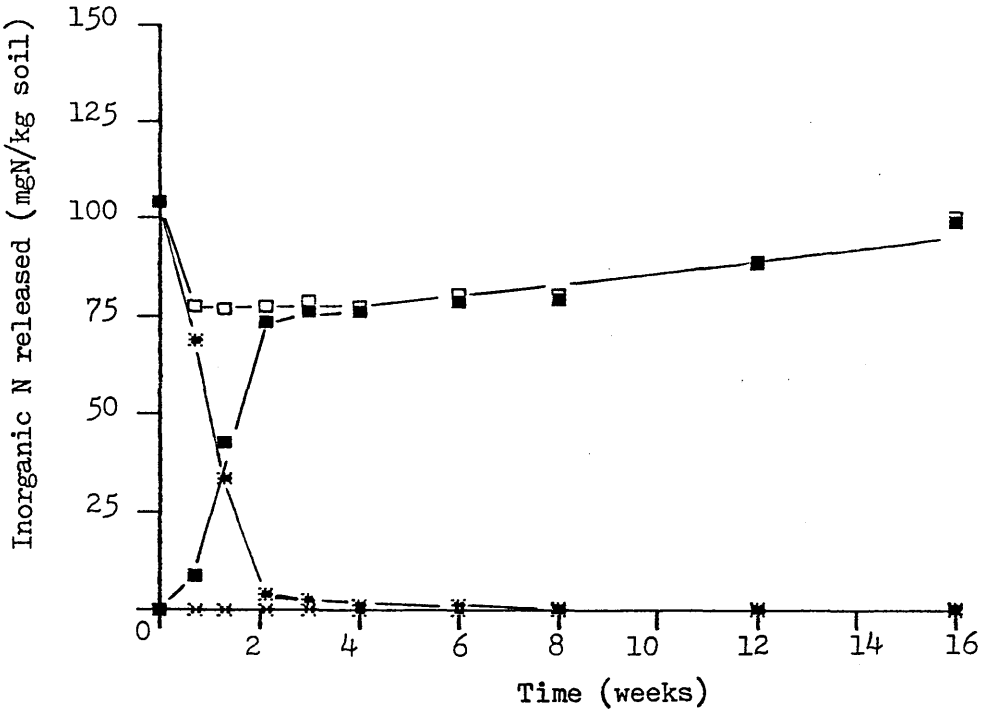


Fig. 4.7 N mineralized by urea treated soil BD 12.
Total inorganic N (□), NH_4-N (*), NO_3-N (■),
NO_2-N (×)

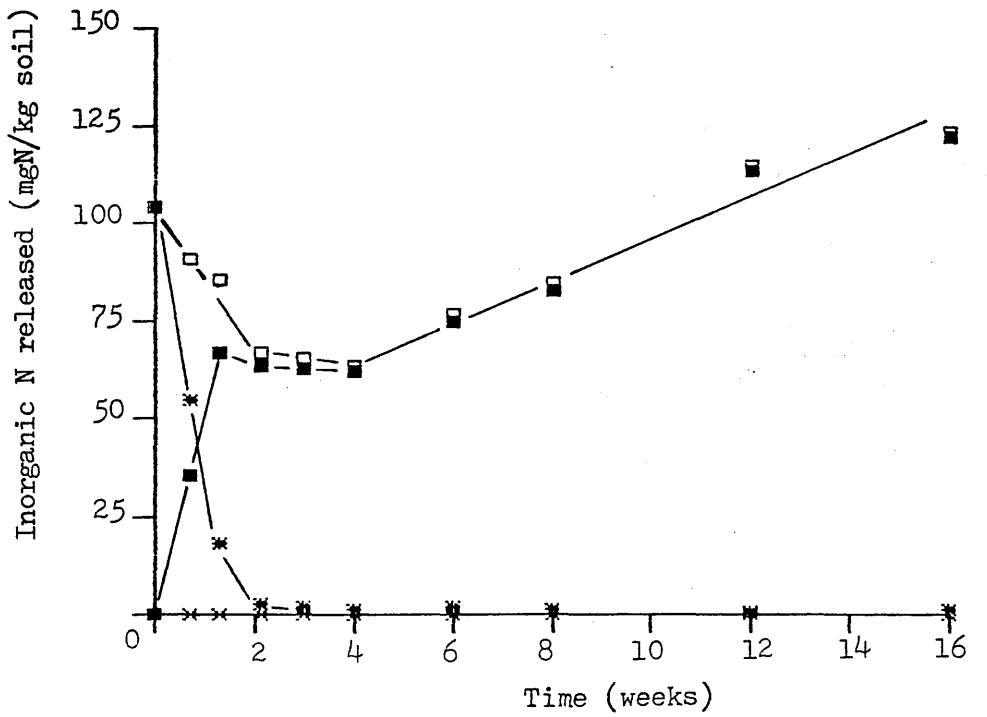


Fig. 4.8 N mineralized by chicken manure treated soil BD 12.
Total inorganic N (□), NH_4-N (*), NO_3-N (■),
NO_2-N (×)

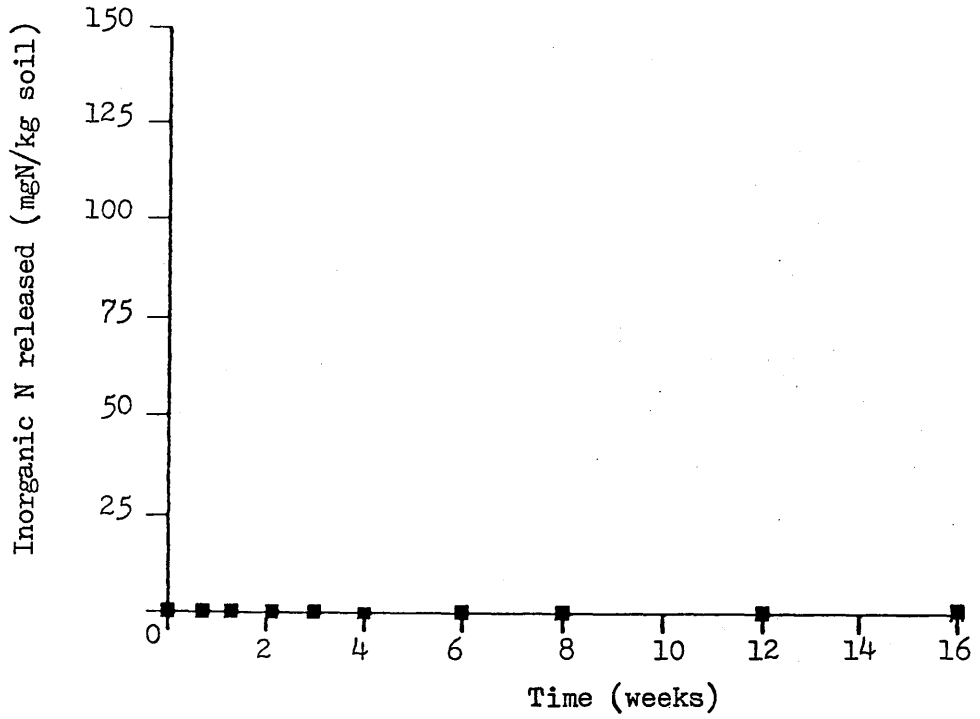


Fig. 4.9 N mineralized by untreated soil BD 13.
Total inorganic N (□), NH_4-N (*), NO_3-N (■),
NO_2-N (×)

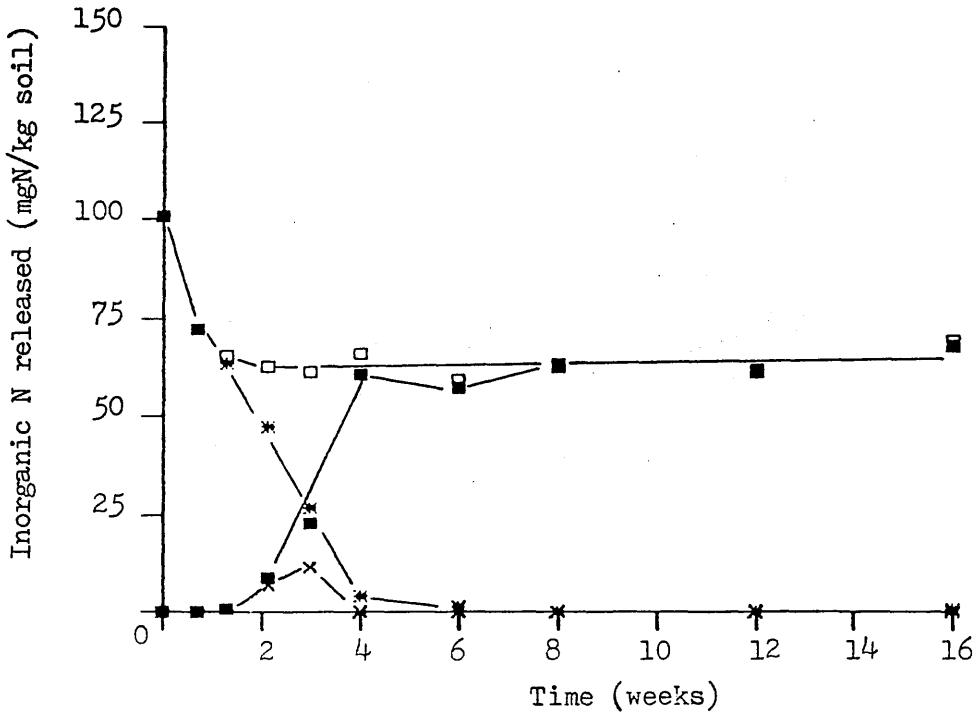


Fig. 4.10 N mineralized by ammonium sulphate treated soil BD 13.
Total inorganic N (□), NH_4-N (*), NO_3-N (■),
NO_2-N (×)

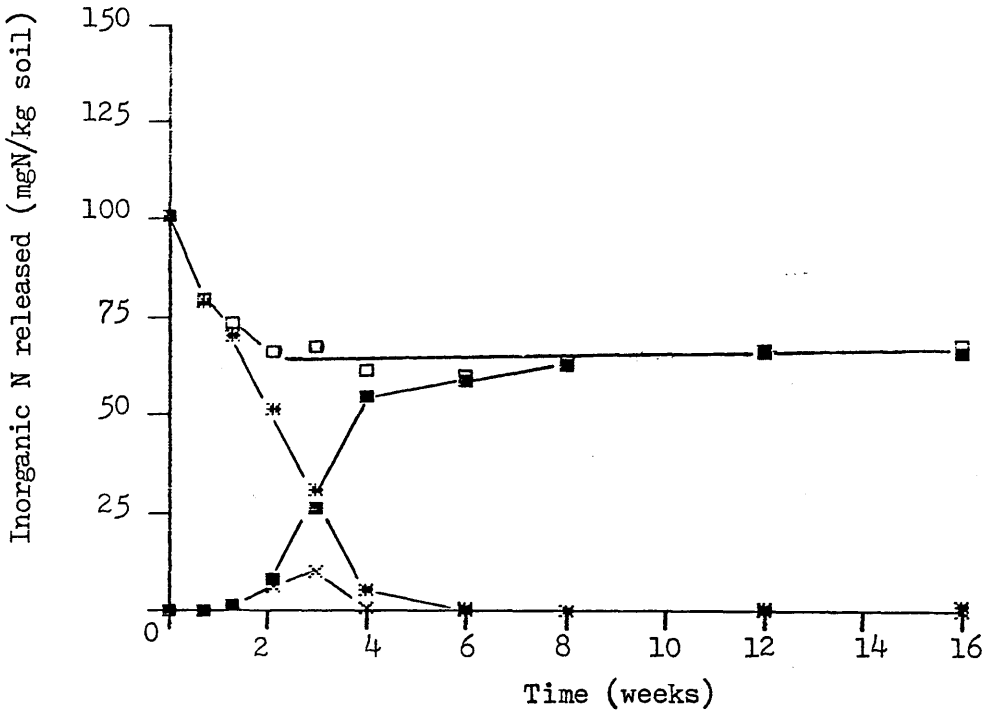


Fig. 4.11 N mineralized by urea treated soil BD 13.
Total inorganic N (□), NH_4-N (*), NO_3-N (■),
NO_2-N (×)

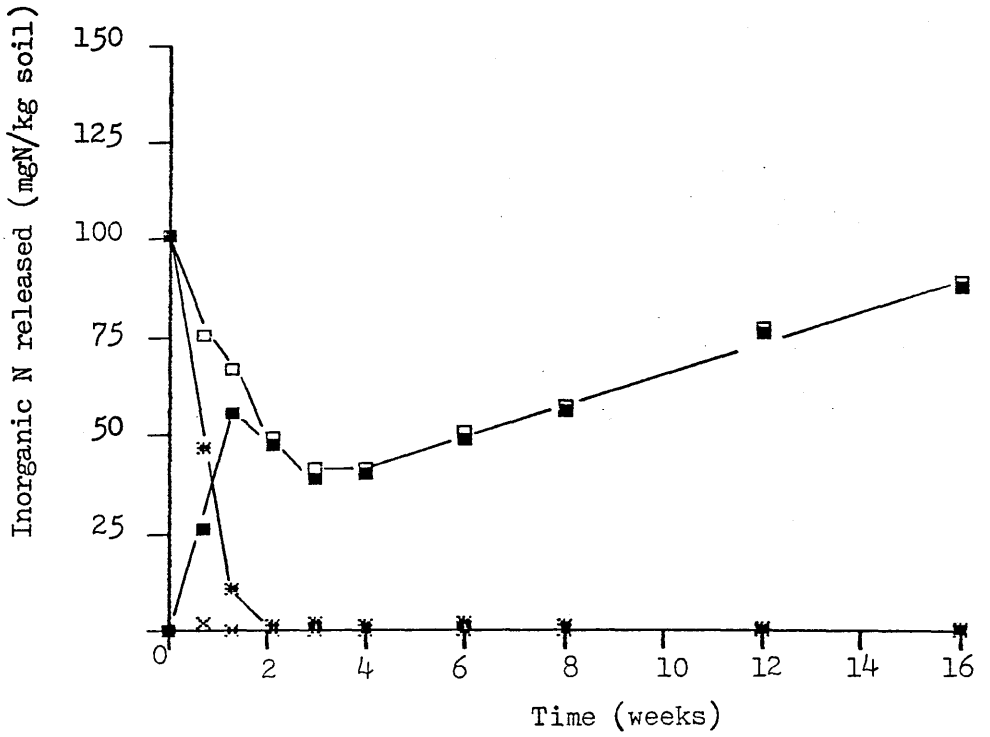


Fig. 4.12 N mineralized by chicken manure treated soil BD 13.
Total inorganic N (□), NH_4-N (*), NO_3-N (■),
NO_2-N (×)

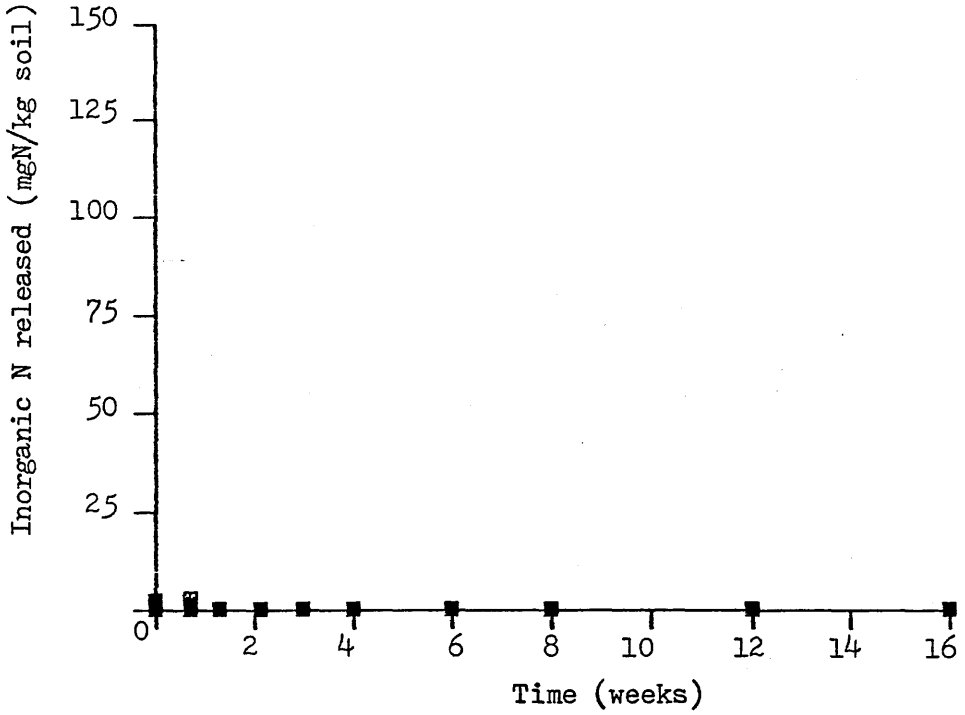


Fig. 4.13 N mineralized by untreated soil BD 14.
Total inorganic N (□), NH_4-N (*), NO_3-N (■), NO_2-N (×)

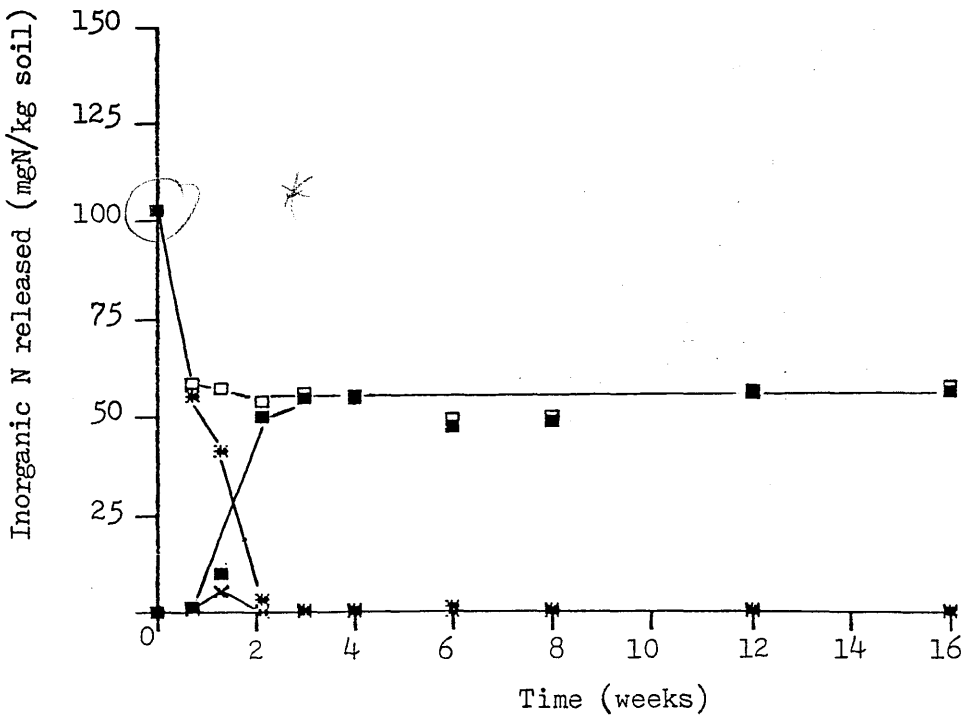


Fig. 4.14 N mineralized by ammonium sulphate treated soil BD 14.
Total inorganic N (□), NH_4-N (*), NO_3-N (■), NO_2-N (×)

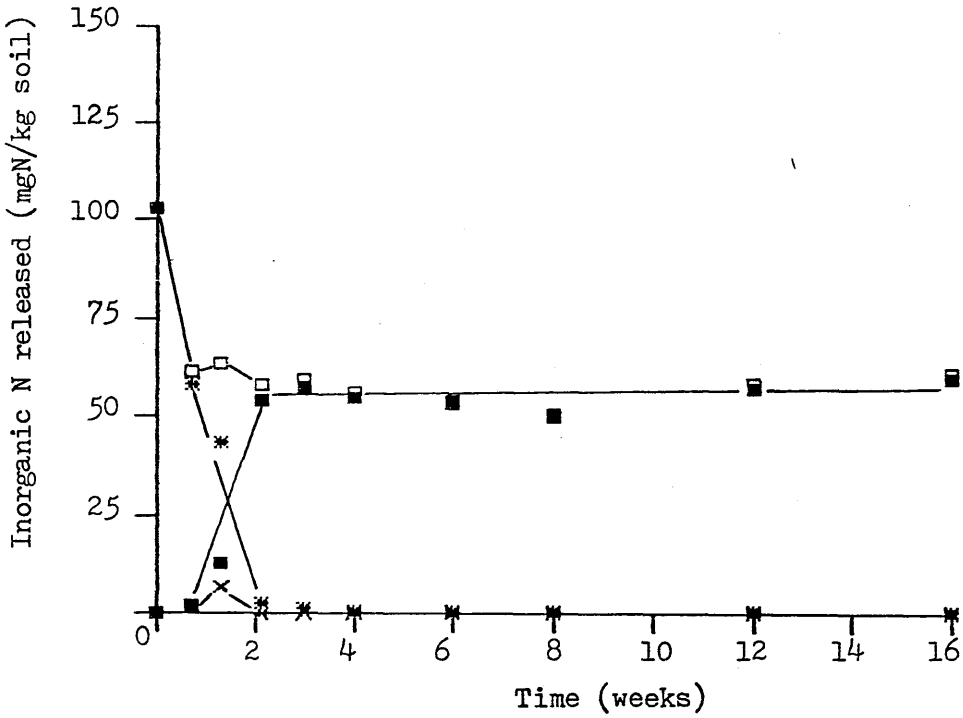


Fig. 4.15 N mineralized by urea treated soil BD 14.
Total inorganic N (□), NH_4-N (*), NO_3-N (■), NO_2-N (×)

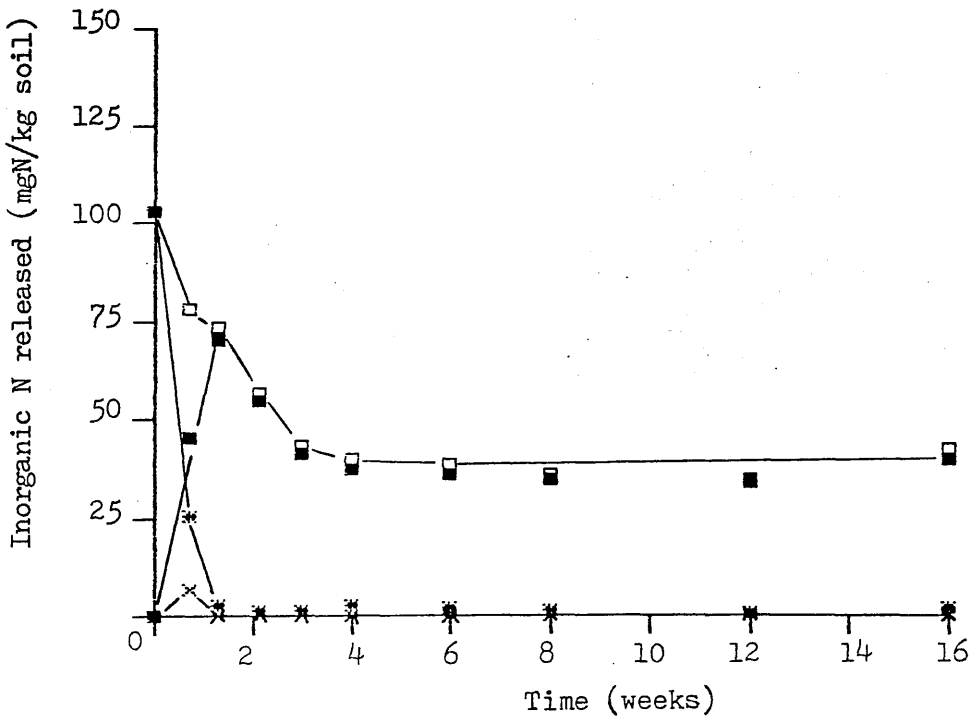


Fig. 4.16 N mineralized by chicken manure treated soil BD 14.
Total inorganic N (□), NH_4-N (*), NO_3-N (■), NO_2-N (×)

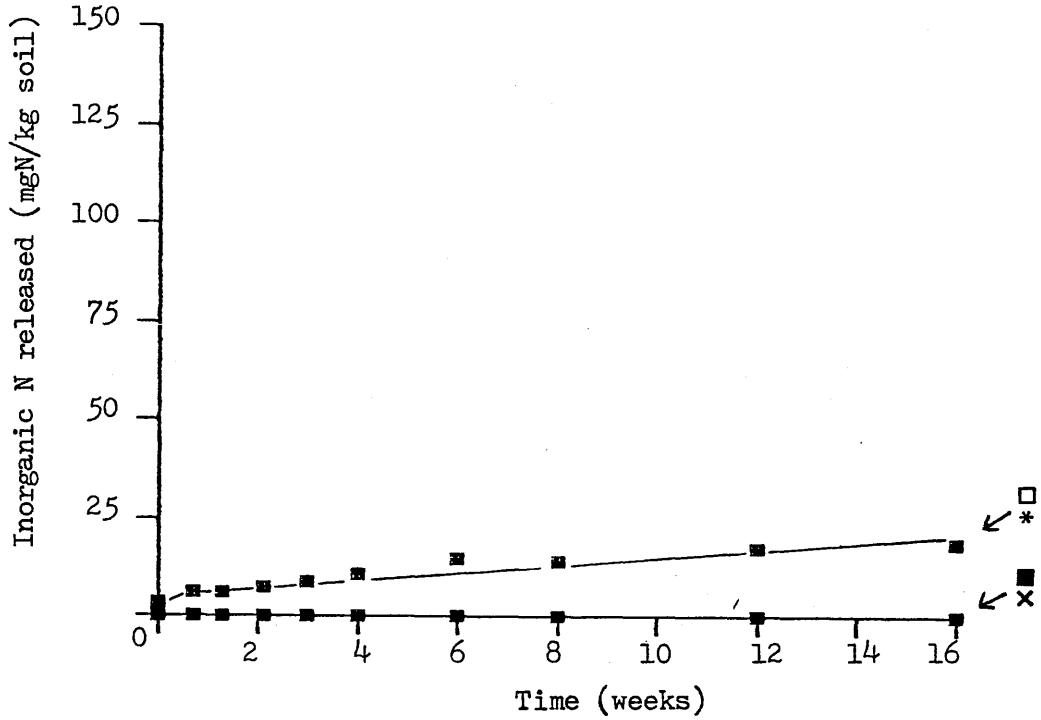


Fig. 4.17 N mineralized by untreated soil BD 15.
Total inorganic N (□), NH_4-N (*), NO_3-N (■), NO_2-N (×)

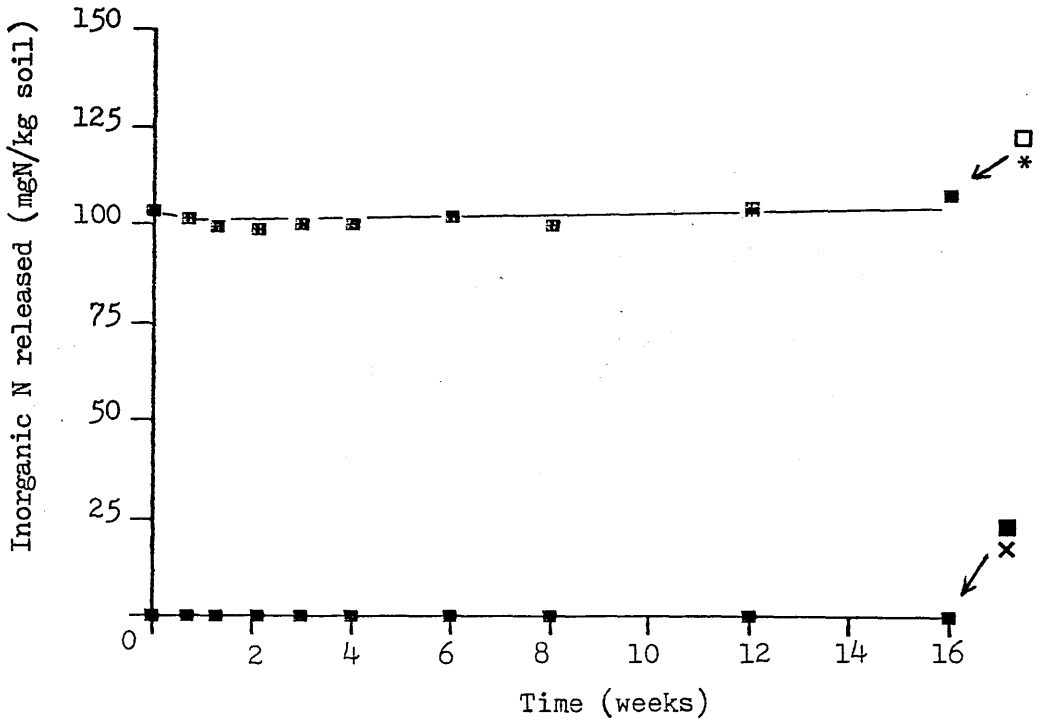


Fig. 4.18 N mineralized by ammonium sulphate treated soil BD 15.
Total inorganic N (□), NH_4-N (*), NO_3-N (■), NO_2-N (×)

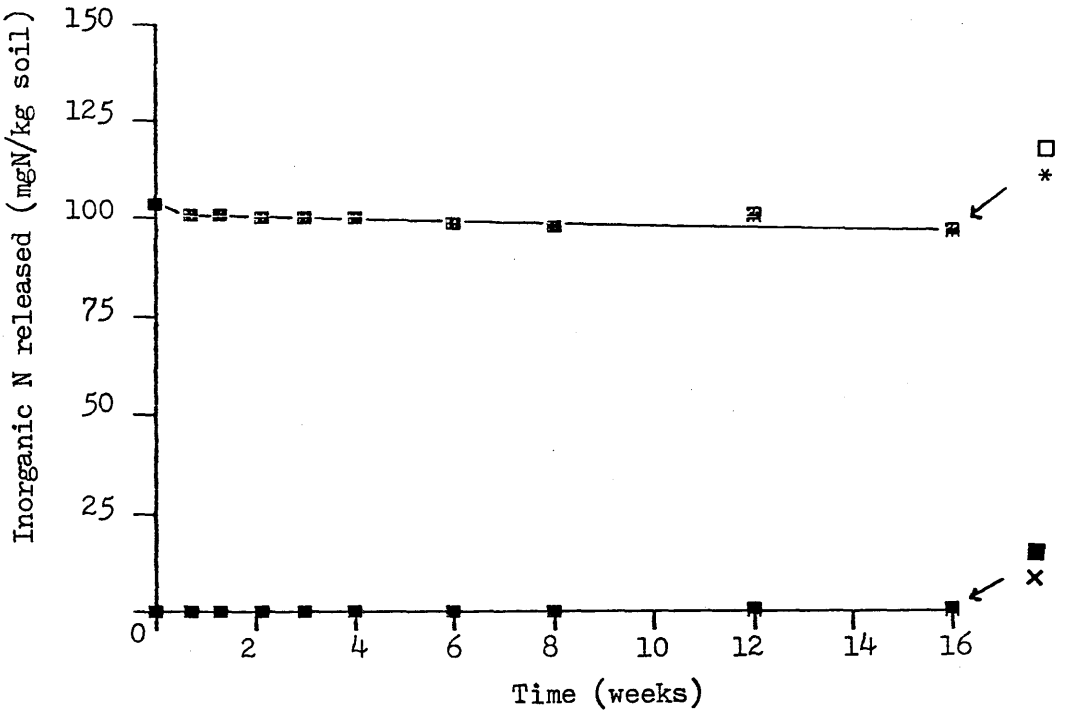


Fig. 4.19 N mineralized by urea treated soil BD 15.
Total inorganic N (□), NH_4-N (*), NO_3-N (■),
NO_2-N (×)

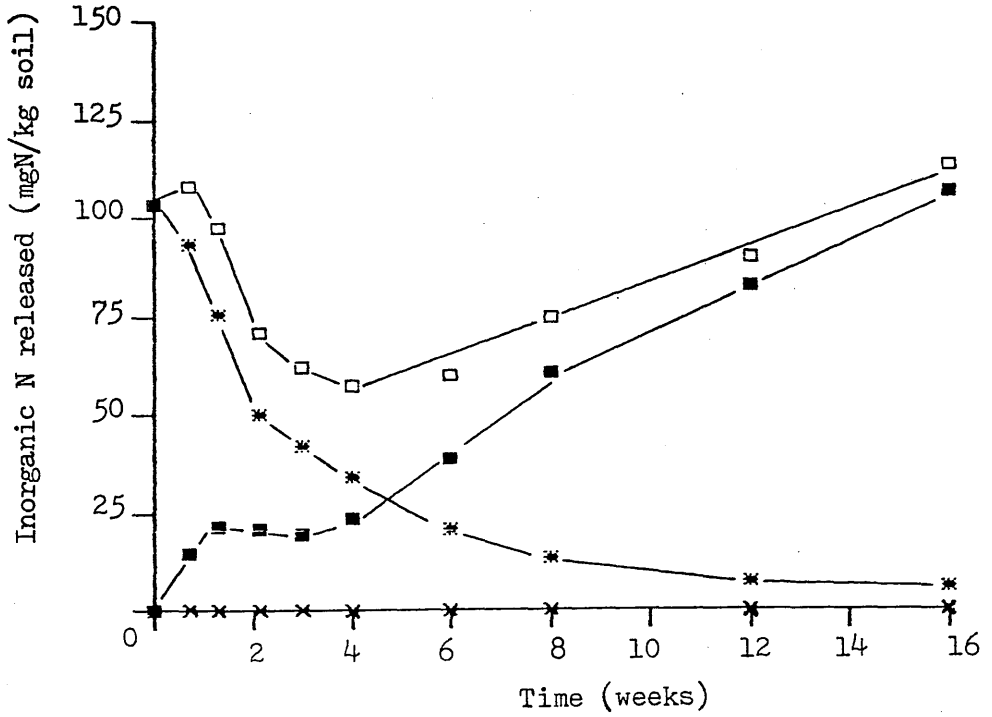


Fig. 4.20 N mineralized by chicken manure treated soil BD 15.
Total inorganic N (□), NH_4-N (*), NO_3-N (■),
NO_2-N (×)

4.3.3 CHANGES IN pH OF COAL MINE SOILS DURING INCUBATION

The changes in the pH of coal mine soils during incubation shown in Table 4.2 indicate that the addition of ammonium sulphate or urea (100 mg N/kg soil) did not affect the pH of coal mine soils to a great extent. The only obvious change in pH due to ammonium sulphate addition was an increase in the initial pH from 7.0 to 7.4 after 2 days of incubation in coal mine soil BD 13. Similarly the initial increase in pH due to urea addition after the first day of incubation was generally not more than 0.1 to 0.4 of a pH unit in the first four coal mine soils. No effect of urea addition on the pH of acid spoil BD 15 was noticed.

Chicken manure incorporation elevated the pH of some coal mine soils, especially of the acid soil BD 15. The pH value of the soil, immediately after manure incorporation was 5.7 (initial soil pH = 3.8). The increase in pH due to manure addition was 0.5 pH unit in the case of BD 11.

Nitrification of 100 mg N/kg soil added as ammonium sulphate, urea or chicken manure resulted in a slight fall of 0.2 to 0.3 pH units in the case of coal mine soil BD 11. There was no measurable change in the pH of soil BD 12 and BD 14, while BD 13 showed a slight upward trend in pH during 112 days of incubation in all treatments. Unlike a small decline in pH from 3.8 to 3.5 in the case of the control, ammonium sulphate and urea treatments of acid soil BD 15, there was a decrease from pH 5.7 to 4.9 in the chicken manure treatment of this soil during 112 days of incubation due to nitrification.

Soil	Treatment	Soil pH							
		Incubation time (days)							
		0	1	2	7	15	28	56	112
BD 11	Control	6.2	6.3	6.3	6.3	6.4	6.4	6.3	6.3
	$(NH_4)_2SO_4$	6.3	6.3	6.3	6.3	6.3	6.2	6.1	6.1
	Urea	6.5	6.6	6.6	6.4	6.4	6.3	6.1	6.2
	Manure	6.7	6.7	6.7	6.5	6.5	6.6	6.4	6.4
BD 12	Control	7.3	7.4	7.4	7.3	7.4	7.4	7.4	7.5
	$(NH_4)_2SO_4$	7.4	7.4	7.4	7.3	7.4	7.4	7.5	7.4
	Urea	7.5	7.5	7.5	7.4	7.5	7.5	7.5	7.4
	Manure	7.5	7.6	7.5	7.4	7.4	7.5	7.5	7.6
BD 13	Control	7.0	7.0	7.2	7.2	7.2	7.4	7.4	7.5
	$(NH_4)_2SO_4$	7.1	7.1	7.4	7.3	7.3	7.3	7.3	7.5
	Urea	7.1	7.3	7.4	7.4	7.4	7.3	7.4	7.5
	Manure	7.3	7.4	7.4	7.2	7.2	7.4	7.4	7.4
BD 14	Control	7.4	7.4	7.4	7.3	7.4	7.5	7.5	7.5
	$(NH_4)_2SO_4$	7.4	7.4	7.4	7.4	7.4	7.5	7.4	7.4
	Urea	7.5	7.5	7.5	7.4	7.4	7.5	7.5	7.5
	Manure	7.5	7.5	7.5	7.4	7.5	7.5	7.5	7.5
BD 15	Control	3.8	3.7	3.7	3.6	3.7	3.6	3.6	3.5
	$(NH_4)_2SO_4$	3.7	3.6	3.6	3.6	3.7	3.7	3.6	3.5
	Urea	3.8	3.7	3.8	3.7	3.8	3.7	3.6	3.5
	Manure	5.7	5.3	5.2	5.0	4.9	4.9	4.8	4.9

Table 4.2. Changes in coal mine soils pH during incubation experiment.

4.4 DISCUSSION

Several factors may affect mineralization of organic nitrogen in soils during incubation studies. These include temperature, moisture, rate of oxygen replenishment, pH, amount and nature of plant residues and level of other nutrients (Stanford and Smith, 1972). Nitrogen mineralization is also dependent upon the fresh or air-dried condition of the soil (Khan, 1987).

Fresh samples of coal mine soils were used during the present incubation study. Care was taken to ensure that the soil received the minimum amount of air drying and disturbance in order to minimize any reduction in the microbial population of soil, especially nitrifying bacteria.

An optimum moisture content (-0.5 bar soil moisture potential) of coal mine soils was maintained, and the moisture lost through evaporation was replaced at appropriate intervals during incubation. Moreover, the incubation temperature of 25 ^{o}C was within the generally accepted range of 25-35 ^{o}C for optimum nitrogen mineralization. The supply of oxygen was not a limiting factor. It was replenished at each interval of incubation. In the later stages of incubation when these intervals were longer the air was replenished weekly.

4.4.1 LOSS IN TOTAL INORGANIC NITROGEN DURING INCUBATION

There was an initial decline in total inorganic nitrogen in the ammonium sulphate, urea or chicken manure treatments of all soils. The length of this phase was different in the 5 coal mine soils and even in the different treatments of the same soil. However, it was generally greater in the chicken manure treatments. The loss in total inorganic nitrogen was estimated at the end of this phase, by subtracting the minimum total inorganic nitrogen from that initially present in the coal mine soil samples plus that added (100 mg N/kg soil) as ammonium sulphate, urea or chicken manure. This loss of total inorganic nitrogen during incubation also includes the loss of ammonium-N due to the fixation by the clay minerals of the shale (Table 4.1). The losses in total inorganic nitrogen are shown in Table 4.3.

Treatment	Coal mine soils				
	BD 11	BD 12	BD 13	BD 14	BD 15
Ammonium sulphate	35.4a	32.2a	41.7a	54.0a	5.9a
Urea	32.3a	30.5a	36.7a	52.5a	6.8a
Chicken manure	53.4b	40.2b	59.3b	68.5b	46.6b

Table 4.3. Loss in total inorganic nitrogen (mg N/kg soil) of coal mine soils during incubation.

Mean values in the same column not followed by a similar letter are significantly different at 1%, using Fisher's LSD test.

The data in Table 4.3 indicate that there was no significant difference in the losses of nitrogen applied as ammonium sulphate or urea. The losses were significantly higher in the manure treatments of all coal mine soils. High losses of inorganic nitrogen were shown by soil BD 14 where about 53 to 69% of the applied nitrogen was immobilized.

Losses of inorganic nitrogen in the ammonium sulphate or urea treatments of the acid coal mine soil (BD 15) were very low compared to other soils. However, a considerable loss of nitrogen added as chicken manure was shown by this soil, suggesting that this immobilization may be due to the decomposition of a readily-decomposable fraction of the manure organic matter. The chicken manure used in this study was a combination of excreta and litter (wood shavings). The carbonaceous excreta material in the manure may have caused immobilization of available nitrogen. An organic material of this composition would be expected to have a different pattern of nitrogen transformation in coal mine soils compared to fertilizer nitrogen sources.

The lag phase after the initial flush of nitrification in the manure treatments of coal mine soils, and the sudden fall in nitrate-N along with decrease in ammonium-N (Figs. 4.12 and 4.16) suggest the immobilization of both ammonium and nitrate. Winsor and Pollard (1956) who noticed nitrate assimilation during incubation study of agricultural soils, found that soil organisms concerned in the immobilization of nitrogen usually prefer ammonium-N, but at higher C:N ratios these

organisms also assimilate nitrate-N. These coal mine soils have generally high ratios of C:N mineralized as reported in chapter 3 (Table 3.2). Flowers and Arnold (1983) also observed immobilization of nitrate along with ammonium in one soil (Dunkeswick series) during their incubation study of ammonium sulphate and pig slurry amended agricultural soils.

Loss of nitrogen due to immobilization can cause a decrease in net available nitrogen for some time. However, this is different from those losses via leaching, ammonia volatilization or denitrification. Because immobilization only fixes the available nitrogen temporarily into organic bound nitrogen, the total nitrogen reserves are not reduced. Moreover, the immobilized nitrogen may be released again by the decay of microbial cells (Kanamori and Yasuda, 1979; Anderson and Domsch, 1980). Possible loss of nitrogen by denitrification was not monitored. However, the soil moisture level (Table 4.1) and the proper aeration during incubation should not favour denitrification. Keeping in view the clay fixation of added ammonium figures of these coal mine soils (Table 4.1) these losses of inorganic nitrogen during incubation, therefore, could be attributed to a combination of ammonium fixation and immobilization.

Nitrogen added as urea was immobilized to virtually the same extent as N added as ammonium sulphate. This was presumably because urea hydrolysis was complete in all soils before the first sampling at 5 days, in view of the measured urease activities of the soils (Table 4.1).

4.4.2 MINERALIZATION RATE CONSTANTS

In the treatments of coal mine soils which showed some evidence of nitrogen mineralization, the cumulative amount of nitrogen mineralized was linear with time of incubation. Best-fit lines were fitted to the linear part of the curves of total inorganic nitrogen plotted against time, and mineralization rate constants for nitrogen release were calculated.

Treatment	Coal mine soils				
	BD 11	BD 12	BD 13	BD 14	BD 15
Control	0.37a	0.39a	0.02a	-0.01a	0.11a
Ammonium sulphate	0.57b	0.33a	0.03a	0.04ab	0.01a
Urea	0.61b	0.33a	0.00a	0.08b	-0.02a
Chicken manure	1.26c	0.66b	0.50b	0.08b	0.72b

Table 4.4. Mineralization rate constants (mg N/kg soil/day) of coal mine soils.
Mean values in the same column not followed by a similar letter are significantly different at 1% level, using Fisher's LSD test.

The zero order rate constants for nitrogen released by different coal mine soils are shown in Table 4.4. These rates in the control, ammonium sulphate or urea treatments of soil samples BD 13, BD 14 and BD 15 were very low compared to the same treatments of soils BD 11 and BD 12. The added nitrogen in the form of ammonium sulphate or

urea stimulated mineralization of organic nitrogen in coal mine soil BD 11, but had no effect on the soil BD 12, BD 13 and BD 15. Although urea and manure treatments of soil BD 14 enhanced the rate of mineralization over control treatment, these rates in all treatments of the soil were negligible.

Conflicting results have been reported in the literature on the effects of ammonium sulphate or urea on soil organic nitrogen mineralization. For example, Williams (1975) incubated fresh samples of coal mine soils at 27 ^{o}C, and reported that the addition of ammonium sulphate (50 mg N/kg soil) decreased the quantity of mineralizable nitrogen. He attributed this decrease to the increase in acidity that resulted from the oxidation of ammonium sulphate.

Wickramasinghe et al. (1985) observed during an incubation study at 25 ^{o}C that nitrogen added as ammonium sulphate, urea or potassium nitrate (100 mg N/kg soil) slightly stimulated mineralization of indigenous organic nitrogen in an acid tropical tea soil and in a neutral temperate grassland soil.

The mineralization rate constants in the chicken manure treatments of all coal mine soils were significantly greater than other treatments except in soil BD 14. The increased rates of nitrogen mineralization in manure treatments over the control, ammonium sulphate or urea treatments can be attributed to mineralization of the chicken manure organic nitrogen.

The higher rates of mineralization in the control

treatments of coal mine soil BD 11 and BD 12 at pH 6.2 and 7.2 respectively, can be attributed to greater microbial activity than in the soil BD 15 at pH 3.8.

The lack of measurable mineralization in soils BD 13 and BD 14 indicate that either the heterotrophic micro-organisms assimilated the available nitrogen produced through ammonification or the organic nitrogen in such materials is relatively inert to biological decomposition (Power et al., 1974).

It should be emphasized that direct comparison of nitrogen transformation rates are questionable unless the same methods of incubation and rate calculations are used. Therefore, the findings during the present study cannot be compared to that reported by Reeder and Berg (1977b) and Fyles and McGill (1987) due to the different methods they used. However, the present findings can be compared with those reported by Pulford et al. (1988), who studied nitrogen mineralization in fresh coal mine soils collected from an organic manure trial at Baads during an incubation experiment at 22 ^{o}C and moisture content at - 0.5 bar soil moisture potential. They reported mineralization rates ranging from 0.68 mg N/kg soil/week in soil from the control treatment to 3.80 mg N/kg soil/week in the soil from the chicken manure treatment.

Information regarding the transformation of different sources of nitrogen in coal mine soils is lacking. For comparison the nitrogen mineralizaton rates in agricultural soils reported by various researchers are presented. Flowers and Arnold (1983) studied

immobilization and mineralization of nitrogen in fresh soils incubated at 30 ^{o}C with 100 mg ammonium-N as ammonium sulphate or pig slurry. The mineralization rate constants (mg N/kg soil/day) for two different soils reported were, 0.71 and 1.04 for untreated soil; 0.48 and 0.93 for ammonium sulphate treated soil and 0.69 and 1.16 for pig slurry treated soil. They further stated that in one soil the nitrogen mineralization rate of slurry treatment was significantly greater than in the untreated control.

Tabatabai and Alkhafaji (1980) used 12 different soils in the fresh condition during incubation study at 20 ^{o}C and reported nitrogen mineralization rates from 1.7 to 4.2 mg N /kg soil/week (0.24 to 0.60 mg N/kg soil /day). Addiscott (1983) studied mineralization of soil organic nitrogen in a laboratory incubation at 25 ^{o}C and reported mineralization rate constants for Rothamsted soils ranging from 0.206 to 0.401 mg N/kg/day. Similarly, Khan (1987) who incubated 9 soils at 10 ^{o}C, reported nitrogen mineralization rates from 0.87 to 1.93 mg N/kg soil/week (0.12-0.28 mg N/kg soil/day).

It is evident that the coal mine soils which were obtained from the experimental plots of an established organic manure trial showed comparable mineralization rate constants. But the other three samples seemed to be very low compared to values reported for normal agricultural soils. These results reflect the favourable effect of the organic manures received by these soil.

4.4.3 NITRIFICATION RATE CONSTANTS

Nitrification of added nitrogen occurred in incubated coal mine soils concurrently with mineralization and immobilization and exhibited different patterns, especially in chicken manure treatments. In most of the treated soils it commenced after an initial lag period and was then linear with time of incubation. Regression lines were fitted to the linear part of the nitrate nitrogen graph, where the increase in nitrate concentration and decrease in ammonium occurred concurrently. Care was taken to avoid the initial part of the graph where immobilization was a problem and last part where the substrate ammonium was a limiting factor. The zero order rate constants calculated are presented in Table 4.5.

Treatment	Coal mine soils				
	BD 11	BD 12	BD 13	BD 14	BD 15
Control	NA	NA	NA	NA	NA
Ammonium sulphate	1.81a	5.91a	3.97a	5.02a	0.00a
Urea	2.04a	6.28a	4.01a	5.38b	0.00a
Chicken manure	1.83a	7.26b	6.22b	7.85c	1.13b

Table 4.5. Nitrification rate constants (mg N/kg soil/day) of coal mine soils.

NA = not applicable.

Mean values in the same column not followed by a similar letter are significantly different at 1% level, using Fisher's LSD test.

The data in Table 4.5 indicate that the rates of nitrification in all coal mine soils except BD 14 were similar whether the addition was made as ammonium sulphate or as urea. However, addition of chicken manure with the same amount of nitrogen resulted in a significantly greater nitrification rate than that in ammonium sulphate or urea treatments, except soil BD 11, where the rate of nitrification was similar in all treatments.

Tam (1987) conducted a laboratory incubation study at 26 ^{o}C and 15 % moisture content of three different coal mine soils treated with 100 mg N/kg soil as sewage sludge, animal manures (chicken and turkey manures) and ammonium sulphate. He reported an increased rate of nitrification in the animal manure treatments compared to the ammonium sulphate treatment and found a significant increase in the pH of acidic coal mine soils with the addition of animal manures. The addition of markedly alkaline organic manure can increase the pH of acid soil. In this study the chicken manure had an alkaline pH of 8.8 which elevated the initial pH of the acid coal mine soil. During incubation the pH values declined gradually presumably due to the nitrification process (Cooper, 1975).

The lack of nitrification of added ammonium sulphate or urea nitrogen in the coal mine soil BD 15 may be due to its low pH (3.8), because of the sensitivity of nitrifying bacteria to pH. At this pH the toxic effect of aluminium ions on nitrification can also be expected (Russell, 1973). Nitrification of added ammonium-N in the form of

chicken manure could be attributed to the increase in pH of the coal mine soil due to manure addition (Table 4.2), which favoured the activity of nitrifying bacteria in this strongly acid soil. Moreover chicken manure could also be considered as a source of nitrifying bacteria.

Cooper (1975) studied nitrification of added ammonium-N ranging between 0 and 1,038 mg N/kg soil as ammonium sulphate or pig slurry in an acid and a neutral agricultural soil during an incubation study at 30 ^{o}C. He reported different nitrification patterns in soils receiving equivalent quantities of ammonium-N as ammonium sulphate or pig slurry and found that slurry ammonium-N was nitrified to a greater extent than ammonium sulphate in the acid soil. He attributed this difference in nitrification to the rise in soil pH caused by slurry addition.

Flowers and O'Callaghan (1983) who incubated two soils at 15 ^{o}C and 45% water holding capacity, reported nitrification rates for soils treated with 100 mg N/kg soil as ammonium sulphate, ranging from 2.5 to 5.6 mg N/kg soil/day. They also compared the nitrification rates in soils treated with 50 and 250 mg ammonium-N/kg soil as ammonium sulphate or pig slurry, and found that slurry addition caused a rise in the pH of the soil but ammonium sulphate did not affect the initial soil pH. Addiscott (1983) incubated fresh soils treated with 80 mg N/kg soil as ammonium chloride at 15 ^{o}C, and reported nitrification rates from 5.32 to 12.73 mg N/kg soil/day.

The nitrification rates of added nitrogen seemed to be reasonable in the coal mine soils having a suitable pH for this process. The coal mine soil BD 15 with pH 3.8 did not show any nitrification of added chemical fertilizer. However, the significant effect on nitrogen mineralization and nitrification of the chicken manure addition to this soil emphasizes the value of chicken manure addition to coal mine soils.

4.5 CONCLUSIONS

In conclusion, the salient points to emerge from this incubation study are:

1. There were large losses of nitrogen in the first weeks of incubation, which were significantly higher in the manure treatments than fertilizer treatments of all soils, with up to 69% of added nitrogen lost. However, in the later stages of incubation there was a clear contribution to mineralization from chicken manure.

2. Only, two samples BD 11 and BD 12, out of five coal mine soils (control, ammonium sulphate and urea treatments) showed reasonable nitrogen mineralization. These samples were collected from the plots of an established organic manure trial (1980). This indicates the favourable effect of organic amendments in the long run on the establishment of nitrogen cycling.

3. Two coal mine soil samples with neutral pHs (BD 13 and BD 14), although they nitrified the nitrogen added as ammonium sulphate or urea, did not mineralize nitrogen during incubation.

4. Chicken manure addition significantly improved the mineralization and nitrification rates in all coal mine soil samples, especially of the acid soil where these processes did not occur with ammonium sulphate or urea addition.

5. Addition of ammonium sulphate or urea had no effect on the initial soil pH, whereas chicken manure addition with the same level of ammonium-N increased the pH of the acid

soil from 3.8 to 5.7 with a favourable effect on the nitrifying bacteria.

6. Urea and ammonium sulphate behaved similarly, whether the coal mine soil was acid or neutral. The measured activities of urease in these coal mine soils suggest that the hydrolysis of applied urea was complete before the first sampling at day 5 of incubation.

CHAPTER 5 RESPONSE OF VEGETATION TO NITROGEN FERTILIZER IN RECLAIMED COAL MINE SOILS

5.1 INTRODUCTION

The establishment and maintenance of a vegetation cover on reclaimed derelict land involves the regeneration of a functioning soil ecosystem containing adequate amounts of major nutrients. Reclaimed coal mine soils are generally deficient in nitrogen and phosphorus due to lack of humified organic matter, but reasonably sufficient in potassium (Fitter and Bradshaw, 1974; Pulford and Duncan, 1978b; Bloomfield et al., 1982; Pulford et al., 1988). Amongst the macronutrients, nitrogen deficiency is a major factor limiting the growth of plants on such soils. They supply little nitrogen for plant growth even though some shales may contain appreciable quantities of fossil nitrogen (Power et al., 1974).

The low levels of extractable mineral nitrogen and high ratio of C:N mineralized in the reclaimed coal mine soils of Central Scotland, reported in chapter 3, suggest a deficiency of plant available nitrogen. Such results indicate the need to ensure an adequate supply of nitrogen to the growing plants on such sites. While the importance of the supply of nitrogen to vegetation on derelict land is generally accepted (Davison and Jefferies, 1966; Bloomfield et al., 1982; Bradshaw, 1983), little is known

about the response relationships between vegetation and different rates of nitrogen application.

A search of the literature shows that a great deal of work has been done on the response of grass plus clover swards to nitrogen application on grasslands (Reid, 1970; Laidlaw, 1980; Wilman and Hollington, 1985; Rangeley and Bolton, 1986; Frame, 1987; Frame and Boyd, 1987). Such information could hardly be fully applied on coal mine soils due to differences in the management practices and nature of the materials. It has also been found that fertilizer nitrogen added to spoils may be less available to plants than the same amount of N added to agricultural soils (Reeder and Berg, 1977a).

Davison and Jefferies (1966) conducted laboratory and field studies on plant nutrition using freshly exposed and weathered, burnt and unburnt spoil with values ranging between pH 3.2 and 7.8. They reported that these spoils were severely deficient in nitrogen and phosphorus, and found a massive increase in the growth and productivity of vegetation on such soils when these nutrients were added. Bloomfield et al. (1982) carried out N, P and K fertilizer trials on reclaimed areas of colliery spoils, and observed a dominating effect of nitrogen fertilizer on the dry matter yield of herbage on all sites and a small effect of phosphorus application on some sites.

Pulford et al. (1988) applied various combinations of N, P and K fertilizer treatments to vegetation on reclaimed coal mine soils at Baads. They found a positive response to nitrogen fertilizer but no response to added

phosphorus or potassium fertilizers. More knowledge of rates of addition would be valuable since they compared only two rates of nitrogen fertilizer (50 and 100 kg N/ha) with no nitrogen applied. In order to have a more detailed look at the response of vegetation to nitrogen on colliery spoil at Baads, a field experiment was designed with varying levels of fertilizer nitrogen during 1986 and 1987.

The objective of this study was to determine the response of vegetation to application of nitrogen fertilizer as measured by dry matter yield and chemical analysis for major nutrients (N, P and K) of vegetation. In addition, attempts were also made to compare the effect of nitrogen in both the presence and absence of added phosphorus and potassium, and as ammonium nitrate or urea, by including some extra treatments in each year.

5.2 MATERIALS AND METHODS

5.2.1 EXPERIMENTAL SITE

An area with a uniform and well established vegetation, adjacent to the established manure trial, was chosen on a reclaimed area of Baads colliery bing (see section 3.2.1). This area had received 50 t/ha of limestone and 300 kg/ha of a compound NPK (15:10:10) fertilizer before being seeded with a grass-white clover mixture in June 1980, and no subsequent fertilizer. Care was taken to keep away from the steeper slopes, sites of old manure heaps and acid patches.

In order to have the experimental plots on an area of relatively uniform pH, 12 grid samples of surface (0-15 cm) soils were taken from the site and tested for pH. The acidic parts of the area, having pH below 6.0 were avoided, and 27 plots of 2m x 2m size were marked out in the vicinity of those samples having pH greater than 6.0. By this means, it was hoped that the pH's of the plots would be uniformly above 6.0. In practice this turned out not to be the case (see section 5.2.3 and Table 5.2).

5.2.2 TREATMENTS AND DESIGN

The various treatments of N, P and K fertilizers applied in the years 1986 and 1987 are given in Table 5.1. In 1986, nitrogen was supplied as ammonium nitrate in varying amounts equivalent to 0, 25, 50, 75, 100, 125, or 150 kg N/ha to 7 plots. Each plot also received a basal dose of 60 kg/ha of P_2O_5 (26.2kg P/ha) and 80 kg/ha of

K_2O (66.4 kg K/ha), so that these elements would not limit the herbage performance (MAAF, 1982). Potassium dihydrogen phosphate was applied as the source of P and some K. Potassium chloride was used to supply the K required above that supplied by the phosphate fertilizer. Two extra treatments without added P and K, one blank with 0 kg N/ha and the other with 100 kg N/ha were also included to study the effect of nitrogen on the yield of herbage both in the presence and absence of added P and K. These 9 treatments altogether were replicated 3 times in a randomised block design.

The experiment was repeated in 1987, and the same treatments were applied to the same plots, except that the last two treatments without added P and K (0 and 100 kg N/ha) were changed into 50 and 150 kg N/ha using urea as a source of nitrogen instead of ammonium nitrate. Phosphate and potassium fertilizers were also added to these two urea plots.

TREATMENTS IN 1986			TREATMENTS IN 1987		
N	P	K	N	P	K
	(kg/ha)			(kg/ha)	
Ammonium nitrate-N			Ammonium nitrate-N		
0	26	66	0	26	66
25	26	66	25	26	66
50	26	66	50	26	66
75	26	66	75	26	66
100	26	66	100	26	66
125	26	66	125	26	66
150	26	66	150	26	66
Ammonium nitrate-N			Urea-N		
0	0	0	50	26	66
100	0	0	150	26	66

Table 5.1. Treatments of nitrogen response experiments in 1986 and 1987.

5.2.3 EXPERIMENTAL PROCEDURES

Appropriate weights of fertilizers were mixed for each plot and were spread by hand as a single dressing.

Nine cores (30 cm, spade depth) of coal mine soils were taken at random from the experimental site outwith the 2m x 2m plots. The soil samples were partially air

dried just to permit sieving through a 4mm sieve. pH, extractable mineral nitrogen (ammonium-N, nitrite-N and nitrate-N), and urease and amidase activities were determined in each soil sample.

Despite the attempt made to have the experimental plots in an area having pH above 6.0 as mentioned in section 5.2.1, this was not achieved due to the heterogeneous nature of the coal mine waste material (See Table 5.2).

The fertilizers were applied on 29 May 1986 and 24 April 1987 and harvesting of vegetation was done on 20 August 1986, and 24 July 1987 respectively.

The herbage was cropped with hand shears leaving about 2-3 cm stubble. Fresh weight of herbage was recorded and a representative sample of 200 to 300 g was taken from each treatment. The samples were dried for 48 hours at 80 ^{o}C, weighed and the dry matter yield was calculated.

The dried herbage was ground using a food grinder. Care was taken to clean the grinder cup before grinding each sample.

Sample	pH	Inorganic-N			Enzyme activity	
		NH_4-N	NO_3-N	Total	Urease	Amidase
		(mg N/kg soil)			(mgN/kg soil/hour)	
A	7.3	0.5	0.1	0.6	14.0	6.8
B	6.0	0.8	0.0	0.8	9.9	5.0
C	5.6	3.3	0.0	3.3	7.7	5.0
D	6.1	5.0	0.0	5.0	7.5	8.0
E	5.8	4.5	0.0	4.5	6.5	4.9
F	4.1	3.2	0.0	3.2	4.8	5.2
G	5.4	2.8	0.1	2.9	10.8	12.4
H	7.1	1.2	0.2	1.4	13.7	7.7
I	5.4	4.1	0.1	4.2	12.1	10.9

Table 5.2. Analysis of 9 core samples from the experimental plot at Baads.

5.2.4 ANALYTICAL METHODS

pH and moisture content in soil core samples were measured using methods in sections 2.1.1 and 2.1.2 respectively. Mineral nitrogen was extracted with 0.5 M potassium sulphate solution by following the method given in section 2.3.4. The extracts were analysed for ammonium-N, nitrite-N and nitrate-N by colorimetric methods using a Technicon AutoAnalyzer II (see section 2.1.5). Urease and amidase activities were determined by the methods described in sections 2.7.4 and 2.7.7

respectively.

Digestion of the ground herbage was carried out with concentrated sulphuric acid following the method described in section 2.8, and the digest was analysed for its nitrogen, phosphorus and potassium contents. Potassium was analysed by flame photometry, ammonium-N and phosphate-P by automated colorimetric methods. Total nitrogen as ammonium-N was measured using the method given in section 2.1.5.1. Phosphate-P was determined as a phospho-vanado-molybdate yellow complex at 420 nm based on a Technicon AutoAnalyzer method (Technicon corporation, 1972)

5.2.5 STATISTICAL ANALYSIS

Data on the dry matter yield and N, P, and K contents of herbage were subjected to analysis of variance. The ratio of the treatment mean square over error mean square was compared to tabulated F values for testing any significant effect of the treatments as a whole and then Fisher's least significant difference (LSD) was used for comparing means (P=0.01) of individual treatments.

In order to calculate the amount of change in dry matter yield and herbage N, P, and K content per kg of fertilizer nitrogen added, a simple linear regression analysis was computed using the following equation.

$$Y = A + B \times N$$

Where Y is a dependent variable e.g. yield which increases with for each unit change in N (independent variable e.g. nitrogen rates). A is the intercept of the line on the Y

axis and B, the linear regression coefficient, is the slope of the line or the amount of change in Y for each unit change in N.

The regression equation was applied to the data of the seven treatments of ammonium nitrate-N with added P and K. The last two treatments with no added P and K during 1986 or with urea-N during 1987 were omitted.

Student´s t values computed by using the regression statistical programme were compared to the tabulated t value at 5%, 1% and 0.1% levels of significance with (n-2) degrees of freedom. A regression line was drawn in those figures where the t value was found to be significant.

5.3 RESULTS AND DISCUSSION

The experiment was performed over two successive years in 1986 and 1987. The dry matter yield and N, P, and K concentration of herbage resulting from different rates of nitrogen fertilizer during the years 1986 and 1987 are presented in Tables 5.3 and 5.4 respectively. The response of herbage to N application is also shown in Figures 5.1 to 5.8. Linear regression lines were fitted only where the response of nitrogen was found to be significant. The equation $Y = A + B \times N$ (as explained in section 5.2.5) was used for calculating the increase in dry matter or N, P, and K content per unit weight of nitrogen fertilizer applied. As the results are mainly concerned with the effects of nitrogen application rates, the regression equation was only applied to the data for the treatments of ammonium nitrate-N with added P and K. The results of the extra treatments during each year are discussed separately.

The data in Tables 5.3 and 5.4 indicate that the dry matter yield of vegetation responded positively to added nitrogen, with the greatest response from the 150 kg N/ha treatment during both years. The yield ranged from 3.6 t/ha with no nitrogen to 5.8 t/ha at 150 kg N/ha in 1986 (Table 5.3). From the data in Table 5.4 it can be seen that the dry matter production varied from an average of 3.4 t/ha with no N to 6.1 t/ha at 150 kg N/ha in the year 1987.

TREATMENT			HERBAGE YIELD	HERBAGE CONTENT		
N	P	K		N	P	K
(kg/ha)			(t/ha)	(%)		
With added P and K						
0	26	66	3.6 AB	1.52 ABC	0.28 BC	1.17 AB
25	26	66	3.7 AB	1.71 BC	0.27 BC	1.46 BC
50	26	66	5.4 C	1.40 AB	0.22 ABC	1.29 ABC
75	26	66	4.9 BC	1.53 ABC	0.26 BC	1.45 BC
100	26	66	5.1 BC	1.43 AB	0.25 ABC	1.26 ABC
125	26	66	5.4 C	1.81 BC	0.28 BC	1.66 C
150	26	66	5.8 C	2.04 C	0.29 C	1.67 C
Without added P and K						
0	0	0	2.8 A	1.08 A	0.21 AB	0.97 A
100	0	0	4.8 BC	1.72 BC	0.18 A	1.12 AB

Table 5.3. Response of dry matter yield and N, P, K content of herbage to nitrogen application on colliery spoil at Baads during 1986.

Mean values followed by same letter within a column are not significantly different at 1% level using Fisher's LSD test.

TREATMENT			HERBAGE YIELD	HERBAGE CONTENT		
N	P	K		N	P	K
(kg/ha)			(t/ha)		(%)	
Ammonium nitrate-N						
0	26	66	3.4 A	1.60 A	0.23 AB	1.59 ABC
25	26	66	3.7 A	1.57 A	0.24 ABC	1.51 AB
50	26	66	4.5 ABC	1.55 A	0.26 CD	1.67 BC
75	26	66	4.5 ABC	1.48 A	0.25 BCD	1.65 BC
100	26	66	5.1 BCD	1.56 A	0.27 D	1.65 BC
125	26	66	5.5 CD	1.49 A	0.27 D	1.77 C
150	26	66	6.1 D	1.67 A	0.27 D	1.73 C
urea-N						
50	26	66	4.1 AB	1.42 A	0.22 A	1.45 A
150	26	66	5.2 BCD	1.44 A	0.23 AB	1.62 ABC

Table 5.4. Response of dry matter yield and N, P, K content of herbage to nitrogen application on colliery spoil at Baads during 1987.

Mean values followed by same letter within a column are not significantly different at 1% level using Fisher's LSD test.

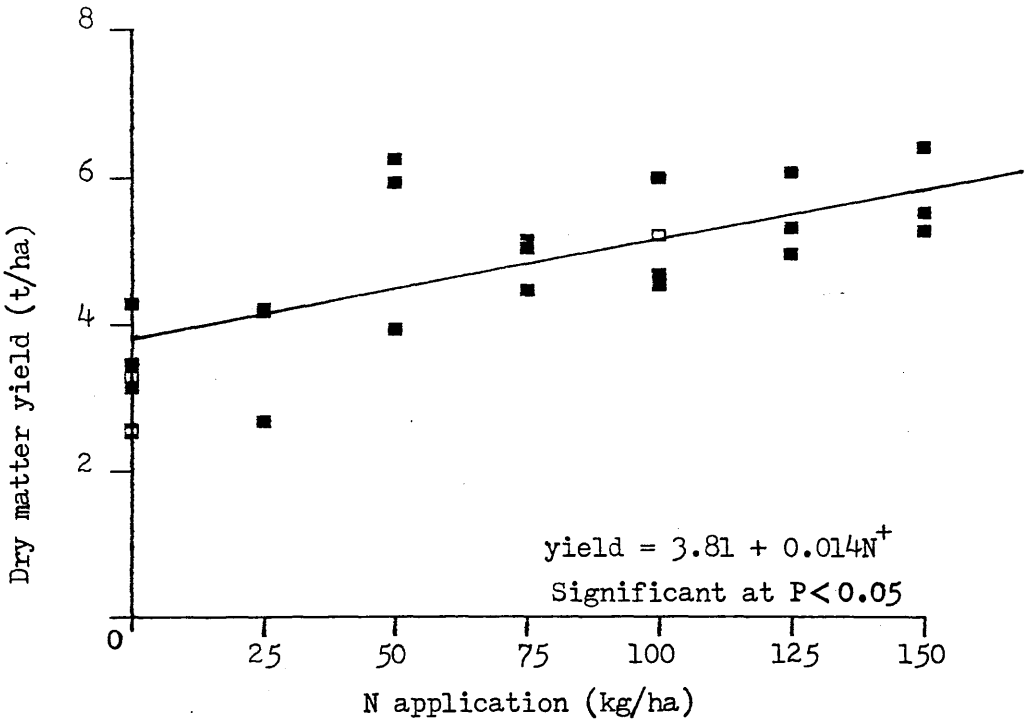


Fig. 5.1 Response of herbage dry matter yield to application of nitrogen (1986).
Ammonium nitrate-N with added P and K (■)
Ammonium nitrate-N without added P and K (□)

† Equation refers to ammonium nitrate-N with added P and K only

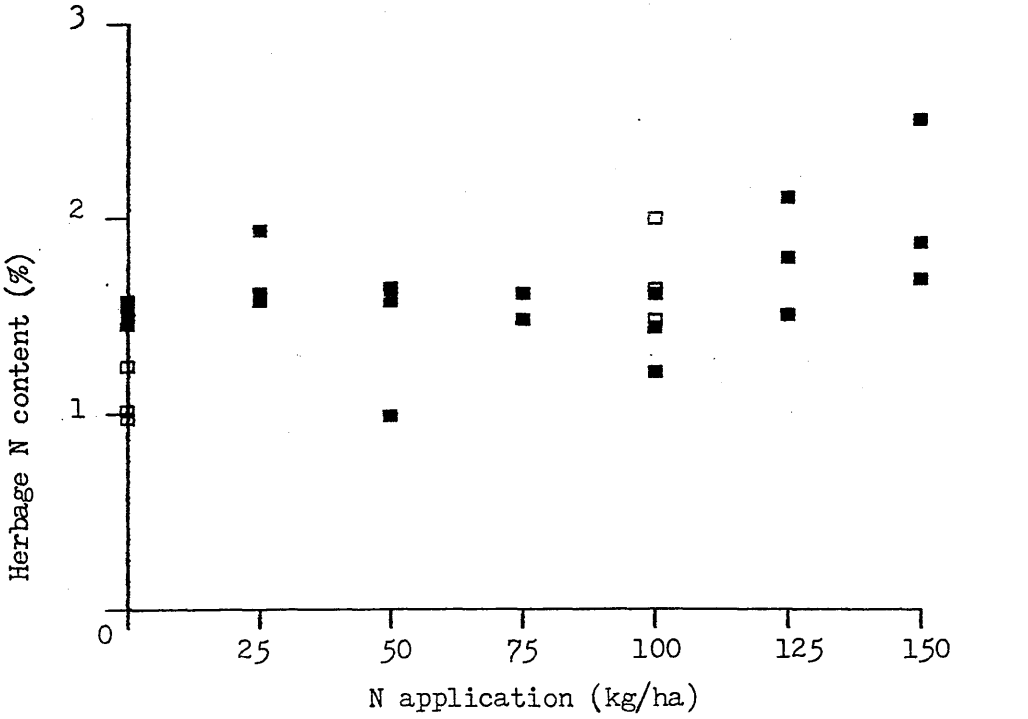


Fig. 5.2 Response of herbage nitrogen content to application of nitrogen (1986).
Ammonium nitrate-N with added P and K (■)
Ammonium nitrate-N without added P and K (□)

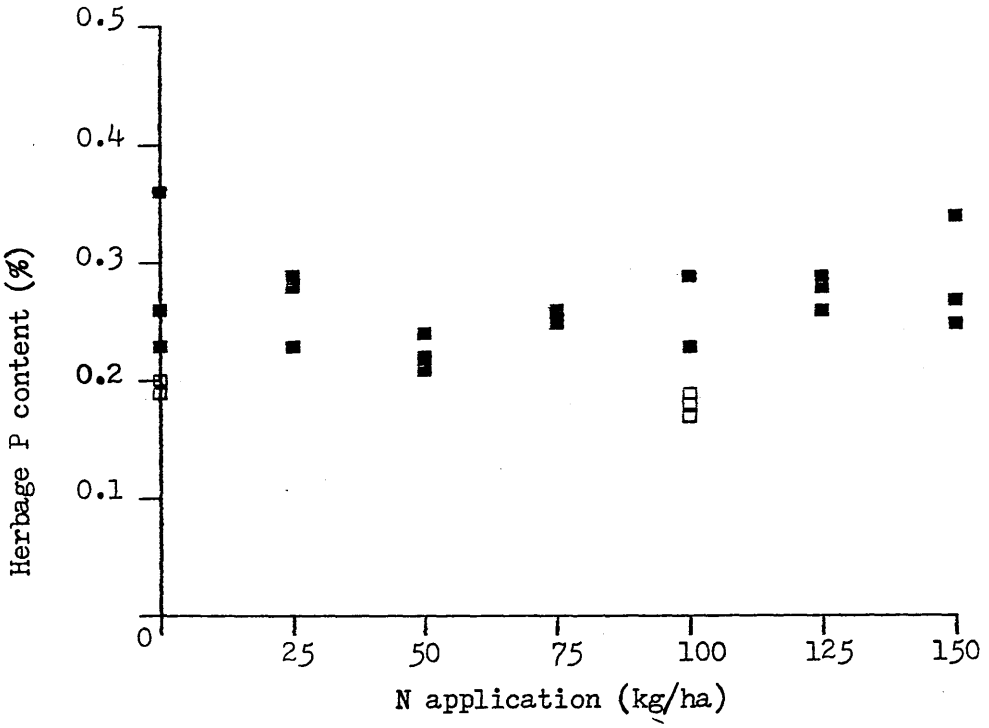


Fig. 5.3 Response of herbage phosphorus content to application of nitrogen (1986).
Ammonium nitrate-N with added P and K (■)
Ammonium nitrate-N without added P and K (□)

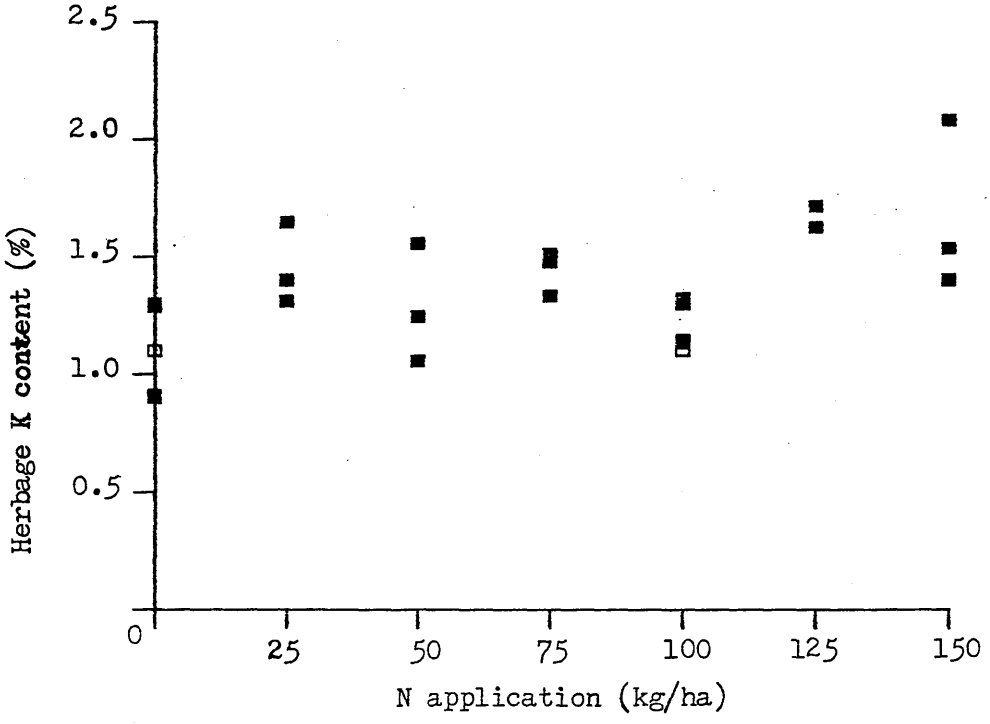


Fig. 5.4 Response of herbage potassium content to application of nitrogen (1986).
Ammonium nitrate-N with added P and K (■)
Ammonium nitrate-N without added P and K (□)

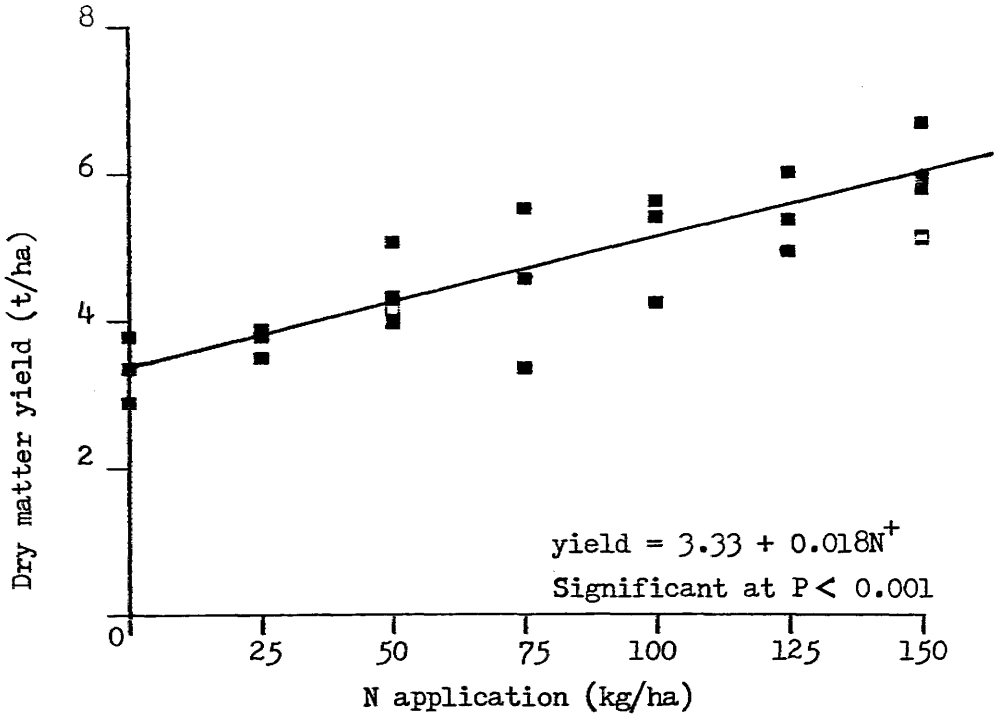


Fig. 5.5 Response of herbage dry matter yield to application of nitrogen (1987).
Ammonium nitrate-N (■), Urea-N (□)
† Equation refers to ammonium nitrate-N only

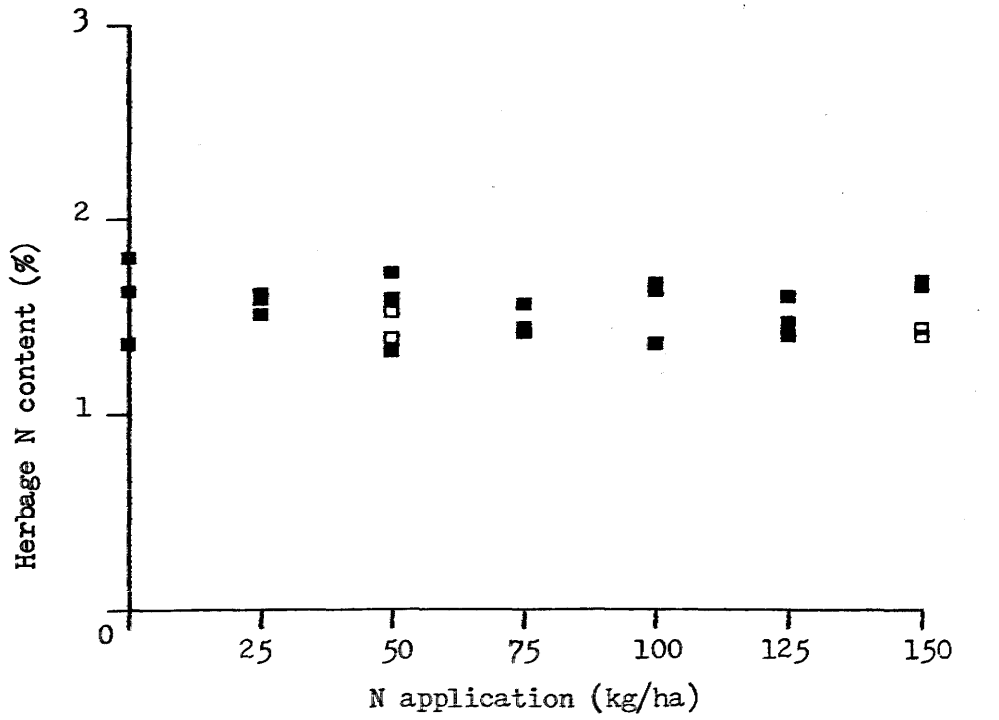


Fig. 5.6 Response of herbage nitrogen content to application of nitrogen (1987).
Ammonium nitrate-N (■), Urea-N (□)

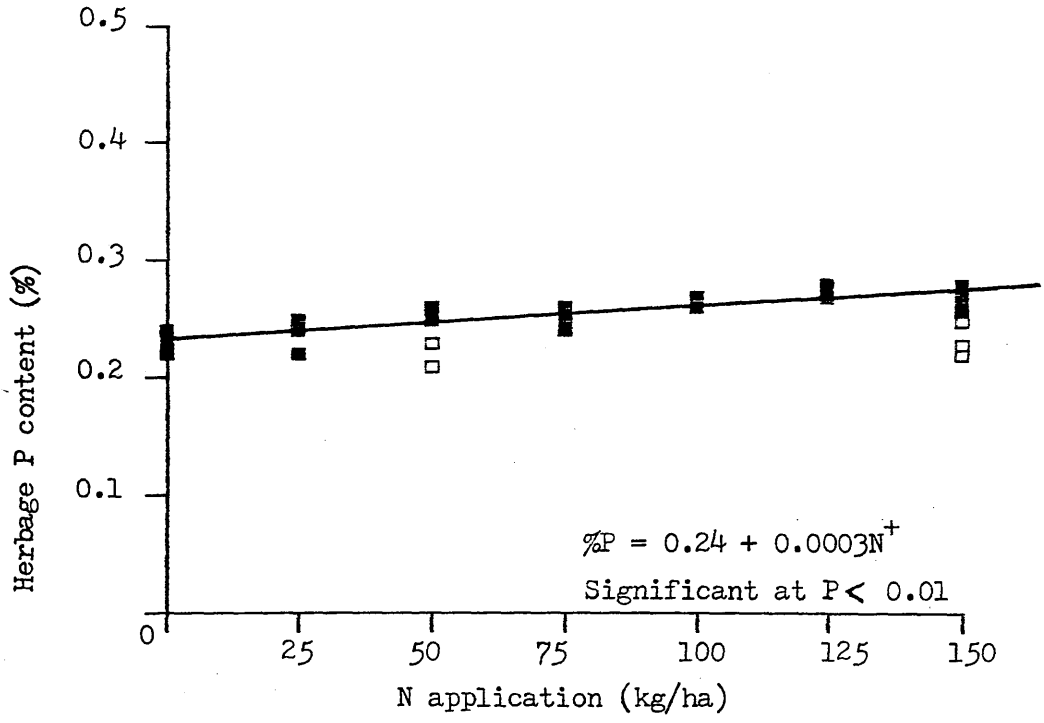


Fig. 5.7 Response of herbage phosphorus content to application of nitrogen (1987).
Ammonium nitrate-N (■), Urea-N (□)
† Equation refers to ammonium nitrate-N only

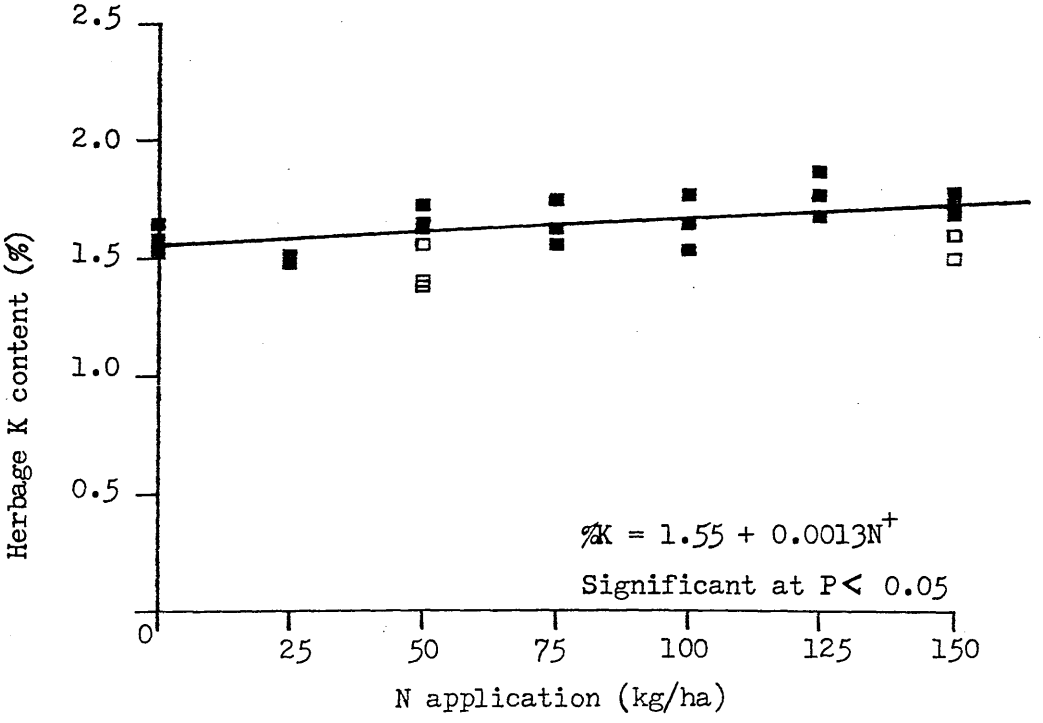


Fig. 5.8 Response of herbage potassium content to application of nitrogen (1987).
Ammonium nitrate-N (■), Urea-N (□)
† Equation refers to ammonium nitrate-N only

The herbage dry matter response curves resulting from the use of seven N treatments, shown in Figs. 5.1 and 5.5, indicate that the increase in yield was almost linear over the entire range of nitrogen applied in both years. Mean herbage yield responses were 14 and 18 kg dry matter/ kg of N applied in 1986 and 1987 respectively. The dry matter yields and N, P, and K contents of the herbage data seem to be relatively more uniform in 1987 than in 1986. This could partly be attributed to the comparatively greater uniformity of the plots as a result of residual effects of previous treatments and cutting of vegetation in 1986.

Davison and Jefferies (1966), Bloomfield et al. (1982), Pulford et al. (1988) and others have shown a positive response of herbage to added nitrogen on coal mine wastes. For example, Pulford et al. (1988) found that the dry weight yield of herbage was increased from 1.7 t/ha with no N to 3.3 t/ha with 100 kg N/ha.

The response of dry matter yield found in the present study seemed to be within the range of those reported by various reseachers for grass plus white clover swards on agricultural grasslands. Reid (1970) reported the herbage response for three successive years ranging from 13.1 to 15.7 kg dry matter/kg of N applied from 0-336 kg N/ha. Wilman and Hollington (1985) reported the effect of seven levels of applied N ranging between 0 and 600 kg N/ha. They found a response of 14 kg dry matter/ kg N by comparing 100 kg N/ha with nil at one site. They have reported no significant response to N application in excess of 400 kg/ha. Frame (1987) reported mean herbage

response of 21.8 and 19.6 kg dry matter/kg N applied for two successive years by comparing 80 kg N/ha with nil.

The herbage analyses data in Tables 5.3 and 5.4 show that nitrogen content of the herbage was generally not affected by nitrogen treatments in both years. This is clearly shown in the year 1987 (Table 5.4), although there was some variation in the data of 1986 (Table 5.3). The effect of added nitrogen on the uptake of P and K was somewhat variable, but P and K concentrations of herbage were generally improved by N application especially in 1987, when the differences were significant at $p< 0.01$ and $p< 0.05$ respectively (Figs. 5.7 and 5.8).

The effect of added nitrogen fertilizer on the uptake of N, P and K by grass plus clover sward on agricultural grasslands has been studied by some researchers. Wilman and Hollington (1985) who studied the effect of seven N levels ranging between 0 and 600 kg N/ha, found an increase in the N content and some decrease in P content of the herbage by applied nitrogen. They reported no consistent effect of applied N on the K content of the herbage. They further stated that the clover was similar to grass in P and K contents and higher than grass in Ca and N. Frame and Boyd (1987) observed that increasing fertilizer N rate reduced N concentration from 30.4 g/kg dry matter at no N to 28.7 g/kg dry matter at 75 kg N/ha rate. In a similar type of study Frame and Paterson (1987) found that N concentration of grass plus clover herbage was not affected by fertilizer N treatments between 0 and 75 kg N/ha.

By comparing the dry matter yield data of treatments receiving the same amount of nitrogen with and without added P and K (Table 5.3), it is evident that addition of P and K tended to increase dry matter yield but this effect was not statistically significant. It has been reported that addition of K alone has shown no effect on the dry matter yield of grass and white clover mixed vegetation in a non-calcareous gley of the Scottish uplands (Rangeley and Bolton (1986). This small increase in dry matter yield may possibly be due to the favourable effect of added P on the growth of the white clover component of the mixed sward (Rangeley and Newbould, 1985; and Rangeley and Bolton, 1986). The addition of P and K also seemed to have some favourable effect on the uptake of P and K (Table 5.3). The effect of P on herbage yield in coal mine soils is not clear. Fitter and Bradshaw (1974) reported an increase in vegetation yield with added P. On the other hand Pulford et al. (1988) found no effect of added P on vegetation yield, but they observed a favourable effect of 60 kg P/ha alone or with N and K on the P content of the herbage.

As far as the efficiency of urea or ammonium nitrate fertilizer was concerned, results in Table 5.4 indicate that dry weight of herbage responded equally to both sources of nitrogen. Although the herbage yields from urea plots were comparatively lower than those produced by the same levels of nitrogen applied as ammonium nitrate, the differences were statistically not significant. The relatively higher dry matter yields on ammonium nitrate-N

plots could be due to the residual effect of added P and K received by these plots in the year 1986. The urea plots did not receive P and K fertilizers that year, which resulted in the low content of these nutrients in the herbage from these plots. The reasonable values of urease activity of coal mine soils from the plot area suggest that the added urea would be rapidly hydrolysed to release ammonium ions. The pH values of the experimental plot area indicate that loss of ammonia due to volatilization would not be a problem (Table 5.2).

There are some variations in data regarding dry matter yield and N, P and K contents of herbage data in both years, especially in 1986. Such variation could be expected on coal mine wastes, which are not even considered as soils (Chadwick, 1987). It is clear from Table 5.2 that the pH of the experimental area was very variable. It was below pH 6.0 on five out of the nine sampling points, in spite of the fact that care was taken in the selection of the site before fertilizer application during 1986 (see section 5.2.1). This indicates the heterogeneous nature of the coal mine waste material. The site is said to be pyritic and the distribution of pyrite is not homogeneous (Backes, 1984).

Moreover, variation in data may also partly be due to the mixed nature of the vegetation. Addition of fertilizer nitrogen has a major influence on grass-white clover relationships in the mixed sward, because it intensifies competition to the clover from the grass. The white clover component of a mixed sward usually reduces the effects on

total dry matter yield of nitrogen application rates (Reid, 1986; and Frame and Boyd, 1987).

However, in spite of great heterogeneity of the substrate material and mixed nature of the sward the results show a clear evidence of nitrogen deficiency in coal mine soils and a positive response of vegetation to added nitrogen fertilizer.

5.4 CONCLUSIONS

The results obtained from the nitrogen response experiment confirmed the deficiency of nitrogen on reclaimed coal mine soils, and emphasized the importance of maintaining an adequate level of this nutrient for optimum growth of vegetation, especially grass.

Added nitrogen fertilizer significantly improved the herbage yield, which was also reflected in improved concentrations of N, P, and K in the herbage. In addition phosphorus and potassium tended to increase the yield of herbage when applied with nitrogen, but their effect was not significant. Vegetation responded equally to both nitrogen fertilizers with no significant difference in herbage yield between ammonium nitrate or urea treated plots.

The main purpose of the experiment was not to maximize yield but to determine the level of nitrogen required to maintain an adequate, green vegetation cover on the reclaimed spoils. This cover will not only be aesthetically appealing, but will also minimize pollution of streams by reducing erosion and leaching. It is suggested that until a self-supporting vegetation cover is established, addition of nitrogen to plants growing on reclaimed spoils is necessary. Nitrogen will become more readily available to plants in the longer term when sufficient has accumulated in an organic fraction. In this experiment, the optimum dose of nitrogen would appear to be in the range of 50-100 kg/ha per year.

CHAPTER 6 GENERAL CONCLUSIONS

The investigations carried out have been concerned with the study of some aspects of nitrogen cycling and its availability to plants in coal mine soils of Central Scotland.

During preliminary tests for the assessment and modification of the various methods used in this study, investigations carried out included; cleaning of filter papers, selection of an extractant for inorganic nitrogen, Mn ion interference in the nitrate nitrogen analysis and methods used for assaying urease and amidase activities in coal mine soils. It was found that Whatman No. 2 filter papers can be used for the extraction of mineral nitrogen, provided they are cleaned of their nitrogen contaminants. This can be done by washing with 0.5 M sulphuric acid in 2 lots each of 25 cm^3, rinsing 3 times with deionized water and drying for 4 hours at 70 ^oC before use.

Three potassium salt solutions, potassium sulphate (0.5M), potassium chloride (1M) and potassium acetate (1M) were compared for the extraction of mineral nitrogen from coal mine soils. Potassium sulphate solution was selected as the most suitable extractant, which was also compatible with the automated colorimetric methods for the analysis of ammonium-N, nitrite-N and nitrate-N.

Mn ions and some other unidentified components in the potassium sulphate extracts from acid coal mine soil

samples (pH below 5.0) were found to interfere in the colorimetric analysis of nitrate nitrogen. This interference was negative leading to an underestimation of the nitrate-N concentration. Ten fold dilution of the extracts effectively removed the negative effect of the Mn ions and the other interfering components and resulted in a satisfactory recovery of nitrate nitrogen. It is suggested that during analysis for nitrate nitrogen of coal mine soils the extracts from acid samples must be checked with 10 fold dilution. Some further work is needed to find out the other components besides Mn ions of the acid coal mine soils causing problems in the nitrate nitrogen analysis. Although 10 fold dilution could minimize the effect of the interfering components, due to the low levels of nitrate nitrogen normally found in the coal mine soils, it would be preferable if a method of nitrate nitrogen measurement not involving this large dilution could be found.

The various reagents used during assaying of urease and amidase activities were found to interfere in the automated colorimetric determination of ammonium-N released by these enzymes. The method could be successfully employed for the measurement of ammonium-N by using a 21-fold dilution step built into the Technicon ammonium-N manifold and by reducing the sodium hypochlorite concentration to one fourth of its original concentration. Shaking of soil samples in a $KCl-Ag_2SO_4$ mixture for one hour at 2 ^{o}C seems to be a satisfactory method of inhibiting amidase activity in the soil during

extraction of ammonium-N.

A detailed survey was carried out to study various chemical and biological transformations in the nitrogen cycle on 90 samples of coal mine soils from sites throughout Central Scotland, representing a wide range of properties. It is important to evaluate such properties of coal mine soils to determine which parts of the nitrogen cycle are functioning. The introduction and increase in the number of organisms involved in nitrogen transformations are particularly interesting because nitrogen is often the limiting plant nutrient in the revegetation and maintenance of reclaimed coal mine soils. An increase in the number of organisms involved in nitrogen transformation could aid in the establishment and survival of plants on such material. Stimulation of soil microorganisms in the reclaimed coal mine soils is possible through the addition of topsoil or organic wastes. Rapid nitrification in coal mine soils may lead to losses of nitrate nitrogen from the system through leaching and denitrification and thus the absence of nitrifying organisms need not be considered a major problem. However, rapid nitrification could be controlled by the use of slow release nitrogen fertilizers.

It was concluded from this survey that the extractable mineral nitrogen status of the waste material was very low. The rate of carbon dioxide evolution was very high compared to the low rate of nitrogen mineralization. Nitrification of added ammonium was measurable only on 50 % of the sites studied and was, as

expected, highly pH dependent. In coal mine soils having pH below pH 5.0 nitrification of added ammonium was absent. However, even on sites above this pH, nitrification was not always measured, which clearly indicated the absence of nitrifying bacteria.

It was found that a considerable amount of applied ammonium nitrogen could be lost due to ammonium fixation by the clay minerals in these waste materials, or assimilated by the heterotrophic population present due to their wide mineralizable C:N ratio. In the soils which showed the highest degree of ammonium fixation, up to 23 % of the 100 mg/kg ammonium-N added was lost in this way. Immobilization of nitrogen in the microbial cell material may be temporary if the nitrogen is released again by the decay of microbial cells. However, fixation of ammonium ion by clay minerals may render the nitrogen unavailable for plant growth and may lead to a poor response to fertilizer nitrogen.

An encouraging result from this survey was the reasonably high values for the activities of the two soil enzymes, urease and amidase. Such activities not only reflect microbial activity but also suggest that the use of urea or amide nitrogen fertilizers may be possible on the coal mine soils studied. These enzyme activities were measured on reclaimed coal mine soils with well established vegetation and such levels would not be expected on freshly exposed coal mine soils. Significant correlations of carbon turnover, urease activity, amidase activity and nitrification rate with each other were

found. This suggests the importance of organic matter for both microbial activity and enzyme stabilization.

In order to improve the nutrient status of coal mine soils, it is obvious that nitrogen must be introduced into the system. From the laboratory incubation study, various conclusions can be drawn as to the fate of nitrogen, added as ammonium sulphate, urea or chicken manure to 5 samples of coal mine soil with different properties. There was no difference in the behaviour of nitrogen applied as urea or ammonium sulphate in almost all the samples. Reasonable values of urease activity in all the soils suggest that the hydrolysis of added urea was complete before the first sampling at day 5 of the incubation experiment. Two soil samples collected from an established organic manure field trial showed nitrogen mineralization rates comparable to those reported for agricultural soils. This indicates the favourable effect of organic amendments in the long term on the establishment of a nitrogen cycle.

Two coal mine soil samples with neutral pH values, although they nitrified the added ammonium nitrogen, did not exhibit mineralization of organic nitrogen during incubation in the control, ammonium sulphate or urea treatments. This indicates that the soil samples were either lacking organic nitrogen or the organic nitrogen present was relatively resistant to microbial decomposition (Power et al., 1974). In an acid soil sample with pH 3.8 no net mineralization or nitrification was measured.

Addition of chicken manure generally improved the

mineralization and nitrification rates in all coal mine soils, especially of the acid soil where these processes did not occur with ammonium sulphate or urea addition.

The losses of total inorganic nitrogen in the ammonium sulphate and urea treatments of the acid soil sample were smaller than in the corresponding treatments of neutral coal mine soils. Such losses were attributed to a combination of ammonium fixation and immobilization. Losses were significantly higher in the manure treatments than in urea or ammonium sulphate treatments of all soils. Following the addition of chicken manure, up to 69% of the added nitrogen was lost during the first few weeks of incubation. The length of this period was generally 3-4 weeks, except in one soil where it lasted up to the final week of incubation (16 weeks). These large losses were attributed to immobilization due to the wide C:N ratio of the chicken manure. However, there was a clear contribution towards mineralization from chicken manure addition during the last weeks of incubations of all except one soil.

Addition of ammonium sulphate or urea had no effect on the initial soil pH, whereas chicken manure addition with the same level of ammonium nitrogen increased the pH of the acid soil from 3.8 to 5.7, with a favourable effect on the nitrifying bacteria. Addition of manure may also have resulted in introduction of nitrifying bacteria to the coal mine soils, especially to the acidic soil.

In order to study the response of established vegetation to added nitrogen, a field experiment was

conducted with varying levels of fertilizer nitrogen in the range of 0 to 150 kg N/ha. In addition, the effects of nitrogen in both the presence and absence of added P and K, and as ammonium nitrate or urea were also investigated. The results obtained from this experiment confirmed the deficiency of nitrogen on reclaimed coal mine soils, and emphasized the importance of maintaining an adequate level of this nutrient for optimum growth of vegetation, especially grass. Added nitrogen fertilizer significantly improved the herbage yield, and also resulted generally in higher N, P, and K contents in the herbage. In addition phosphorus and potassium tended to increase the yield of herbage when applied with nitrogen but their effect was not significant. Vegetation responded equally to added nitrogen with no significant difference in the herbage yield between corresponding plots treated either with ammonium nitrate or urea.

It is evident from the results of the survey of nitrogen status that the coal mine soils of Central Scotland are generally very low in extractable mineral nitrogen. The rate of carbon turnover is very high compared to the low rates of nitrogen mineralization. Such results suggest a deficiency of plant available nitrogen. This was confirmed by the results obtained from the nitrogen response experiment. These findings emphasize the need for the addition of an adequate level of nitrogen for the maintenance of a reasonable plant cover on such sites. A good plant cover on coal mine waste material is not only needed to make them aesthetically attractive but also to

minimize pollution of streams due to erosion and leaching.

A considerable input of nitrogen is always necessary to build up a nitrogen capital of about 1600 kg N/ha to sustain plant growth on reclaimed coal mine soils (Bradshaw, 1983). This cannot be done in one operation without providing more than the plants can take up. If it is applied in this way, a great deal will leach away and will be wasted.

Nitrogen could be applied as inorganic fertilizer in the ammonium form, as nitrate or as a mixture of both, or in the amide form as urea, or as organic manures. Nitrogen fertilizer in the nitrate form would be susceptible to leaching and denitrification losses. Lack of nitrification in most of the coal mine soils would probably help to retain added ammonium on the exchange sites, although a considerable amount of this ammonium could be fixed by the clay minerals of the waste material, and so become unavailable to plants. The added ammonium or nitrate could also be assimilated by the heterotrophic population which would make it temporarily unavailable for plant uptake.

The measured values of urease and amidase activities would suggest the possibility of the use of urea or amide nitrogen in the aftercare management of reclaimed sites. However, the use of such types of fertilizers on freshly exposed reclamation sites would depend on the capacity of the material to hydrolyse urea or amide nitrogen.

There are many waste materials which may contain reasonable levels of nitrogen and other plant nutrients. They include chicken manure, farm yard manure or sewage

sludge all of which can be used successfully on reclamation sites. They contain nitrogen in a soluble form, mainly ammonium, and in the organic form as well. The organic nitrogen is ultimately mineralized and becomes plant available. However, problems may arise sometimes over the release of nitrogen, and other nutrients as well, due to the high C:N ratio of coal mine soils and of the manure used. The bacteria using the carbon can lock up the available nitrogen temporarily but it is released later by mineralization. The major factor regarding the use of manures is their availability and cost. Unless a source is available close to the reclamation site, transport costs may be so high that its use is precluded.

High rates of organic manures like chicken manure or sewage sludge can be added to give a high initial input of nitrogen capital, which will also provide P and organic matter. However the results obtained from an established organic manure trial conducted by Agricultural Chemistry Department, Glassgow University indicate that the use of high rates of organic manures may not be suitable for the maintenance of the legume component in a mixed sward. Good yields of herbage were obtained for the first few years using such types of manures, but after 7 years there was no noticeable effect due to any of the treatments and the yields were almost equal to the control plots (Pulford et al., 1988). Legumes may not be compatible with large initial additions of organic manures intended to build up organic matter and nutrient cycling, because legumes tend to disappear due to the shading effects of tall grasses

encouraged by high levels of applied nitrogen.

Fertilizers or organic manures, and lime if necessary, are applied at seeding to produce a short term good effect for the establishment of vegetation. The need is then for a moderate input continued for a number of years.

When the land is reclaimed for agricultural purposes the farmers concerned will take care of the reclaimed land and apply the nutrients required to keep the land productive. However, management of the established vegetation is needed even if the reclamation is just for cosmetic purposes. The nitrogen status of the coal mine soils must be maintained to promote a build up of organic matter and nutrient cycling. This could be possible by the addition of a nitrogen fertilizer at intervals after the initial reclamation or by proper maintenance of legumes in the established sward. Results from the fertilizer response experiment suggest the use of 50 to 100 kg N/ha/year in the aftercare management. Every site would have to be independently assessed to determine the optimum nitrogen requirements which would also depend on the intended land use.

An over application of nitrogen in swards containing legumes can be as damaging as too little because it will not only result in inhibiting the nitrogen fixing capacity of legumes, but will also kill legumes by shading effects of tall grasses. However, up to 50 kg/ha/year of nitrogen (not necessarily every year) can be applied without affecting the nitrogen fixing rate of legumes like

Trifolium repens (Skeffington and Bradshaw, 1980). The maintenance of legumes is also important to ensure an efficient transfer of nitrogen from legumes to grass components of a sward. After a number of years, it is hoped that the system will become self-sustaining, as the nitrogen will be recycled through the vegetation and further additions may not be necessary.

However, application of nitrogen fertilizer seems to be an expensive solution to the problem and is not likely to be acceptable to the authorities funding reclamation schemes. So a good way of developing the nitrogen status of the coal mine soils is to rely entirely on legumes for the maintenance input of nitrogen. The value of legumes in maintaining the nitrogen status of coal mine soils has been generally accepted (Jefferies et al., 1981b; Palmer, 1982; Palmer and Chadwick, 1985). Legumes are an important component in almost all grass mixtures because they contribute and maintain adequate nitrogen supplies and ensure the build-up of an adequate capital of organic nitrogen in the newly forming soil. They can eliminate the need for aftercare treatment of nitrogen by increasing the amount of mineralizable nitrogen. The choice of a suitable legume would depend on the soil conditions and on climate, but the most valuable legumes are those which are used in agriculture, particularly clovers, since they have generally high rates of nitrogen fixation. Rates of the order of 50-150 kg N/ha/year have been measured on reclaimed sites.

FURTHER DEVELOPMENT OF THIS WORK

The study of soil enzymes could play a useful role in reclamation management of coal mine soils (Klein et al., 1985). There is an obvious need for research to investigate urease and amidase activities in freshly exposed coal mine soils as this study concentrated on vegetated, reclaimed sites only. Moreover, testing of the non-buffer method of urease activity is also needed, this would provide an index of the ability of the urease in coal mine soils to hydrolyse urea under natural conditions (Zantua and Bremner, 1975).

During the incubation and survey studies it was observed that most of the coal mine soils did not show any mineralization of organic nitrogen in the control, ammonium sulphate or urea treatments. In one soil even the chicken manure treatment did not accumulate any mineralizable nitrogen and showed a considerable loss of inorganic nitrogen during the whole 16 weeks of incubation. Further studies are required to investigate the reasons for the lack of mineralization and recycling of added nitrogen. As the various nitrogen transformations were studied under controlled laboratory conditions, it is suggested that a study of these transformations is also made under natural field conditions.

It may also be worthwhile to study and compare the effect of inorganic nitrogen fertilizers, organic manures and legumes on the accumulation of mineralizable nitrogen in the reclaimed coal mine soils of Scotland.

The use of inorganic fertilizers and manures as coal mine soil ameliorants provides an initial means by which the nitrogen status of the material may be improved and may help in the establishment of a vegetation cover. The longer term strategy for producing a self-sustaining system on such types of new soils is to ensure a continuous nitrogen supply by sowing legumes.

Application of high rates of organic manures to reclaimed coal mine soils of Baads has shown that the use of such types of organic manures are not helpful for the maintenance of legumes on reclaimed sites in the long term. On the other hand on sites sown with legumes or receiving inorganic fertilizer only, a reasonable plant cover of grass and legumes could be found. Tolerance of legumes to high levels of manure addition needs to be tested in order to determine levels of organic manures which can be used without affecting the establishment of the legumes. It would also be useful to determine the level of inorganic fertilizer nitrogen which would not affect the normal growth and nitrogen fixing capacity of the legumes. As most of the coal mine soils are acidic and lack essential plant nutrients, soil conditions like suitable pH and adequate supply of Ca and P are also important factors to be considered for the successful growth of legumes. Legumes are generally more sensitive to the harsh environmental conditions of coal mine soils compared to grasses. It may be better to test legumes and find species which are more tolerant of acid and low levels of plant nutrients.

Testing of nitrogen fixing abilities of different species of legumes on coal mine soils is also an important factor to be considered. The selection of more effective strains of Rhizobium adapted to the harsh environmental soil conditions can improve the potential benefits of legumes on coal mine soils. Moreover, it would obviously be of great interest to measure and compare the amounts of atmospheric nitrogen fixed by the legume species introduced and naturally occurring on the reclaimed sites. Some wild species of legumes, such as meadow vetchling (Lathyrus pratensis), tufted vetch (Vicia cracca), and broom (Cytisus scoparius) have been found on the reclaimed site of Baads colliery. The acetylene reduction method, which is generally used for this purpose could be used to compare fixation rates under field conditions. ^{15}N methods which are more expensive could be used to determine N fixation rates. Such types of studies would be helpful to show whether the legumes grown are fixing significant amounts of atmospheric nitrogen.

The use of some leguminous and non-leguminous trees as reclamation tools could be tested on reclaimed coal mine soils in Scotland. For example, alder which has the symbiotic nitrogen fixing actinomycete Frankia in root nodules, can accumulate as much as 100 kg N/ha/annum to soil from leaf fall alone (Postgate, 1982). Such trees are usually less effective in stabilising the spoil surface against erosion but could be grown along with grass clover mixed swards to improve the overall appearance of the reclaimed site.

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