INCUBATION STUDY ON THE BIODYNAMICS OF NITROGEN IN TEA SOILS OF EAST PAKISTAN

By

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ABSTRACT

Top-soil samples from acid high-flat and low-flat areas of East Pakistan where tea bushes are currently being grown were studied with respect to changes in mineral nitrogen forms during incubation.

Mineral nitrogen changes were studied during incubation in relation to soil moisture content, temperature, pH and the addition of inorganic nitrogen sources and organic materials. Other experiments were concerned with the effects of addition of major and trace elements, gamma irradiation treatments and period of air-dry storage of soils on mineral nitrogen changes during subsequent incubation. The denitrifying potential of the soils and the nitrification of fixed ammonium in the clay fraction were also determined.

The occurrence of nitrification under conditions (low pH, high temperature and high moisture content) which would be expected to preclude autotrophic nitrification suggests that a heterotrophic process(es) is responsible for nitrification in these soils.

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Dedicated to :

Dr. A.H.Cornfield
Department of Chemistry,
Imperial College of Science & Technology,
London, S.W. 7

My Beloved Father, My Wife
and
My Children.

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CHAPTER 1

INTRODUCTION

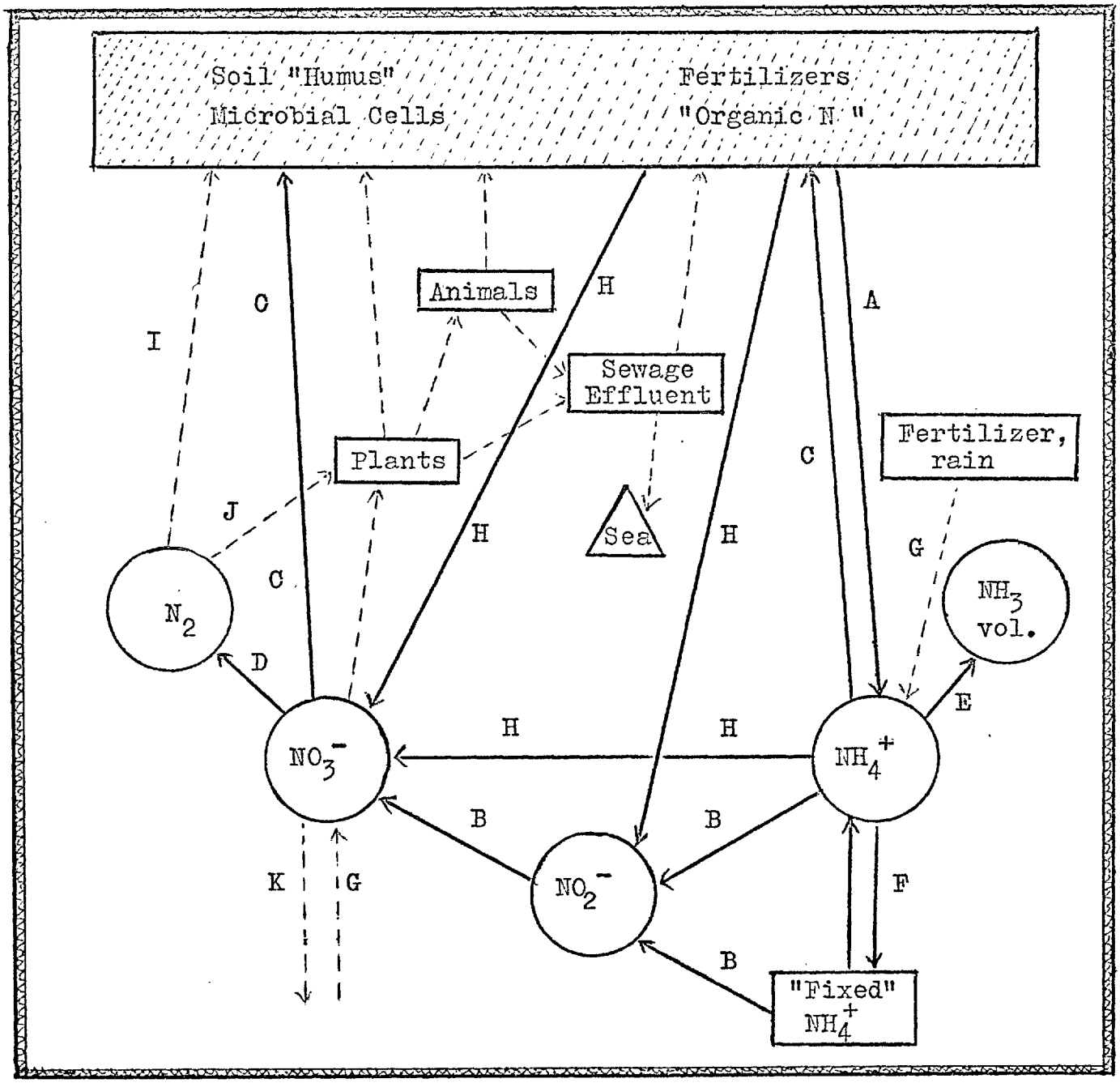
The work reported in this thesis is concerned with some aspects of nitrogen transformations in "Tea Soils" from East Pakistan.

The nitrogen cycle in nature is shown in Figure 1. The aspects of nitrogen changes which were studied are shown by the heavy lines. All these aspects were studied using incubation techniques. This allowed for accurate control of temperature and moisture content.

The "Tea Soils" used were typical of those used for growing tea in East Pakistan. These are acid soils on which no work appears to have been reported on nitrogen changes with respect to various imposed treatements. The literature review reported in the next section will cover mainly those factors affecting nitrogen changes which were studied in this work.

Figure 1

The Nitrogen Cycle



(The deep lines A,B,C,D,E,F & H show the processes studied)

A. Ammonification (Mineralization)

B. Nitrification (autotrophic)

C. Immobilization

D. Denitrification

E. Ammonia volatilization

F. Fixation of ammonium

G. Gain by fertilizer, rain etc.

H. "Heterotrophic nitrification" converting organic N direct to NO_2^- or NO_3^-

I. Non-Symbiotic nitrogen fixation

J. Symbiotic nitrogen fixation

K. Loss of nitrate by leaching

LITERATURE REVIEW

GENERAL

Most of the nitrogen present in soils is in organic combination (proteinaceous compounds and their derivatives) (1,2). Plants cannot utilize this form of nitrogen. A wide range of heterotrophic microorganisms can attack these organic nitrogenous compounds and convert part of this nitrogen to ammonia. This process is called AMMONIFICATION or MINERALIZATION. A large variety of bacteria, fungi and actinomycetes e.g.,*Achromobacter*, *Bacillus*, *Mucor*, *Penicillum*, *Aspergillus*, etc., can accomplish this process (1,3,4,5). The actual conversion takes place in a series of enzymatic reactions involving conversion of proteins through proteoses, peptones, polypeptides and amino acids. The amino acids are then degraded to simpler units with the formation of ammonia (1,2,6,7,8).

Providing conditions are satisfactory, the ammonia formed is converted by autotrophic bacteria (*Nitrosomonas*, *Nitrobacter*) into nitrate via nitrite (9,10,11,12,13). This process is called NITRIFICATION. Recent work has suggested the possibility that organic nitrogen compounds can be converted to nitrite or nitrate by heterotrophic process, i.e., without the traditional nitrifying organisms being implicated (14,15).

When energy rich material is present in soil,

decomposition of this material by heterotrophic micro-organisms results in assimilation of inorganic nutrients including ammonia and nitrate (1). The process is called IMMOBILIZATION.

Under conditions of restricted aeration such as induced by water-logging nitrate can be decomposed by certain bacteria (1) to produce elementary nitrogen and oxides of nitrogen. This process is called DENITRIFICATION. The process is stimulated by the presence of energy rich material in combination with anaerobic conditions.

In soils of high pH ammonia may be lost by volatilization, particularly where heavy applications of ammonium or ammonia-forming fertilizers (e.g. urea) are applied (1,16,17,18,19,20). The process is called AMMONIA VOLATILIZATION.

Ammonium (NH_4) can be held in soils in fixed form. This, by definition is that ammonium which cannot be removed from soils by cation exchange reagents. The layer lattice clay minerals are implicated in this AMMONIUM FIXATION (21,22,23,24,25).

Elementary nitrogen can be "fixed" or converted to organic form by (a) microorganisms associated with higher plants e.g., legumes (SYMBIOTIC NITROGEN FIXATION), (b) free-living bacteria and algae in soils (NON-SYMBIOTIC NITROGEN FIXATION) (1).

LITERATURE REVIEW

FACTORS AFFECTING NITROGEN CHANGES IN SOILS

AMMONIFICATION

Moisture and Aeration

The optimum moisture content for ammonification in soils is usually between 50 and 75% of the maximum water holding capacity (mwhc) (26). At high moisture levels the process is not prevented, hence in soils of high moisture content, including water-logged soils ammonia will accumulate, since lack of oxygen would prevent nitrification (26,27,28). Ammonification rate declines with decreasing soil moisture content below 50% mwhc, but can occur, although to a very limited extent even at the wilting point moisture content (pF 4.2) (29,30,31,32,33).

pH

Ammonification occurs over a wide range of soil pH (34,35). However, in general, the process is more rapid in approximately neutral than in acid soils, whilst under alkaline condition the rate tends to fall off (1,15,36, 37,38,39).

Temperature

Ammonification increases with increase of temperature to a maximum ranging from 40°to 60°C (1,40,41,42,43,44,45).

Thermophyllic microorganisms are active at the higher temperature (46).

NITRIFICATION

The transformation of ammonia to nitrate is mainly by autotrophic processes involving firstly conversion of ammonia to nitrite by Nitrosomonas followed by conversion of nitrite to nitrate by Nitrobacter (9,10,11,12,13,47,48). However, the possibility that some heterotrophic bacteria can convert ammonia to nitrite has been reported (1,49,50, 51,52). In addition, some fungi, Fusarium, Aspergillus flavus etc., (51,53,54) can produce nitrite from organic nitrogenous compounds in pure culture. However, the rate of formation of nitrite and nitrate from ammonium or organic nitrogenous compounds by the heterotrophic process is very low compared with that produced by the autotrophic process (51,53).

Moisture and Aeration

Since oxygen is required during nitrification of ammonium those factors (high moisture content or compacted soil; 1,55,56) which restrict the movement of oxygen will decrease nitrification rate. Nitrification is restricted at low moisture contents even where aeration is adequate (1). As soil moisture content increases so nitrification rate increases. The moisture content for maximum nitrification

varies considerably amongst different soils, ranging from 20% to 80% of the water holding capacity (1,30,31, 57,58,59,60,61,62,63,64).

pH

Nitrification is usually absent or slight at low pH. pH values below which no nitrification occurs range from 3.7 to 5.5 (65,66,67,68,69,70). These are very early references to nitrification in the literature (1929 or earlier). The only recent reference (1962) to nitrification in acid soils is that by Weber and Gainey (71) who found nitrification in four soils of pH 3.9 - 4.7. Nitrification increases with pH and the optimum pH for nitrification has been reported as 6.5 to 9.0 (35,72,73,74,75). The maximum pH above which no nitrification occurs has been reported as 7.8 to 10.3 (66,76,77,78,79,80,81,82,83).

Temperature

Nitrification rate is very slow below 5°C, increases with temperature to a maximum ranging from 25°C to 37°C, and falls off very rapidly with increasing temperature above 40°C (1, 57,84,85,86,87,88,89,90,91,92,93,94,95,96 97,98,99).

Changes in N when ammonium salts and urea are applied to soils

The nitrogenous fertilizers commonly applied to the

soil are ammonium salts and urea. Addition of ammonium sulphate increases the acidity of the soil in the absence of sufficient buffer or base, due to the formation of nitric acid from the oxidation of ammonium and the accumulation of residual sulphuric acid. Nitrate accumulation will proceed at a reduced rate until the soil pH has fallen to 5 or less. Under these conditions, the amount of nitrate formed depends upon the initial pH of the soil and its buffer capacity (65,100).

The autotrophic oxidation of ammonium to nitrite and then nitrate takes place on the surface of the soil particles. Nitrification rate is roughly proportional to the base exchange capacity of the soil (56). The continuous use of ammonium sulphate as a fertilizer without the addition of lime will, therefore, lead to a gradual decrease in pH. However, nitrates may be found even in very acid soils. This was explained by Hall and associates (101) as due to the fact that, under acid conditions, nitrate formation takes place in films surrounding the small isolated particles of calcium carbonate. When the process of nitrate formation from ammonium salts and amino acids are compared, the latter is found to take place more slowly (102). This is probably due to the fact that the amino acids have to be changed first to ammonia and also to the fact that some of the nitrogen will be stored away in the microbial cells which use the carbon of the amino compounds as a source of energy. In acid

enviornments, the nitrification of ammonia and organic nitrogen (such as urea and amino acids) is faster than that of ammonium sulphate. The process of nitrification is favoured here due to the increase of pH resulting from ammonium formed from urea and amino acids (103,104,105, 106,107,108,109).

In calcareous soils the nitrification of ammonium sulphate takes place rapidly as the soil pH remains near neutral and even high applications do not disturb nitrification. The addition of calcium carbonate has a decided stimulating effect on nitrate formation, particularly in acid soils (110, 111, 112). In alkaline soils which are deficient in humus, calcium carbonate may have the opposite effect since it tends to liberate free ammonia from ammonium salts and urea, which retards the nitrite $\longrightarrow$ nitrate stage. Thus nitrite tends to accumulate and this is also toxic to nitrate-forming bacteria (113,114,115,116,117,118,119). The inhibitory action of nitrite and free ammonia may be temporary, as with time the levels of nitrite and ammonia will decrease due to loss through leaching and volatilization and the pH will then drop and eventually nitrification may become normal. Nitrite is formed only when ammonium, ammonia or urea are applied in high concentrations to soils of high pH (99,120,121,122).

When urea is applied to soil it is readily hydrolysed and as much as 200 to 400 ppm urea-N may be transformed to

ammonium even at 10°C in periods of less than one week (1,123). The conversion is even more rapid at higher temperatures. The pH of soils receiving urea rises as ammonium is produced, and the conversion may proceed so rapidly that free ammonia is lost to the atmosphere. This volatilization is appreciable only where the buffering capacity is low, a situation common where urea is applied on the soil surface. Following the initial increase, the pH falls as the ammonia is oxidised to nitric acid and conditions for nitrate formation becomes favourable (1). Urea hydrolysis is favoured by warm temperature and is quite rapid at 37°C, but the transformations proceeds at a good rate even at 2°C. The conversion of urea to ammonium in soil far exceeds the nitrate appearance so that ammonium continues to accumulate as long as urea is present (1).

Many soil microorganisms possess the enzyme urease, the catalyst responsible for hydrolysing urea. Ammonium carbamate is an intermediate in the hydrolysis (1). The reaction is as follows:-

$$\underset{\text{Urea}}{CO(NH_2)_2} + \underset{\text{Water}}{H_2O} \xrightarrow{\text{urease}} \underset{\text{Ammon. carbamate}}{H_2NCOONH_4} \longrightarrow \underset{\text{Ammonia}}{2\ NH_3} + \underset{\text{Carbon dioxide}}{CO_2}$$

NITROGEN IMMOBILIZATION

The microbial assimilation of mineral-N during decomposition of organic materials in soils depends mainly on the carbon nitrogen ratio (C/N) of the added materials.

The critical C/N ratio above which immobilization occurs has been reported as ranging from 20:1 to 25:1 (1,35,124). The rate of nitrogen immobilization is rapid where easily oxidisable carbohydrates are added and very slow where resistant material such as lignin or well-rotted manure is added. The rate and extent of immobilization is dependent on the degree of resistance of the organic matter to microbial attack (125, 126, 127, 128). The preference for one form of mineral-N over another seems to vary. Some workers (129,130,131,132,133,134,135) found that NH_4-N was preferred to NO_3-N by the soil microorganisms when both forms of nitrogen were present, but others (126,136,137) found the reverse to be the case.

AMMONIA VOLATILIZATION

pH

Soil pH is the major factor determining the extent of loss of nitrogen through ammonia volatilization. In general, negligible loss occurs in this way at pH levels less than about 7, but loss increases considerably with increasing pH above 7 (16,17,117,138,139,140,141,142,143, 144,145).

Moisture Content

In general, volatilization of ammonia, where pH is sufficiently high to induce this loss, is high at low moisture contents and decreases with increasing moisture

content of soils (62,146,147,148). However, it has been reported that ammonia can volatilize even from water-logged soils (149,150).

Temperature

Volatilization of ammonia increases with increase in soil temperature (62,140,141,146,147,148,151,152). Increasing soil temperature favours the shift from

Adsorbed (NH_4) $\rightleftharpoons$ Ammonium (NH_4) in

solution and this favours the loss of ammonia by volatilization.

Ammonia volatilization is greater where urea than where ammonium sulphate is applied at the same rate of N. This is presumably because when urea hydrolyses to ammonium carbonate soil pH increases thus encouraging ammonia volatilization, whereas ammonium sulphate has no initial effect on soil pH (17,62,109,123,139,140,141,146,147,148, 149,153). When these sources of nitrogen are applied to soils of high pH ammonia volatilization losses are less when these materials are applied at some depth than when applied to the soil surface (20,140,141,153,154,155,156,157, 158). In addition, ammonia losses usually decrease with increasing fineness of soil texture (17,109,159,160).

OTHER FACTORS AFFECTING AMMONIFICATION AND NITRIFICATION

Effect of major and trace elements

The ammonifying and nitrifying organisms require the major elements and in addition some of the trace elements.

Copper, iron, manganese and molybdenum sometimes stimulated both the processes when compounds of these elements were added to soils in low concentrations but were toxic at high concentrations. Zinc was toxic to both processes even at low added concentrations (161,162,163,164,165,166, 167,168,169,170,171,172,173,174,175,176,177).

Effect of period of air-dry storage on subsequent mineralization of nitrogen (mineralizable-N)

Variable results have been reported on the effects of period of air-dry storage of soil samples on mineralizable-N (mineral-N produced on subsequent wetting and incubation). Results obtained ranged from considerable increases in mineralizable-N with increasing period of air-dry storage (178), initial decreases followed by increases(179), negligible effects, and decreased production of mineral-N (179,180, 181,182,183).

Fixation of added ammonium (NH_4^+) and nitrification of the fixed ammonium (NH_4^+) in soils

The fixation of ammonium by soils against extraction with cation exchange reagents is due to penetration of ammonium into the clay lattices (184,185). The extent of fixation varies with the amount and type of clay present and decreases in the order: illite, montmorillonite and kaolinite (186,187,188,189,190).

Fixation of added ammonium is generally negligible

in moist soil, but is increased on drying the soil at ordinary and, in particular, elevated temperatures (191, 192,193,194). The greatest fixation occurred when soils or clays were oven dried at 100°C (191,192,195,196,197, 198,199,200,201,202,203,204,205,206,207).

The extent of ammonium fixation is not related to pH in general though some workers found decreases in ammonium fixation as the pH decreased (191,199,208,209,210). Fixation decreases with increasing organic matter content of soils, probably due to preferential adsorption of ammonium on organic matter (which does not fix ammonium) or blocking of the clay exchange positions by the organic matter (211, 212,213,214).

Contradictory results have been obtained on the extent of nitrification of fixed ammonium, ranging from negligible to almost complete nitrification (196,197,215, 216). Ammonium fixed by montmorillonite was nitrified much more readily than that fixed by vermiculite (192,197,215, 217,218,219).

SCOPE OF THE PRESENT STUDY

The work reported in this thesis is concerned with laboratory studies of factors affecting ammonification, nitrification, nitrogen immobilization, NH_4-fixation,

ammonia volatilization, and denitrification in two acid tea soils from East Pakistan.

The main factors studied were the effects of pH, moisture content and temperature, and the addition of nitrogen fertilizers, organic materials and major and trace elements.

Incubation methods were used since with these technique good control of temperature and moisture conditions were attained.

CHAPTER 2

MATERIALS AND METHODS

CHAPTER 2

CHARACTERISTICS OF THE SOIL

Two lots of soils from a typical tea growing area in the north-east part of East Pakistan were used in this study. The samples represented a "HIGH FLAT" and a "LOW FLAT" area as described below. The samples were air-dried and ground to pass a 1 mm sieve before despatch in polythene bags to the United Kingdom.

HIGH FLAT (HF)

This was an abandoned old tea area which was rehabilitated before replanting was undertaken. It is an undulating high flat and "Dulia" variety of tea was replanted in the year 1961 at a spacing of 4 ft. x 2 ft., and shaded with Albizzia procera, A. odoratissima, A. lebbek, A. stipulata, and Derris robusta. Thedrainage system of the area is satisfactory. The soil samples were collected from different spots over about 2 acres from the 0-9 inches depth and bulked to a representative sample.

LOW FLAT (LF)

This was also an abandoned tea area which was rehabilitated before replanting. The area was replanted with dark leaf "Manipuri" variety of tea in the year 1960-1961 at a spacing of 4 ft. x 2 ft. This area is also shaded with the same species of shade trees as described above. The samples were collected from different spots over an area of

5 acres and bulked to a representative sample. The drainage system of the area is satisfactory but during the monsoon the water table rises within 3 ft. of the surface.

From 1962-66 both areas were fertilised with an NPK mixture in the ratio of 1:2:2 (ammonium sulphate, superphosphate and potassium chloride) with N at 80 lbs per acre, and P and K at 160 lbs per acre. Afterwards only sulphate of ammonia was applied once in every year just before the monsoon (March-April). During the dry season (December to March) the areas were irrigated when necessary.

Physical, chemical and mineralogical characteristics of the two soils are shown in the Table 1 and major and trace elements extracted from the two soils by various reagents are shown in Table 2.

Analytical methods used to determine soil characteristics

Mechanical analysis was done by pipette and sieving method after destruction of organic matter with sodium hypochlorite solution and dispersion after addition of sodium hydroxide to bring the suspension to pH 8.0.

Cation exchange capacity was determined by leaching with N-ammonium acetate pH 7.0, removing excess ammonium acetate with 80% aqueous ethyl alcohol, leaching out the exchangeable ammonium with N-potassium chloride and determining this by distillation after adding excess sodium hydroxide.

TABLE 1.

PHYSICAL, CHEMICAL AND MINERALOGICAL CHARACTERISTICS OF LOW FLAT AND HIGH FLAT SOILS

	LOW FLAT	HIGH FLAT
Coarse Sand (>200 μ) %	9.1	20.0
Fine Sand (200-50 μ) %	48.5	39.5
Silt (50-2 μ) %	27.6	26.2
Clay (<2 μ) %	13.0	12.3
Difference(including organic matter)	1.8	2.0
Cation exchange capacity (m.e./ 100 g.)	6.10	7.25
pH	4.1	4.2
Total N (%)	0.061	0.073
Total P (%)	0.048	0.024
Total Organic Carbon (%)	0.84	0.92
Walkley- Black Carbon(%)	0.54	0.60

MINERALOGICAL ANALYSIS

LOW FLAT: The clay fraction consists mainly of kaolinite, mica, smectite and vermiculite with minor amounts of mixed layer minerals, chlorite, quartz, goethite and feldspar.

HIGH FLAT: The clay fraction consists mainly of kaolinite, mica, interstratified smectite-vermiculite and vermiculite, with minor mixed-layer minerals, chlorite, quartz and goethite.

TABLE 2

MAJOR AND TRACE ELEMENTS EXTRACTED FROM LOW FLAT AND HIGH FLAT SOILS BY VARIOUS REAGENTS

(Results are given in ppm on dry soil basis)

E.R.	Soil	K	Ca	Mg	Cu	Fe	Mn	Zn	Al
W	LF	13.0	19.30	2.13	0.00	1.00	0.76	0.24	
W	HF	6.9	7.04	1.93	0.00	1.28	2.00	0.38	
NA	LF	24.2	34.70	6.30	0.00	0.00	1.48	0.21	
NA	HF	22.2	38.10	6.73	0.00	0.12	5.36	0.25	
MR	LF	46.5	9.10	6.60	0.36	16.80	2.46	1.27	147
MR	HF	26.8	29.70	6.83	0.00	8.02	9.36	0.45	117
EN	LF	15.4	0.18	6.35	0.72	49.30	2.81	1.23	
EN	HF	14.8	0.36	9.40	1.70	47.00	10.00	0.50	
BH	LF	500.0	140.00	425.00	5.50	5875.00	450.00	16.30	
BH	HF	300.0	135.00	475.00	9.00	9375.00	1200.00	10.60	

Key to Table 2

E.R.	=	Extracting Reagent	K	=	Potassium
W	=	Water	Ca	=	Calcium
NA	=	N-Ammonium Acetate pH 7.0	Mg	=	Magnesium
MR	=	Morgan Reagent	Cu	=	Copper
EN	=	0.1 N EDTA-Na pH 4.0	Fe	=	Iron
BH	=	Boiling 6N HCL	Mn	=	Manganese
LF	=	LOW FLAT	Zn	=	Zinc
HF	=	HIGH FLAT	Al	=	Aluminium

pH was determined, after shaking 10 grams of soil with 10 ml of water, using the Pye Model 79 pH meter.

Total nitrogen was determined by digestion with sulphuric acid, potassium sulphate and copper sulphate (as catalyst) and the ammonia formed was determined by steam distillation following addition of excess sodium hydroxide.

Total phosphorus was determined after digestion with nitric and perchloric acid and, after dilution followed by filtration, a suitable aliquot was analysed for phosphorus by colorimetric method (220,221).

Total organic carbon was determined by a dry combustion method (the author is indebted to Micro-Analytical Laboratory of the Organic Chemistry Department of Imperial College for these analyses).

"Walkley-Black" Carbon was determined by standard Walkley and Black procedure(222,223).

Extractable metallic elements shown in Table 2 were determined by shaking 10 g. of soil with 20 ml of reagent for 2 minutes, centrifuging and determining the metals by Atomic Absorption Spectroscopy (A.A.S.) in the supernatant liquid. Reagents used were water, N-ammonium acetate pH 7.0, Morgan reagent (0.5 N-acetic acid- 0.75 N-sodium acetate), and 0.1 N EDTA-Na (pH 4.0).

Boiling 6N HCl

"Total" extractable metals were determined by boiling 5g. soil with 20 ml 6N-HCl for 20 minutes. After dilution, filtering and washing, the combined filtrate was brought to a fixed volume and the metals (except aluminium) were determined by A.A.S. Aluminium was determined by an Aluminon method (224,225).

The mineralogical analyses were done by Dr. A.H.Weir and Mr. E.C.Ormerod of Rothamsted Experimental Station.

Incubation, extraction and analytical methods used in incubation experiments

Incubation

The incubation was done in 4" x 1" diameter flat bottomed glass tubes using 10 grams of air-dried, 1 mm sieved soil. Water was added to give a particular moisture content based on the maximum water holding capacity (mwhc) of each soil. The maximum water holding capacity was determined by weighing 10g. of soil into a dry porcelain crucible with sintered bottom and weighing. The crucible was then placed in a dish of water so that the water in the dish was at the same level as the sintered bottom. The water was absorbed into the soil by capillary action and 30 minutes after the top of the soil appeared moist the crucible was removed. The outside was quickly dried and the crucible was then weighed. To allow for water absorbed by the sintered bottom an empty crucible was subjected to

the same treatment. The amount of water absorbed by 10g. of soil represents 100% mwhc, and this represents a pF of zero.

In those experiments involving the addition of materials to the soil before incubation, these materials (e.g. ammonium sulphate, urea, dried blood, straw etc.) in finely ground form were mixed with the dry soil bulk at suitable levels, before weighing out 10g. portions in to the incubation tubes. Water was then added to the required moisture content (usually 50% mwhc for aerobic incubation and 133% mwhc for anaerobic incubation). For aerobic incubation the barium peroxide method (226) was used for aeration and to absorb carbon dioxide. The tubes were then closed with rubber bungs and placed in the incubator.

Extraction

After incubation ~~of~~ 10 ml water was added to the tube and the contents shaking for 2 minutes with the rubber bung in position. Then 10 ml of one of the following reagents were added in double strength (double strength reagents were used in order to allow for the dilution with the 10 ml of water added for the pH determination) :-

i) the Morgan reagent (0.5N-acetic acid-0.75N-sodium acetate) or N-sodium acetate was usually used.

ii) where trace elements were being determined 0.1N EDTA-Na (pH 4.0) was used.

iii) where nitrite was to be determined N-potassium chloride was used.

Analytical methods

Ammonium and nitrate-N were determined in suitable aliquots of the extracts using the micro-diffusion method (227,228). Ammonium-N was determined by using magnesium hydroxide alone and ammonium plus nitrate-N using magnesium hydroxide plus 0.5M ferrous sulphate in 0.1N sulphuric acid with two drops of saturated solution of silver sulphate:-

$$NO_3^- + 8\ Fe(OH)_2 + 6\ H_2O \longrightarrow 8\ Fe(OH)_3 + OH^- + NH_3$$

When nitrite was present in the extract (the extracts were analysed for nitrite before the determination of the other mineral-N forms) two drops of N-potassium permanganate were added to the extract in the micro-diffusion cell and allowed to react for 1 minute to oxidise nitrite to nitrate. It was shown that nitrite was not quantitatively reduced to ammonia by ferrous hydroxide, hence this preliminary procedure was necessary. The magnesium hydroxide, ferrous sulphate and silver sulphate were then added.

2 ml of 2% boric acid were added to the central chamber of the micro-diffusion cell to absorb ammonia. The boric acid in the central chamber was then titrated with standard 0.005N sulphuric acid using a methyl red-methylene blue mixed indicator.

Nitrite was determined in a separate aliquot of the extract by a colorimetric method using sulphanilic acid and N-(1-naphthyl)-ethylenediamine hydrochloride as described

by Barnes and Folkard (229).

In those studies where NH_3 volatilization was determined a small vial containing 1 ml of 0.1N sulphuric acid was placed in the incubation tubes to absorb ammonia. The ammonia absorbed was determined by a colorimetric Nessler method (230).

Major and trace element cations were determined in the extracts by Atomic Absorption Spectroscopy.

Details of the methods used for studying ammonium fixation and nitrification of fixed ammonium will be given in the appropriate chapter.

Total nitrogen (N) contents of organic materials used in this study

All these materials were oven-dried (60°C) and ground to fine powder in a Christie and Norris mill.

Total N was determined by Kjeldhal digestion followed by distillation of ammonia after making the digest alkaline. Results for total N were as follows:-

Straw 0.525% ; composted grass 2.52% ; pea seed 3.40% ; dried blood 15.3% ; green grass 0.921% ; urea 47% ; oxamide 32% N.

CHAPTER 3

EFFECT OF SOIL MOISTURE CONTENT DURING INCUBATION ON MINERALIZATION OF NITROGEN AND NITRIFICATION

Experiment 1

Effects of varying static moisture content (20% to 100% mwhc) on mineral-N levels during incubation (2,5 and 12 weeks) of LOW FLAT soil alone and with added dried blood (200 ppm N on soil basis).

Experiment 2

Effects of varying static moisture content (20% to 100% mwhc) on mineral-N levels during incubation (2,5 and 12 weeks) of HIGH FLAT soil alone and with added dried blood (200 ppm N on soil basis)

Experiment 3

Effects of varying static moisture contents (60% to 133% mwhc) on mineral-N levels during incubation (5 and 13 weeks) of HIGH FLAT soil without and with added ammonium sulphate (100 ppm N)

CHAPTER 3

EFFECT OF SOIL MOISTURE CONTENT DURING INCUBATION ON MINERALIZATION OF NITROGEN AND NITRIFICATION

This chapter reports on experiments dealing with the effects of varying static moisture contents during incubation on nitrogen mineralization and nitrification of low flat and high flat soils. The soils were incubated by themselves or with addition of dried blood or ammonium sulphate.

Experiments 1 and 2 were exactly the same except that the low flat soil was used in Expt. 1 and the high flat soil in Expt. 2.

EXPERIMENT 1

Effects of varying static moisture content (20% to 100% mwhc) on mineral-N levels during incubation (2,5 and 12 weeks) of LOW FLAT soil alone and with added dried blood (200 ppm N on soil basis)

METHODS

Where dried blood was added this was mixed in finely ground form in bulk with the soil. Sufficient incubation

tubes using 10g. portions of soil were set up with untreated and dried blood treated soil to allow for analysis in duplicate initially and after 3 periods of incubation. The moisture contents used were 20, 40, 60, 80 and 100% mwhc. The tubes were closed with rubber bungs and the barium peroxide method was used for aeration and absorption of carbon dioxide. Incubation periods were for 2, 5 and 12 weeks and incubation temperature was 30°C. Initially and after each incubation period 10 ml of water was added for pH measurement and then 0.5N-sodium acetate pH 7.0 was used for extraction of mineral-N. Aliquots of the filtrate were analysed for NH_4- and NO_3-N.

RESULTS

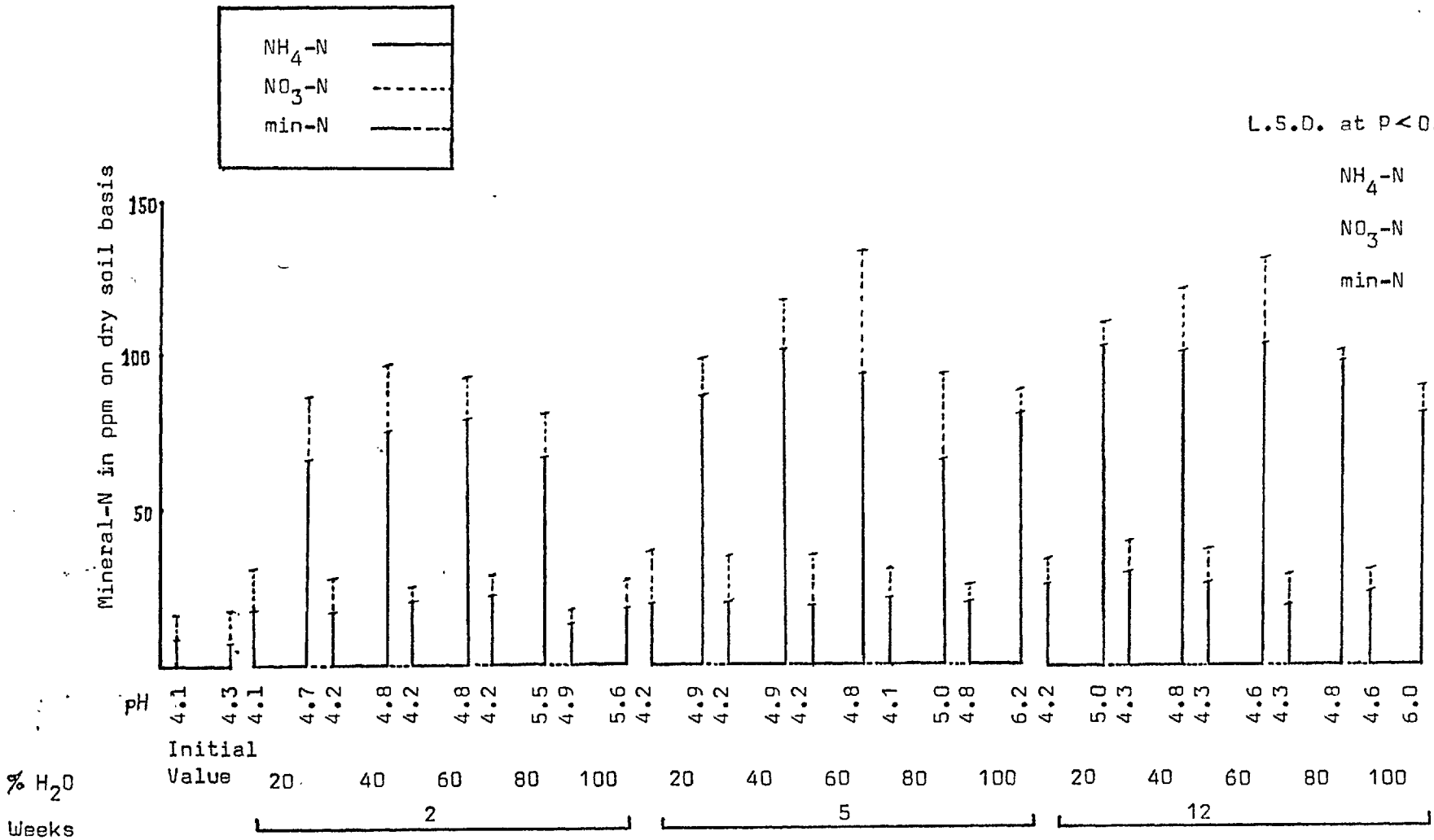
Figure 2 and Table 3 show results for pH and NH_4-, NO_3- and min-N levels initially and after each incubation period. Table 4 shows min-N accumulation values, obtained by subtracting initial values from those after each incubation period. Negative values indicate disappearance of min-N present initially.

No dried blood added

After 2 weeks of incubation there was no accumulation of min-N (NH_4- + NO_3-N) at 100% mwhc but there was significant accumulation at all other moisture contents to about the same extent. After 5 weeks incubation there was further min-N accumulation at all moisture contents including

FIGURE 2

EFFECTS OF VARYING STATIC MOISTURE CONTENT (20 to 100 % mwhc) ON MINERAL-N LEVELS DURING INCUBATION (2 to 12 weeks) OF LOW FLAT SOIL WITHOUT AND WITH ADDED "N" (200 ppm "N" AS DRIED BLOOD)



For each pair of values the left hand one represents no added N and the right hand one represents addition of N (200 ppm "N" from dried blood)

TABLE 3

EFFECTS OF VARYING STATIC MOISTURE CONTENT(20 to 100 %) ON MINERAL-N LEVELS DURING INCUBATION (2,5 and 12 weeks) OF LOW FLAT SOIL ALONE AND WITH ADDED DRIED BLOOD (200 ppm 'N' on soil basis)

(Results are given in ppm on dry soil basis)

	NH_4-N	NO_3-N	min-N	pH
Initial values(no N added)	8.8	7.4	16.2	4.1
Initial values(dried blood added)	8.0	10.2	18.2	4.3

After 2 weeks incubation

% mwhc	NH_4-N		NO_3-N		min-N		pH	
	Nil N	Added N	Nil N	Added N	Nil N	Added N	Nil N	Added N
20	16.8	67.2	13.7	16.8	30.5	84.0	4.1	4.7
40	17.5	75.6	9.4	20.3	26.9	95.9	4.2	4.8
60	21.7	80.2	3.5	13.0	25.2	93.2	4.2	4.8
80	23.1	67.2	6.0	14.0	29.1	81.2	4.2	5.5
100	14.0	18.2	3.6	9.8	17.6	28.0	4.9	5.6

After 5 weeks incubation

20	21.0	87.5	16.3	12.6	37.3	100.1	4.2	4.9
40	19.6	102.2	16.5	15.0	36.1	117.2	4.2	4.9
60	18.6	95.2	17.5	40.0	36.1	135.2	4.2	4.8
80	21.7	64.4	10.2	30.1	31.9	94.5	4.1	5.0
100	20.3	81.9	5.3	2.1	25.6	84.0	4.8	6.2

After 12 weeks incubation

20	25.9	104.7	7.0	4.9	32.9	109.6	4.2	5.0
40	29.8	102.2	10.2	19.6	40.0	121.8	4.3	4.8
60	27.3	103.6	10.5	28.5	37.8	132.1	4.3	4.6
80	19.6	99.8	10.5	2.8	30.1	102.6	4.3	4.8
100	24.5	83.0	5.3	6.0	29.8	89.0	4.6	6.0

L.S.D. $P<0.05$ NH_4-N 3.6, NO_3-N 5.3, min-N 3.7 ppm

For accumulation values see Table 4

TABLE 4

MIN-N ACCUMULATION VALUES FOR EXPT.1 AFTER EACH INCUBATION PERIOD

LOW FLAT SOIL (See Table 3)

(Results are in ppm N on dry soil basis)

% mwhc	NH_4-N		NO_3-N		min-N	
	Nil N	Added N	Nil N	Added N	Nil N	Added N
After 2 weeks incubation						
20	8.0	59.2	6.3	6.6	14.3	65.8
40	8.7	67.6	2.0	10.1	10.7	77.7
60	12.9	72.2	-3.9	2.8	9.0	75.0
80	14.3	59.2	-1.4	3.8	12.9	63.0
100	5.2	10.2	-3.8	-0.4	1.4	9.8
After 5 weeks incubation						
20	12.2	79.5	8.9	2.4	21.1	81.9
40	10.8	94.2	9.1	4.8	19.9	99.0
60	9.8	87.2	10.1	29.8	19.9	117.0
80	12.9	56.4	2.8	19.9	15.7	76.3
100	11.5	73.9	-2.1	-8.1	9.4	65.8
After 12 weeks incubation						
20	17.1	96.7	-0.4	-5.3	16.7	91.4
40	21.0	94.2	2.8	9.4	23.8	103.6
60	18.5	95.6	3.1	18.3	21.6	113.9
80	10.8	91.8	3.1	-7.4	13.9	84.4
100	15.7	75.0	-2.1	-4.2	13.6	70.8

L.S.D. $P<0.05$ NH_4-N 3.6, NO_3-N 5.3, min-N 3.7 ppm

100% mwhc. After 12 weeks of incubation there were no further significant changes in accumulation of min-N at 60 and 80% mwhc, increases at 40 and 100% mwhc and a decrease at 20% mwhc.

There was significant nitrate accumulation only at 20% mwhc after 2 weeks and at 20, 40, 60 and 80% mwhc after 5 weeks. After 12 weeks there was no significant nitrate accumulation.

There were slight changes in pH due to incubation with maximum changes at 100% mwhc. Even under the latter condition pH did not rise above 4.9.

Dried blood added

The greatest increase in min-N levels occurred during the first 2 weeks of incubation with maximum accumulation at 40 and 60% mwhc, but with only slight accumulation at 100% mwhc. During the 2 - 5 week period there were considerable increases in min-N at 60% and at 100% mwhc and relatively small, though significant, increases at other moisture contents. During the 5 - 12 week period there were further slight increases in min-N at all moisture contents except 60% mwhc.

There was significant accumulation of nitrate at 20 and 40% mwhc after 2 weeks, at 40, 60 and 80% mwhc after 5 weeks, and at 40 and 60% mwhc after 12 weeks of incubation. Maximum nitrate accumulated at 40% mwhc after 2 weeks and at 60% mwhc after 5 and 12 weeks. There was some disappearance

of nitrate during the 5 - 12 week period. At 80% mwhc all the nitrates formed after 5 weeks and that initially present had disappeared. However, there was still significant accumulation at 40 and 60% mwhc even after 12 weeks.

There were increases in pH with increasing moisture content after 2 weeks of incubation but little further change with further incubation except at 100% mwhc. The maximum pH value at 60% mwhc and lower moisture contents was 5.0, at 80% mwhc 5.5, and at 100% mwhc 6.2.

The apparent extent of mineralization of the organic N of dried blood ranged from 5% to 58%.

EXPERIMENT 2

Effects of varying static moisture content (20 to 100% mwhc) on mineral-N levels during incubation (2, 5 and 12 weeks) of HIGH FLAT soil alone and with added dried blood (200 ppm N on soil basis)

METHODS

The methods were exactly as described in Experiment 1 except that the high flat soil was used.

RESULTS

Figure 3 and Table 5 show results for pH and NH_4-, NO_3- and min-N levels initially and after each incubation period. Table 6 shows min-N accumulation values, obtained by subtracting initial values from those after each incubation period. Negative values indicate disappearance of min-N present initially.

No dried blood added

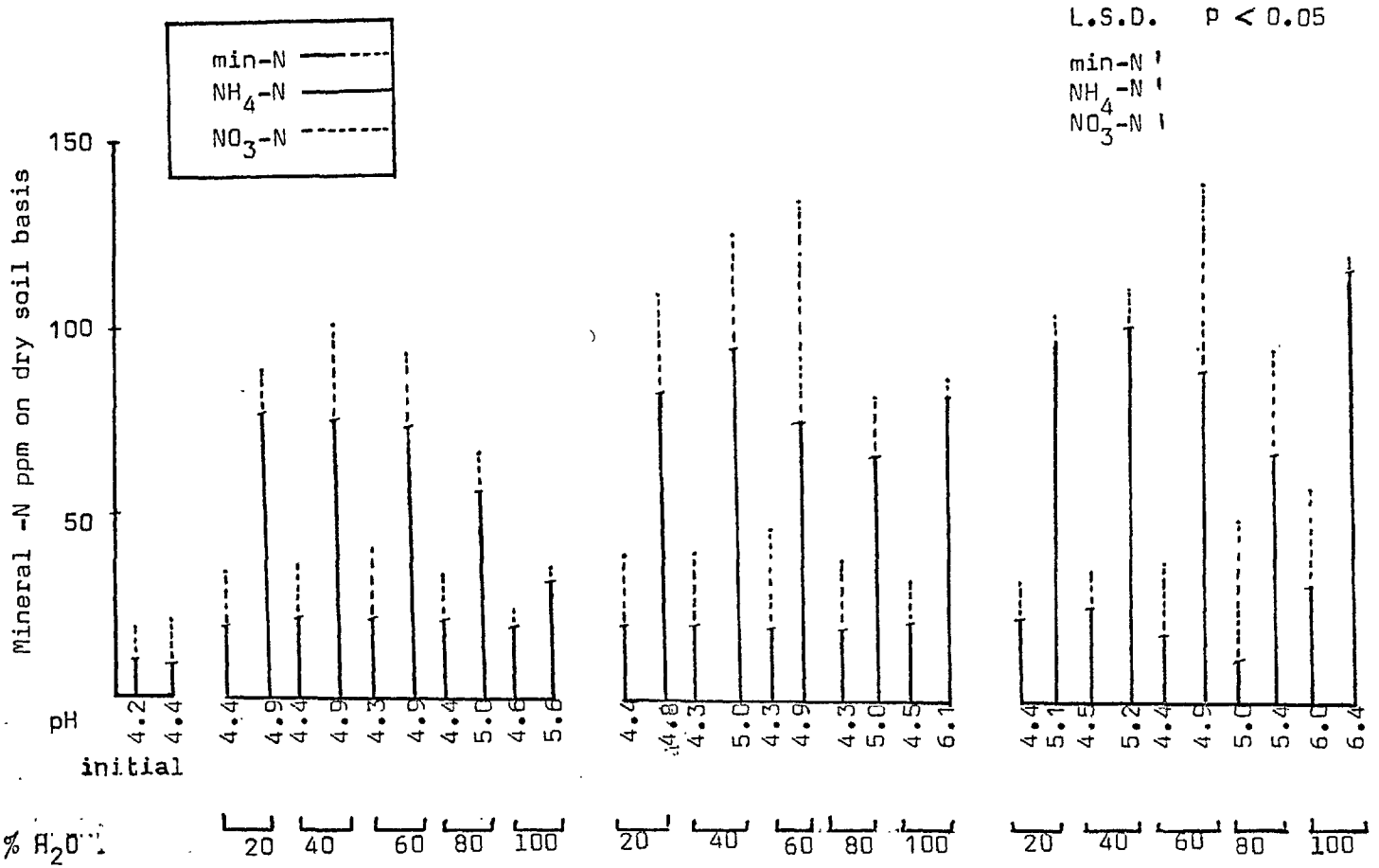
After 2 weeks of incubation there was significant accumulation of min-N at all moisture contents, with maximum accumulation at 60% mwhc. With incubation up to 5 weeks there was some further increase in min-N at all moisture contents, particularly at 100% mwhc. Maximum min-N accumulated at 60% mwhc. During the 5 - 12 week period min-N levels were unaffected at 20 and 40% mwhc, decreased at 60% mwhc, and increased at 80 and 100% mwhc. Maximum min-N occurred at 100% mwhc.

There was significant nitrate accumulation at all moisture contents except 100% mwhc up to 2 weeks of incubation. At 100% mwhc there was significant loss of nitrate. After 5 weeks of incubation nitrate levels increased further at all moisture contents including 100% mwhc. After 12 weeks of incubation nitrate contents decreased at 20, 40 and 60% mwhc, and increased at 80 and 100% mwhc. Maximum nitrate occurred at 60% mwhc after 2 and 5 weeks and at 80% mwhc

FIGURE 3

EFFECTS OF VARYING STATIC MOISTURE CONTENT (20 to 100 % mwhc) ON MINERAL-N LEVELS DURING INCUBATION (2 to 12 weeks) OF HIGH FLAT SOILS WITHOUT AND WITH ADDED "N"

(200 ppm "N" as dried blood)



For each pair of values the left hand one represents no added "N and the right hand one represents added "N".

TABLE 5

EFFECTS OF VARYING STATIC MOISTURE CONTENT (20 to 100% mwhc) ON MINERAL-N LEVELS DURING INCUBATION (2,5 and 12 weeks) OF HIGH FLAT SOIL ALONE AND WITH ADDED DRIED BLOOD (200 ppm N ON SOIL BASIS)

(Results are given in ppm on dry soil basis)

	NH_4-N	NO_3-N	min-N	pH
Initial values (no N added)	9.8	8.4	18.2	4.2
Initial values (dried blood added)	9.8	11.2	21.0	4.4

After 2 weeks incubation

% mwhc	NH_4-N		NO_3-N		min-N		pH	
	Nil N	Added N	Nil N	Added N	Nil N	Added N	Nil N	Added N
20	18.9	76.3	14.0	12.3	32.9	88.6	4.4	4.9
40	21.0	74.6	15.4	26.9	36.4	101.5	4.4	4.9
60	20.2	72.7	19.7	20.4	39.9	93.1	4.3	4.9
80	21.0	55.9	11.9	9.8	32.9	65.7	4.4	5.0
100	19.6	32.2	3.1	2.8	22.7	35.0	4.6	5.6
After 5 weeks incubation								
20	19.6	83.9	17.5	25.9	37.1	109.8	4.4	4.8
40	19.3	95.5	20.7	31.5	40.0	127.0	4.3	5.0
60	21.0	76.0	25.1	59.9	46.1	135.9	4.3	4.9
80	19.3	65.1	19.3	16.8	38.6	81.9	4.3	5.0
100	18.2	84.0	13.9	3.5	32.1	87.5	4.5	6.1
After 12 weeks incubation								
20	22.8	98.7	11.5	7.4	34.3	106.1	4.4	5.1
40	25.2	104.3	13.7	8.1	38.9	112.4	4.5	5.2
60	18.5	90.6	18.2	51.5	36.7	142.1	4.4	4.9
80	10.5	67.5	39.1	28.7	49.6	96.2	5.0	5.4
100	31.5	118.0	26.0	1.2	57.5	119.2	6.0	6.4

L.S.D. at $P<0.05$ NH_4-N 2.4, NO_3-N 3.2, min-N 3.3 ppm

For accumulation values see Table 6

TABLE 6

MIN-N ACCUMULATION VALUES FOR EXPT.2 AFTER EACH INCUBATION PERIOD

HIGH FLAT SOIL (See Table 5)

(Results are given in ppm on dry soil basis)

% mwhc	NH_4-N		NO_3-N		min-N	
	Nil N	Added N	Nil N	Added N	Nil N	Added N
After 2 weeks incubation						
20	9.1	66.5	5.6	1.1	14.7	67.6
40	11.2	64.8	7.0	15.7	18.2	80.5
60	10.4	62.9	11.3	9.2	21.7	72.1
80	11.2	46.1	3.5	-1.4	14.7	44.7
100	9.8	22.4	-5.3	-8.4	4.5	14.0
After 5 weeks incubation						
20	9.8	74.1	9.1	14.7	18.9	88.8
40	9.5	85.7	12.3	20.3	21.8	106.0
60	11.2	66.2	16.7	48.7	27.9	114.9
80	9.5	55.3	10.9	5.6	20.4	60.9
100	8.4	74.2	5.5	-7.7	13.9	66.5
After 12 weeks incubation						
20	13.0	88.9	3.1	-3.8	16.1	85.1
40	15.4	94.5	5.3	-3.1	20.7	91.4
60	8.7	80.8	9.8	40.3	18.5	121.1
80	0.7	57.7	30.7	17.5	31.4	75.2
100	21.7	108.2	17.6	-10.0	39.3	98.2

L.S.D. $P < 0.05$ NH_4-N 2.4, NO_3-N 3.2, min-N 3.3 ppm

after 12 weeks of incubation.

There were only slight differences in pH due to moisture content after 2 and 5 weeks of incubation (pH ranged from 4.3 to 4.6). After 12 weeks of incubation pH increased with increasing moisture content above 60% mwhc (pH ranged from 4.4 to 6.0).

Dried blood added

Min-N accumulation after 2 weeks of incubation increased from 40% to 60% mwhc and decreased with further increasing moisture content. During the 2 - 5 week period min-N increased further at all moisture contents, the greatest increases occurring at 60 and 100% mwhc. During the 5 - 12 week period min-N decreased slightly at 20 and 40% mwhc, increased slightly at 60% mwhc, and increased to a fair extent at 80 and 100% mwhc. After both 5 and 12 weeks maximum min-N accumulation occurred at 60% mwhc.

After 2 weeks of incubation nitrate accumulated only at 40 and 60% mwhc, and there was loss of nitrate at 100% mwhc. With incubation up to 5 weeks nitrate accumulation increased at all moisture contents below 100% mwhc, particularly at 60% mwhc. After 12 weeks of incubation at 20 and 40% mwhc there was complete disappearance of nitrate formed earlier, and slight disappearance occurred at 60% mwhc. At 80% mwhc there was further nitrate accumulation, whilst at 100% mwhc

there was complete loss of nitrate present initially.

There were only slight differences in pH due to moisture content from 20 to 60% mwhc after all incubation periods (pH ranged from 4.8 to 5.2). pH at 80% mwhc and, in particular at 100% mwhc was higher than at lower moisture contents, and increased with length of incubation.

The apparent extent of mineralization of the organic N of dried blood ranged from 8% to 62%.

DISCUSSION OF EXPERIMENTS 1 AND 2

This discussion will be concerned mainly with the differences in results obtained between LOW FLAT and HIGH FLAT soil.

Where dried blood was not added the main differences between the two soils occurred at the higher moisture contents during the later stages of incubation. The high flat soil accumulated much more mineral-N and nitrate at 80 and 100% mwhc during the 5 - 12 week period than did the low flat soil. The high flat soil is higher in total N than the low flat soil (0.073% and 0.061%). The difference was reflected only at the higher moisture contents and indicates differences in the behaviour of mineralizing and nitrifying organisms between the two soils. The considerable accumulation of nitrate in the high flat soil at partially anaerobic conditions of 80% mwhc and even at apparently completely anaerobic conditions of 100% mwhc, compared with no nitrate accumulation in the

low flat soil at these moisture contents is particularly interesting. In the high flat soil nitrate accumulation at these higher moisture contents was greater than at the lower, apparently completely aerobic, moisture contents. It appears that in this soil nitrification is favoured at high moisture content. This may be due to higher moisture requirement for activity of the nitrifying organisms. Alternatively, some other nitrifying process, apart from the usual autotrophic process may be operative at the higher moisture content. The other process may be heterotrophic nitrification, as reported by other workers (14,15). The fact that the significant nitrate accumulation at the higher moisture contents did not occur until the 5 - 12 week period tends to confirm this. During the first 2 weeks of incubation the high flat soil behaved in the same way as temperate region soils with respect to its pattern of nitrogen mineralization, namely, maximum nitrogen mineralization and nitrification at 60% mwhc and loss of nitrate (probably by denitrification) at 100% mwhc. During the 2 - 5 week period the pattern was similar except for nitrate accumulation at 80 and 100% mwhc, indicating that the autotrophic process still contributed mainly to nitrification at 60% mwhc and at low moisture contents, whilst the heterotrophic process became operative at the higher moisture contents. With incubation beyond 5 weeks heterotrophic nitrification appeared to accelerate at 80 and 100% mwhc, whilst there was no further nitrate accumulation at the lower moisture contents.

Where dried blood was added the pattern of nitrogen mineralization with respect to moisture content was similar in both soils during the first 5 weeks of incubation; both soils showed maximum mineral-N accumulation at 40% mwhc up to 2 weeks and at 60% mwhc up to 5 weeks. With incubation up to 12 weeks the two soils differed in that mineral-N accumulation was greater at 80 than at 100% mwhc in the low flat soil. It appears that there was also differences in the microbial pattern between these two soils which only showed up after prolonged incubation and at the higher moisture contents.

With regard to nitrification in the soils treated with dried blood there were significant differences between the two soils after all periods of incubation and at most moisture contents. In particular, after 5 weeks the high flat soil accumulated more nitrate at 20 to 60% mwhc than did the low flat soil at corresponding moisture contents. This was not related to pH since pH values were almost identical between the two soils at each moisture content. It appears that the autotrophic organisms, assuming that only these are operative at these low moisture contents, were stimulated by the presence of the dried blood to a greater extent in the high flat than in the low flat soil. At 80% mwhc differences again became apparent between the two soils, in that in the low flat soil there was some nitrate accumulation after 5 weeks, but most of this

disappeared after 12 weeks, whereas in the high flat soil nitrate continued to accumulate up to 12 weeks. However, at 100% mwhc both soils behaved similarly by showing a decrease in nitrate accumulation with incubation up to 12 weeks.

It is possible that the presence of the dried blood and its metabolic products (including ammonia) prevented heterotrophic nitrification at 100% but not at 80% mwhc. Alternatively, since the soil pH rose to 5.4, due to ammonia accumulation arising from ammonification of dried blood, this pH may have permitted some autotrophic nitrification at 80% mwhc. Later work (see chapter 5) on the effects of changing soil pH showed that in this soil the highest nitrification occurred at pH 5.7 and 7.2, which is very near the pH attained in this experiment. The lack of any nitrate accumulation at 100% mwhc in spite of the high pH of 6.4 attained also indicates that the normal autotrophic process is occurring, and nitrification is being prevented by lack of oxygen at the high moisture content. The virtually complete loss after 12 weeks of nitrate present initially also indicates a normal behaviour for both the low flat and high flat soils in the presence of dried blood.

The fact that ammonium was present in both soils with all treatments after all periods of incubation indicates that at no stage was nitrate formation being limited by lack of ammonium. This applies only to autotrophic nitrification, which requires the presence of ammonium. In the case o

heterotrophic nitrification ammonium is not necessary, since organic nitrogenous compounds containing N at a level of oxidation higher than that of ammonium can be converted to nitrite by many types of soil organisms (bacteria, fungi and actinomycetes) (15,51). Relatively few heterotrophic microorganisms are able to generate nitrate from organic nitrogenous compounds, these being Aspergillus and Pseudomonas (15,51,231). However, providing soil conditions are satisfactory there seems no reason why nitrite produced heterotrophically cannot be converted to nitrate by the normal autotrophic process, i.e., oxidation by nitrobacter.

In the acid tea soils used in these experiments it is possible that nitrosomonas ($NH_4 \longrightarrow NO_2^-$) was being suppressed at the high moisture contents but nitrite was being produced by heterotrophic organisms, and this was then converted to nitrate by nitrobacter, which was not suppressed by the high moisture content and low pH.

EXPERIMENT 3

Effects of varying static moisture contents (60 to 133% mwhc) on mineral-N levels during incubation (5 and 13 weeks) of HIGH FLAT soil without and with added ammonium sulphate (100 ppm N)

In view of the unusual results obtained with the high flat soil at high moisture contents in the previous experiment (Expt.2) a further experiment was set up with the

high flat soil to determine the effects of adding ammonium sulphate. Only the higher moisture contents were studied and in addition a further moisture content of 133% mwhc (representing water-logging) was included.

METHODS

Finely ground ammonium sulphate was mixed with the soil in bulk to supply 100 ppm added N on the dry soil basis. The soils were set up for incubation as previously described and control soils (no ammonium sulphate added) were also set up. Moisture contents used were 60, 80, 100 and 133% mwhc and analysis were done initially and after 5 and 13 weeks of incubation at 30°C. Otherwise, the methods were the same as used in the previous experiments.

RESULTS

Figure 4 and Table 7A show for high flat soil without added ammonium sulphate results for pH and NH_4-, NO_3- and min-N levels initially and after each period of incubation. Table 7B shows accumulation values for NH_4-, NO_3- and min-N. Figure 5 and Table 8A show results for ammonium sulphate-treated soil, and Table 8B shows accumulation values.

Where no ammonium sulphate was added min-N accumulation after 5 weeks of incubation was the same at all moisture contents. After 13 weeks of incubation there was further

FIGURE 4

EFFECTS OF VARYING STATIC MOISTURE CONTENT (60 to 133 % mwhc) ON MINERAL-N LEVELS DURING INCUBATION (5 and 13 Weeks) OF HIGH FLAT SOIL WITHOUT ADDED AMMONIUM SULPHATE

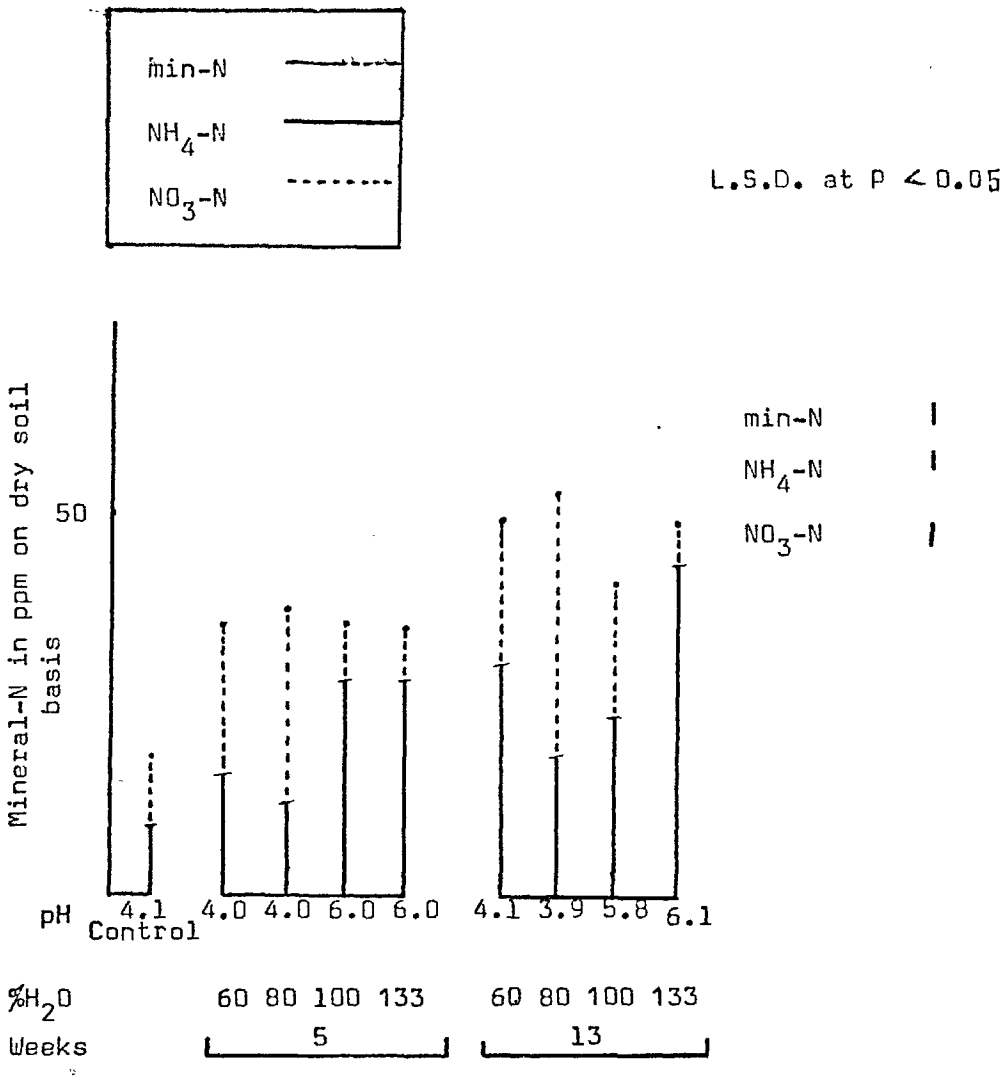


TABLE 7 A

EFFECTS OF VARYING STATIC MOISTURE CONTENT (60 to 133%mwhc) ON MINERAL-N LEVELS DURING INCUBATION (5 and 13 weeks) OF HIGH FLAT SOIL WITHOUT ADDED AMMONIUM SULPHATE

(Results are given in ppm on dry soil basis)

	NH_4-N	NO_3-N	min-N	pH
Initial values	9.3	8.4	17.7	4.2
After 5 weeks incubation				
% mwhc	NH_4-N	NO_3-N	min-N	pH
60	15.8	20.3	36.1	4.1
80	11.9	25.6	37.5	4.0
100	27.7	7.7	35.4	6.0
133	28.4	7.4	35.8	6.0
After 13 weeks incubation				
60	30.1	19.6	49.7	4.1
80	17.5	35.0	52.5	3.9
100	24.2	17.2	41.4	5.8
133	44.1	5.6	49.7	6.1

TABLE 7 B

Mineral-N accumulation values for the above experiment

After 5 weeks incubation				
60	6.5	11.9	18.4	
80	2.6	17.2	19.8	
100	18.4	-0.7	17.7	
133	19.1	-1.0	18.1	
After 13 weeks incubation				
60	20.8	11.2	32.0	
80	8.2	26.6	34.8	
100	14.9	8.8	23.7	
133	34.8	-2.8	32.0	

L.S.D. $P < 0.05$ NH_4-N 1.6, NO_3-N 3.5, min-N 2.8 ppm

FIGURE 5

EFFECTS OF VARYING STATIC MOISTURE CONTENT (60 to 133% mwhc) ON MINERAL-N LEVELS DURING INCUBATION (5 and 13 weeks) OF HIGH FLAT SOIL WITH ADDED AMMONIUM SULPHATE (100 ppm N on soil basis)

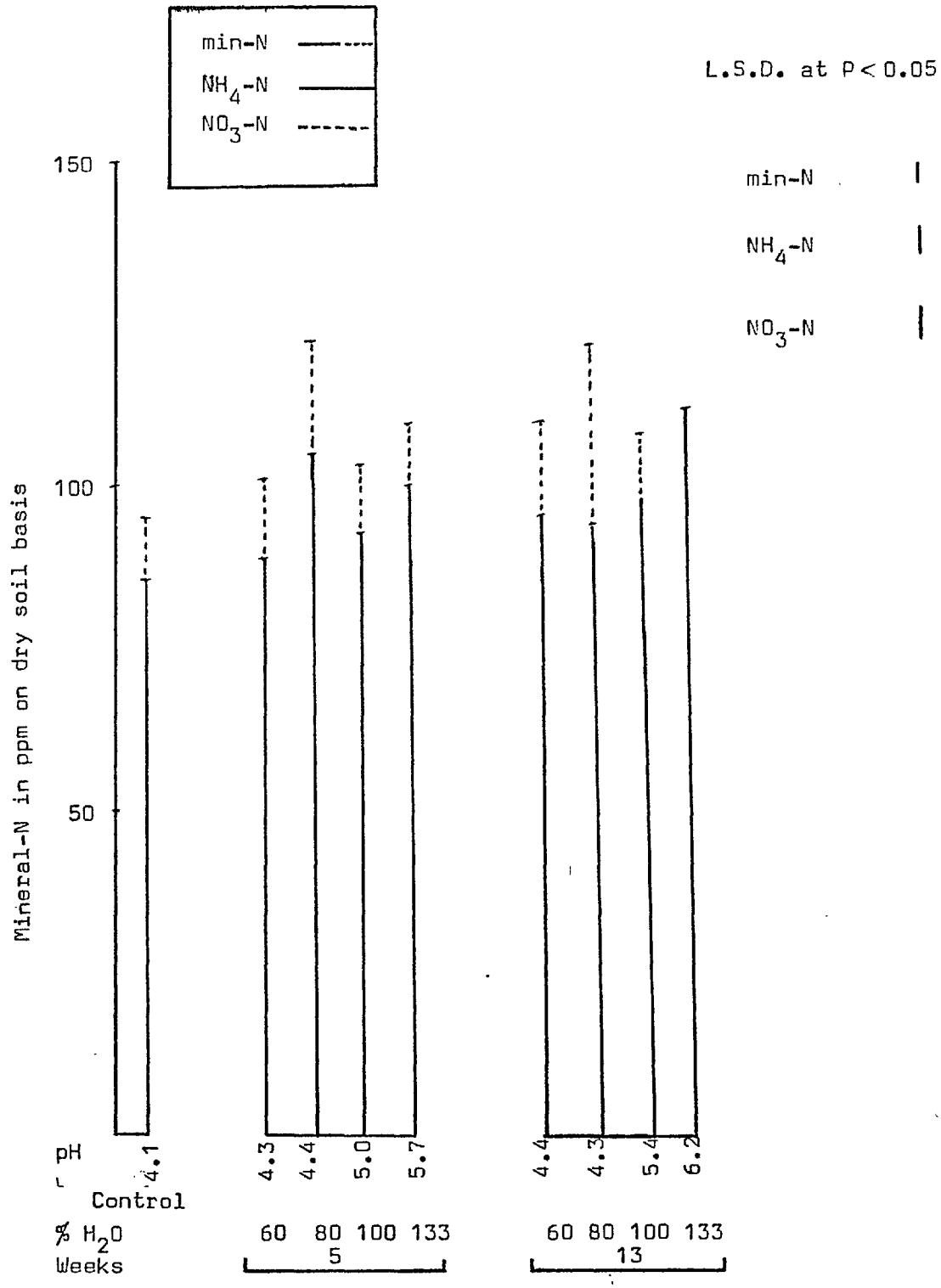


TABLE 8 A

EFFECTS OF VARYING STATIC MOISTURE CONTENT (40to 133%mwhc) ON MINERAL-N LEVELS DURING INCUBATION (5 and 13 weeks) OF HIGH FLAT SOIL WITH ADDED AMMONIUM SULPHATE (100 ppm N on soil basis)

(Results are given in ppm on dry soil basis)

	NH_4-N	NO_3-N	min-N	pH
Initial values	85.4	9.1	94.5	4.1
After 5 weeks incubation				
%mwhc	NH_4-N	NO_3-N	min-N	pH
60	88.2	13.2	101.4	4.3
80	105.0	17.5	122.5	4.4
100	93.1	9.8	102.9	5.0
133	100.4	9.6	110.0	5.7
After 13 weeks incubation				
60	95.9	14.7	110.6	4.4
80	94.5	27.3	121.8	4.3
100	98.0	9.8	107.8	5.4
133	112.0	0.0	112.0	6.2

TABLE 8 B

Mineral-N accumulation values for the above experiment

After 5 weeks incubation				
60	2.8	4.1	6.9	
80	19.6	8.4	28.0	
100	7.7	0.7	8.4	
133	15.0	0.5	15.5	
After 13 weeks incubation				
60	10.5	5.6	16.1	
80	9.1	18.2	27.3	
100	12.6	0.7	13.3	
133	26.6	-9.1	17.5	

L.S.D. $P < 0.05$ NH_4-N 3.7, NO_3-N 4.2, min-N 2.6 ppm

min-N accumulation, with a maximum at 80% and a minimum at 100% mwhc. After 5 weeks there was significant nitrate accumulation only at 60 and 80% mwhc, and after 13 weeks at 60, 80 and 100% mwhc. Nitrate accumulation increased from 5 to 13 weeks at 80 and 100% mwhc and maximum nitrate accumulation occurred at 80% mwhc after both periods of incubation.

Where ammonium sulphate was added maximum min-N levels occurred at 80% mwhc after 5 and 13 weeks. There was a slight increase in min-N from 5 to 13 weeks at 60% mwhc but little change occurred at the higher moisture contents. After 5 weeks there was significant nitrate accumulation only at 80% and after 13 weeks only at 60 and 80% mwhc. At 133% mwhc nitrate present initially was unaffected after 5 weeks, but completely disappeared after 13 weeks of incubation. Maximum nitrate accumulation occurred at 80% mwhc after 5 and 13 weeks.

Soil pH was little affected by either period of incubation at 60 and 80% mwhc, but increased at 100 and 133% mwhc.

DISCUSSION OF EXPERIMENT 3

The lack of any disappearance of nitrate at 133% mwhc even after 13 weeks of incubation of the control soil indicates a low activity of denitrifying organisms in this

soil even at the apparently completely anaerobic conditions of water-logging. However, where ammonium sulphate was applied to the high flat soil denitrification was stimulated to some extent, as shown by complete disappearance of nitrate, but then only after 13 weeks. This may be compared with results obtained for an English soil of similar texture from which initial nitrate completely disappeared even after 3 weeks of incubation at 133% mwhc and even where no ammonium sulphate have been added (232).

The maximum accumulation of nitrate which occurred at 80% mwhc after both periods of incubation where ammonium sulphate was added again indicates that heterotrophic nitrification may be operative at this aerobic-anaerobic moisture content. The fair increase in nitrate from 5 to 13 weeks tends to confirm this. The greater nitrate accumulation at 80% than at 60% mwhc where ammonium sulphate was added compared with the lesser accumulation where dried blood was added indicates that ammonium sulphate inhibited autotrophic nitrification, whereas dried blood tended to inhibit heterotrophic nitrification.

Where either dried blood or ammonium sulphate was added nitrate accumulation was higher at 80% than at 100 or 133% mwhc and this was correlated with increase in soil pH. Nitrate accumulated generally only when soil pH remained low or increased to a slight extent, but not when it increased to a fair extent.

The fair increase in pH (0.9 to 2.1 units) at 100 and 133% mwhc where ammonium sulphate was applied could not always be explained on the basis of increased levels of NH_4-N in the soils due to incubation. Ammonium accumulation tends to increase pH whereas nitrate accumulation will tend to decrease pH; sulphate residues from applied ammonium sulphate would also tend to decrease pH if left unchanged, but would not do so if converted to organic sulpher compounds by immobilization. The variation in the extent of sulphate immobilization in this way could help to explain the considerable increases in pH which occurred, particularly at 133% mwhc.

SUMMARY

The effects of moisture contents ranging from 20% to 100% mwhc on mineral-N levels during incubation (2-12 weeks) of LF and HF soils without and with addition of dried blood (200 ppm N on soil basis) were studied. Where no N was added maximum nitrogen mineralization and nitrate accumulation in the HF soil occurred at 60% mwhc after 5 weeks; after 12 weeks maximum nitrogen mineralization occurred at 100% mwhc and maximum nitrification at 80% mwhc. In the LF soil maximum nitrogen mineralization occurred at 20% mwhc after 5 weeks and at 40% mwhc after 12 weeks. Nitrate accumulation was less in the LF than in the HF

soil at all, particularly the higher, moisture contents. In the LF soil nitrate accumulation was at a maximum at 20% mwhc after 2 weeks and 40% to 60% mwhc after 5 weeks, whilst there was no significant nitrate accumulation at any moisture content after 12 weeks. Where dried blood was added both HF and LF soils behaved similarly, in that maximum nitrogen mineralization and nitrification occurred at 40% mwhc after 2 weeks and at 60% mwhc after 5 and 12 weeks.

The effects of static moisture contents ranging from 60% to 133% mwhc on mineral-N levels during incubation (5 and 13 weeks) of HF soil were studied without and with addition of ammonium sulphate (100 ppm N on soil basis). Where no N was added nitrogen mineralization was similar at all moisture contents after 5 weeks of incubation; after 13 weeks nitrogen mineralization was least at 100% mwhc, but approximately the same at the other moisture contents. Nitrate accumulation was at a maximum at 80% mwhc after 5 and 13 weeks.

The results were discussed in relation to the possibility of the occurrence of heterotrophic nitrification in the HF soil at the higher moisture contents.

CHAPTER 4

MINERALIZATION OF NITROGEN AND NITRIFICATION DURING INCUBATION OF HIGH FLAT AND LOW FLAT SOILS IN RELATION TO pH

Experiment 4

Mineral-N levels during incubation (30°C, 50% mwhc) for 2 to 12 weeks of LOW FLAT (LF) and HIGH FLAT (HF) soils treated with 0.1 - 0.8% calcium carbonate ($CaCO_3$) just before incubation

Experiment 5

Mineral-N levels during incubation (1 to 6 weeks at 30°C, 50% mwhc) of LOW FLAT soil (which had been previously adjusted to different pH levels) treated with 100 ppm N as urea and ammonium sulphate ($(NH_4)_2SO_4$)

CHAPTER 4

MINERALIZATION OF NITROGEN AND NITRIFICATION DURING INCUBATION OF HIGH FLAT AND LOW FLAT SOILS IN RELATION TO pH

This chapter describes two experiments with soil pH as the main variable. The first (Expt. 4) reports on the effects of adding varying levels of calcium carbonate ($CaCO_3$) just before incubation to both high flat and low flat soils on accumulation of ammonium and nitrate-N during 12 weeks of incubation. The second (Expt. 5) uses the low flat soil which had been ajusted to varying pH levels by addition of varying amounts of calcium carbonate and kept moist for two months before being air dried and set up for incubation. These soils were incubated alone and also after addition of 100 ppm N as urea and ammonium sulphate. Incubation was for 6 weeks.

Experiment 4

Mineral-N levels during incubation (30°C, 50% mwhc) for 2 to 12 weeks of LOW FLAT (LF) and HIGH FLAT (HF) soils treated with 0.1 - 0.8% calcium carbonate ($CaCO_3$) just before incubation

METHODS

A bulk of both low flat and high flat soils were

mixed with monocalcium phosphate to supply 200 ppm P. This was to ensure that phosphorus would not be limiting mineral-N changes. Suitable amounts of these soils were then mixed with 0.1, 0.2, 0.4, and 0.8% by weight of finely ground calcium carbonate. Sufficient tubes of each treatment, including controls (no $CaCO_3$ added), were set up for aerobic incubation to allow for analysis in duplicates after 2, 4 and 12 weeks of incubation. Water was added to 50% mwhc and incubation temperature was 30°C. The Morgan reagent was used for extraction of min-N.

RESULTS

Table 9 and Figure 6 show results for NH_4-, NO_3- and min-N levels and pH after each incubation period. Also shown are the initial values for the min-N forms. Table 10 shows accumulation values after each period of incubation.

Mineral-N

After two weeks incubation of both HF and LF soils min-N accumulation increased with addition of calcium carbonate up to 0.4% to about the same extent, whilst 0.8% calcium carbonate resulted in somewhat lower min-N accumulation than with 0.4% calcium carbonate. After four weeks incubation there were further increases in min-N accumulation in both soils, and accumulation increased with calcium carbonate addition up to 0.8%. After 12 weeks incubation of the LF soil there were further increases in min-N accumulation

TABLE 9

MINERAL-N LEVELS DURING INCUBATION (30°C, 50% mwhc) FOR 2 TO 12 WEEKS OF LOW FLAT (LF) AND HIGH FLAT (HF) SOILS TREATED WITH 0.1 - 0.8% CALCIUM CARBONATE (200 ppm P as monocalcium phosphate added to all samples before incubation)

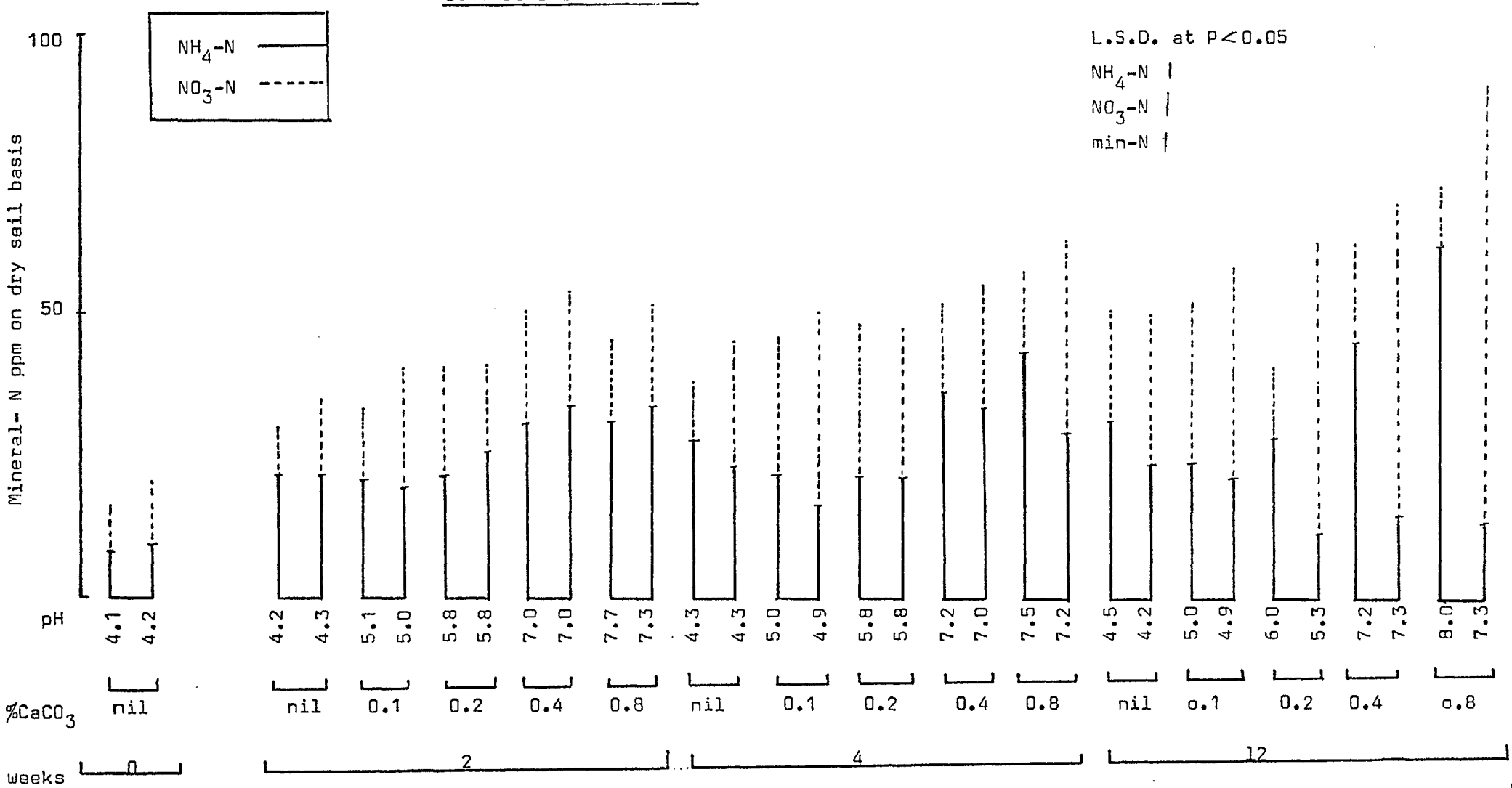
(Results are in ppm on dry soil basis)

	pH		NH_4-N		NO_3-N		min-N	
	LF	HF	LF	HF	LF	HF	LF	HF
Initial values	4.1	4.2	7.7	9.8	8.4	10.5	16.1	20.3
After 2 weeks incubation								
%$CaCO_3$								
0.0	4.2	4.3	21.0	21.0	9.1	14.0	30.1	35.0
0.1	5.1	5.0	20.3	19.6	12.6	21.4	32.9	41.0
0.2	5.8	5.8	22.4	25.2	17.9	15.8	40.3	41.0
0.4	7.0	7.0	31.9	34.6	18.6	19.8	50.5	54.4
0.8	7.7	7.3	31.9	34.4	13.3	17.4	45.2	51.8
After 4 weeks incubation								
0.0	4.3	4.3	28.0	23.1	10.5	22.0	38.5	45.1
0.1	5.0	4.9	21.7	15.4	25.2	35.8	46.9	51.2
0.2	5.8	5.8	22.4	21.4	26.3	27.7	48.7	49.1
0.4	7.2	7.0	37.0	34.7	15.5	21.7	52.5	56.4
0.8	7.5	7.2	44.5	29.4	14.4	35.0	58.9	64.4
After 12 weeks incubation								
0.0	4.5	4.2	32.2	24.5	19.0	26.4	51.2	50.9
0.1	5.0	4.9	23.8	21.7	29.0	37.5	52.8	59.2
0.2	6.0	5.6	28.7	11.2	12.8	53.2	41.5	64.4
0.4	7.2	7.3	45.5	14.0	18.5	56.7	64.0	70.7
0.8	8.0	7.3	63.8	14.0	10.8	78.8	74.6	92.8

L.S.D. at $P < 0.05$ NH_4-N 3.0, NO_3-N 3.8, min-N 3.1 ppm

FIGURE 6

MINERAL-N LEVELS DURING INCUBATION (30^0C, 50% mwhc) FOR 2 to 12 weeks of LOW FLAT (LF) AND HIGH FLAT (HF) SOILS TREATED WITH 0.1 - 0.8% CALCIUM CARBONATE ($CaCO_3$) JUST BEFORE INCUBATION



For each pair of values the left hand side represents values for LOW FLAT soil and the right hand one represents HIGH FLAT soil.

TABLE 10

MINERAL-N ACCUMULATION VALUES FOR EXPT.4 AFTER EACH INCUBATION PERIOD

(Results are in ppm on dry soil basis)

After 2 weeks incubation

% $CaCO_3$	pH		NH_4-N		NO_3-N		min-N	
	L F	H F	L F	H F	L F	H F	L F	H F
0.0	4.2	4.3	13.3	11.2	0.7	3.5	14.0	14.7
0.1	5.1	5.0	10.8	9.8	5.2	12.3	16.0	22.1
0.2	5.8	5.8	12.9	15.4	10.5	6.7	23.4	22.1
0.4	7.0	7.0	22.4	24.8	11.2	10.7	33.6	35.5
0.8	7.7	7.3	22.4	24.6	5.9	8.3	28.3	32.9
After 4 weeks incubation								
0.0	4.3	4.3	20.3	13.3	2.1	11.5	22.4	24.8
0.1	5.0	4.9	12.2	5.6	17.8	26.7	30.0	32.3
0.2	5.8	5.8	12.9	11.6	18.9	18.6	31.8	30.2
0.4	7.2	7.0	27.5	24.9	8.1	12.6	35.6	37.5
0.8	7.5	7.2	35.0	19.6	7.0	25.9	42.0	45.5
After 12 weeks incubation								
0.0	4.5	4.2	24.5	14.7	10.6	15.9	35.1	30.6
0.1	5.0	4.9	14.3	11.9	21.6	28.4	35.9	40.3
0.2	6.0	5.6	19.2	1.4	5.4	44.1	24.6	45.5
0.4	7.2	7.3	36.0	4.2	11.1	47.6	47.1	51.8
0.8	8.0	7.3	54.3	4.2	3.4	69.7	57.7	73.9

L.S.D. $P < 0.05$ NH_4-N 3.0, NO_3-N 3.8, min-N 3.1 ppm

except where 0.2% calcium carbonate was added, where there was a decrease compared with results after 4 weeks incubation. Apart from this exception both soils showed increasing min-N accumulation with addition of calcium carbonate up to 0.8%. At any particular level of added calcium carbonate the HF soil showed higher min-N accumulation than did the LF soil.

Nitrate-N

There were differences in the extent of nitrate accumulation between LF and HF soils and these differences increased with length of incubation. There were no consistent differences in nitrate accumulation between low flat and high flat soils due to calcium carbonate addition up to 2 weeks of incubation. However, after 4 and 12 weeks of incubation nitrate accumulation was higher in the HF than in the LF soil with all levels of calcium carbonate, except where 0.2% calcium carbonate was added. The differences due to soil type were particularly great after 12 weeks of incubation with addition of 0.2 to 0.8% calcium carbonate. At these levels of added calcium carbonate min-N accumulated almost entirely as nitrate in the HF soil, but mainly as ammonium in the LF soil. In the LF soil maximum nitrate accumulation after 12 weeks occurred with 0.1% calcium carbonate, whereas in the HF soil nitrate accumulation increased with addition of calcium carbonate up to 0.8%.

The LF soil treated with 0.2% calcium carbonate was the only one to show some disappearance of min-N and considerable disappearance of nitrate-N during the 4 - 12 week period.

pH

Soil pH values after each period of incubation with any particular level of calcium carbonate were very similar in both soils, except that after 12 weeks of incubation where 0.2 and 0.8% calcium carbonate were added the pH of the LF soils was higher than that of the corresponding HF soil.

DISCUSSION

The pattern of mineralization of N (accumulation of NH_4+NO_3-N) in relation to increasing pH due to calcium carbonat addition was similar in both LF and HF soils. The results were similar to those obtained when acid temperate region soils are treated with calcium carbonate, namely, increasing mineralization of N with increasing pH up to slight alkalinity (1,15,36,37,38,39). Thus the nature of the nitrogen mineralising organisms present in temperate region soils and the two Pakistani soils appear to be similar at least as far as their responses to increasing soil pH are concerned. The somewhat higher N mineralization in the HF than in the LF soil can be accounted for by the higher total N content of the former soil (0.073% N in HF and 0.061% N in the LF soil)

However, responses in nitrification to increasing pH were quite different between the LF and HF soils. The HF soil behaved similarly to temperate region soils in showing increased nitrate accumulation with increasing pH up to slight alkalinity (35,72,73,74,75). The LF soil, on the other hand, showed in general higher nitrification with the lower than with the higher levels of addition of calcium carbonate. The pH at which maximum nitrification occurred decreased with time of incubation from pH 7.0 after two weeks to pH 5.0 after 12 weeks. It appears therefore that the nitrifying characteristics of the LF soil are quite different to those of the HF soil, at least as far as they are affected by changes in pH.

Some reasons for the difference between the two types of soil may be discussed:

i) The LF soil may contain initially smaller numbers of nitrifying bacteria than the HF soil and increasing pH to the higher levels did not provide suitable conditions for proliferation of nitrifying organisms in the LF soil. In fact, the higher levels of calcium carbonate, giving pH values of about 6 or more, may have been toxic to the nitrifiers in this soil. The higher accumulation of ammonium, particularly during the 4 - 12 week incubation period, is a reflection of this and this high ammonium accompanied by high pH may have been toxic to nitrifying bacteria. This is in contrast to low NH_4-N levels in the HF soils of

higher pH after 12 weeks of incubation.

ii) The differences in nitrification patterns between the LF and HF soils may also be considered in relation to the presence of autotrophic and heterotrophic nitrifying organisms. In the previous chapter (chapter 3) where nitrate accumulated in the HF but not LF soil at high moisture contents (80 to 100% mwhc) it was considered that this may have been due to heterotrophic nitrification in the HF soil. In the present experiment where nitrate accumulated only in the HF soil when pH was high it is possible that the activity of both heterotrophic and autotrophic nitrifying organisms in this soil may have been stimulated at the higher pH levels. In the LF soil, on the other hand, where there was no evidence for heterotrophic nitrification in chapter 3 the possible absence of heterotrophic nitrifiers could account for the absence of nitrification at the higher pH levels.

EXPERIMENT 5

Mineral-N levels during incubation (1 to 6 weeks at 30°C, 50% mwhc) of LOW FLAT soil (which had been previously adjusted to different pH levels) treated with 100 ppm N as urea and ammonium sulphate ($(NH_4)_2SO_4$)

METHODS

500 g. portions of the LF soil were mixed with 0.1, 0.2 and 0.4% calcium carbonate (finely ground). Each soil

was placed in glass pot with free drainage. A control soil, receiving no calcium carbonate, ~~were~~ was set up in the same way. Sufficient water was added to each pot to bring the moisture content to 50% mwhc. The pots were left at room temperature (18 - 20°C) for two months, with water being added when necessary to maintain the moisture content at 40 to 50% mwhc. The contents of each pot were then air dried and passed through 1 mm seive. The pH values of the soils were then determined. These were 4.1, 4.7, 5.7 and 7.3 for the nil, 0.1, 0.2, and 0.4% calcium carbonate treatments respectively.

100g. portions of soils of each pH were mixed with (i) finely ground urea and (ii) finely ground ammonium sulphate so as to give a level of 100 ppm N on the dry soil basis. 10g. portions of the control, urea and ammonium sulphate treated soils of each pH were set up in incubation tubes. Sufficient tubes were prepared to allow for analysis in duplicate initially and after 1, 3 and 6 weeks of incubation at 50% mwhc and 30°C. After each period the soils in each tubes were shaken with water for pH measurement and then extracted with Morgan reagent, and the extracts analysed for ammonium and nitrate as described in chapter 2.

RESULTS

Table 11 and Figure 7 shows results for soil pH and min-N (NH_4+NO_3-N) levels initially and after each period

of incubation. Table 12 shows corresponding accumulation values.

In the control soil min-N accumulation increased with time of incubation to about the same extent in soils at all pH levels, except that there was a trend for values to be somewhat low in the soil of initial pH 7.3 than at lower pH values. The only significant accumulation of nitrate occurred after 3 weeks of incubation in the soil of initial pH 5.7 and after 6 weeks of incubation in the soil of initial pH 4.7 and 5.7. However, the extent of this accumulation was low.

Min-N accumulation in the urea-treated soils was greater in soils of initial pH 4.1 and 4.7 than in the soils of higher pH. Most of the urea-N which was converted to NH_4-N did so during the first week of incubation in all soils. The soils of initial pH 5.7 was the only one to show some decrease in min-N during the 3 - 6 week incubation period. This was accounted for almost entirely by disappearance of nitrate during this period. There was some accumulation of nitrate only after 3 weeks in the soil of initial pH 5.7 and after 6 weeks in the soil of initial pH 4.7. However, nitrate accumulation, though significant, was small compared with the extent of accumulation of ammonium.

In the $(NH_4)_2SO_4$ treated soil min-N levels decreased to the greatest extent during the first week of incubation,

TABLE 11

MINERAL-N LEVELS DURING INCUBATION (1 to 6 weeks at 30°C, 50% mwhc) OF LOW FLAT SOIL (WHICH HAD BEEN PREVIOUSLY ADJUSTED TO DIFFERENT pH LEVELS) TREATED WITH 100 ppm N AS UREA AND AMMONIUM SULPHATE ($(NH_4)_2SO_4$)

(Results are given in ppm on dry soil basis)

pH levels	Treatment	Initial values NH_4-N	Initial values NO_3-N	Initial values min-N	Initial values pH	After 1 week incubation NH_4-N	After 1 week incubation NO_3-N	After 1 week incubation min-N	After 1 week incubation pH
4.1	Control	26.3	14.0	40.3	4.0	33.6	10.5	44.1	4.1
	Urea	23.8	17.5	41.3	4.1	101.5	13.7	115.2	4.7
	$(NH_4)_2SO_4$	106.8	18.6	125.4	4.1	110.3	4.6	114.9	4.1
4.7	Control	15.1	26.6	41.7	4.7	21.7	22.4	44.1	4.7
	Urea	16.8	25.9	42.7	4.7	90.3	27.0	117.3	5.2
	$(NH_4)_2SO_4$	98.4	29.8	128.2	4.7	99.4	18.2	117.6	4.7
5.7	Control	20.7	19.6	40.3	5.7	22.4	20.3	42.7	5.7
	Urea	23.6	24.9	48.5	5.8	97.0	19.3	116.3	6.2
	$(NH_4)_2SO_4$	105.7	29.4	135.1	5.4	95.9	21.4	117.3	5.7
7.3	Control	19.3	14.7	34.0	7.3	24.5	9.1	33.6	7.6
	Urea	20.7	20.7	41.4	7.3	93.1	9.1	102.2	8.0
	$(NH_4)_2SO_4$	106.2	13.3	119.5	7.2	99.4	8.8	108.2	7.5

pH levels	Treatment	After 3 weeks incubation NH_4-N	After 3 weeks incubation NO_3-N	After 3 weeks incubation min-N	After 3 weeks incubation pH	After 6 weeks incubation NH_4-N	After 6 weeks incubation NO_3-N	After 6 weeks incubation min-N	After 6 weeks incubation pH
4.1	Control	32.7	12.5	45.2	4.1	37.8	14.7	52.5	4.1
	Urea	103.8	18.3	122.1	4.7	112.7	10.5	123.2	4.6
	$(NH_4)_2SO_4$	101.5	19.3	120.8	4.1	116.9	13.3	130.2	4.2
4.7	Control	19.6	28.0	47.6	4.7	21.0	30.8	51.8	4.7
	Urea	90.0	27.0	117.0	5.1	88.2	42.7	130.9	5.2
	$(NH_4)_2SO_4$	93.5	27.0	120.5	4.6	105.0	30.8	135.8	4.7
5.7	Control	18.9	25.2	44.1	5.7	28.0	23.8	51.8	5.4
	Urea	89.3	33.3	122.6	5.9	93.1	13.3	106.4	5.7
	$(NH_4)_2SO_4$	93.5	26.9	120.4	5.4	99.4	25.2	124.6	5.3
7.3	Control	25.9	10.5	36.4	7.6	36.4	6.7	43.1	7.2
	Urea	85.8	15.4	101.2	7.9	97.3	12.6	109.9	7.7
	$(NH_4)_2SO_4$	95.9	17.9	113.8	7.4	115.5	8.4	123.9	7.2

L.S.D. at $P < 0.05$ NH_4-N 3.5, NO_3-N 4.0, min-N 2.4 ppm

FIGURE 7

MINERAL-N LEVELS DURING INCUBATION (1 to 6 weeks at 30°C, 50% mwhc) OF LOW FLAT SOIL (WHICH HAD BEEN PREVIOUSLY ADJUSTED TO DIFFERENT pH LEVELS TREATED WITH 100 ppm N AS UREA AND AMMONIUM SULPHATE $(NH_4)_2SO_4$]

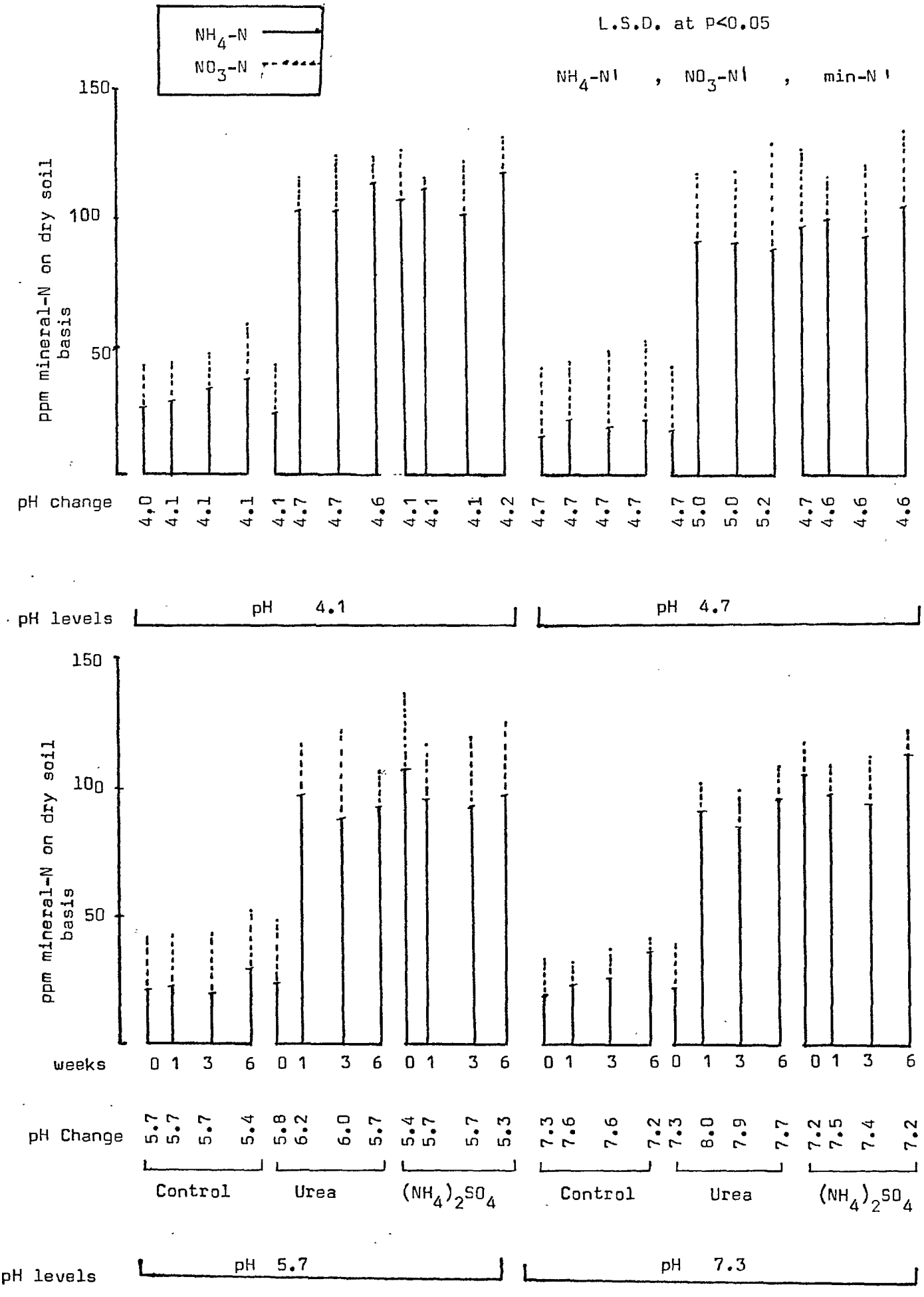


TABLE 12

MINERAL-N ACCUMULATION VALUES FOR EXPT.5 AFTER EACH INCUBATION PERIOD

(Results are given in ppm on dry soil basis)

pH levels	Treatment	After 1 week incubation NH_4-N	NO_3-N	min-N	pH	After 3 weeks incubation NH_4-N	NO_3-N	min-N	pH
4.1	Control	7.3	-3.5	3.8	4.1	6.4	-1.5	4.9	4.1
	Urea	77.7	-3.8	73.9	4.7	80.0	0.8	80.8	4.7
	$(NH_4)_2SO_4$	3.5	-14.0	-10.5	4.1	-5.3	0.7	-4.6	4.1
4.7	Control	6.6	-4.2	2.4	4.7	4.5	1.4	5.9	4.7
	Urea	73.5	1.1	74.6	5.2	73.2	1.1	74.3	5.1
	$(NH_4)_2SO_4$	1.0	-11.6	-10.6	4.7	-4.9	-2.8	-7.7	4.6
5.7	Control	1.7	0.7	2.4	5.7	-1.8	5.6	3.8	5.7
	Urea	73.4	-5.6	67.8	6.2	65.7	8.4	74.1	5.9
	$(NH_4)_2SO_4$	-9.8	-8.o	-17.8	5.7	-12.2	-2.5	-14.7	5.4
7.3	Control	5.2	-5.6	-0.4	7.6	6.6	-4.2	2.4	7.6
	Urea	72.4	-11.6	60.8	8.0	65.1	-5.3	59.8	7.9
	$(NH_4)_2SO_4$	-6.8	-4.5	-11.3	7.5	-10.3	4.6	-5.7	7.4

		After 6 weeks incubation			
4.1	Control	11.5	0.7	12.2	4.1
	Urea	88.9	-7.0	81.9	4.6
	$(NH_4)_2SO_4$	10.1	-5.3	4.8	4.2
4.7	Control	5.9	4.2	10.1	4.7
	Urea	71.4	16.8	88.2	5.2
	$(NH_4)_2SO_4$	6.6	1.0	7.6	4.7
5.7	Control	7.3	4.2	11.5	5.4
	Urea	69.5	-11.6	57.9	5.7
	$(NH_4)_2SO_4$	-6.3	-4.2	-10.5	5.3
7.3	Control	17.1	-8.0	9.1	7.2
	Urea	76.6	-8.1	68.5	7.7
	$(NH_4)_2SO_4$	9.3	-4.9	4.4	7.2

L.S.D. at $P < 0.05$ NH_4-N 3.5, NO_3-N 4.0, min-N 2.4 ppm

and then increased somewhat with further incubation so that after 6 weeks there was a small net accumulation of min-N in all soils except that of initial pH 5.7. After 1 week nitrate disappeared in preference to NH_4 in soils of initial pH 4.1 and 4.7, but both ammonium and nitrate disappeared from the soils of higher pH. After 3 weeks ammonium disappeared in preference to nitrate at all pH levels. After 6 weeks nitrate disappeared in preference to ammonium in all soils except that of initial pH 5.7.

The pH values of all the ammonium sulphate-treated soils were very similar (differing by 0.0 - 0.3 units) to the control soils. The pH values of urea treated soil were consistently higher, by 0.3 - 0.6 units, than those of the control soils after each period of incubation.

DISCUSSION

During the two months neutralization period with varying levels of calcium carbonate it appears that the urease activity of the soil was decreased somewhat when pH was increased above 5.0. This was reflected in the somewhat lower accumulation of mineral-N at the higher pH levels than in the control soil or in the soil where sufficient calcium carbonate was added to raise pH to 4.7. This pH was also optimum for nitrate accumulation where urea was applied.

During the first week of incubation a fair amount of nitrate was immobilized where ammonium sulphate was applied and the extent of immobilization decreased with soil pH. This immobilization is probably due to presence of easily decomposable carbonaceous materials which accumulated during the neutralization period. When the soils were incubated the heterotrophic organisms attacked this carbonaceous materials and immobilized mineral-N in the process. There was a difference in preference by immobilizing organisms for nitrate over ammonium. In the two soils of low pH nitrate was preferred over ammonium, whilst in the two soils of higher pH, ammonium and nitrate were utilized to about the same extent. However, with further incubation (up to 3 weeks) ammonium was preferred to nitrate in soils of all pH levels. With incubation up to 6 weeks there was a trend for the mineral-N which accumulated to do so as ammonium in all soils and for nitrate to disappear in the two soils of higher pH.

In general, it appears that, increasing the pH of these soils to above 5.0 stimulated the activity of heterotrophic organisms to such an extent that carbonaceous materials were being attacked and in the process mineral forms of N, particularly nitrate, were being immobilized.

If the results obtained with the LF soil in this experiment are compared with those in the previous experiment (where calcium carbonate was added just before incubation and where no source of N were added) it is seen that, as

far as nitrate accumulation is concerned, this was at a maximum at approximately the same pH level in both experiments (pH 5.0 to 5.2).

SUMMARY

The effects of addition of 0.1 to 0.8% calcium carbonate (to give pH ranging from 4.2 to 8.0) on min-N levels during incubation (30°C, 50% mwhc) for 2 - 12 weeks of LF and HF soils were studied. Mineral-N accumulation increased with pH in both soils. Except in the soils receiving no calcium carbonate mineral-N accumulated to a greater extent in the HF than in the LF soil. In the HF soil nitrate accumulated to the greatest extent at pH 4.9 to 5.0 early in incubation but at the highest pH after 12 weeks of incubation. In the LF soil maximum nitrate accumulation occurred at pH 7.0 after 2 weeks and at pH 5.0 to 5.8 after 4 - 12 weeks of incubation. After 4 weeks of incubation nitrate accumulated to a greater extent, particularly at the higher pH levels, in the HF than in the LF soil.

The effects of adjusting the pH of LF soil to 4.1 to 7.3 (by adding calcium carbonate to moist soil two months previously) during incubation (1 - 6 weeks at 30°C, 50% mwch) with and without addition of urea and ammonium sulphate

(100 ppm N) on mineral-N levels were studied. Where urea was applied there was a general decrease in mineral-N accumulation with increasing pH. Where ammonium sulphate was applied there was some immobilization of N up to 1 - 3 weeks of incubation at all pH levels; after 6 weeks of incubation there was slight mobilization at all pH levels except at pH 5.7. Maximum nitrate accumulation occurred where urea was applied at pH 5.7 after 3 weeks and at pH 4.7 after 6 weeks of incubation. There were differences in preference by immobilizing organisms for nitrate over ammonium, and the preference varied with pH.

CHAPTER 5

EFFECTS OF TEMPERATURE, pH AND MOISTURE CONTENT ON LEVELS OF MINERAL-N (NH_4-, NO_2-, and NO_3-N), AMMONIA VOLATILIZATION AND CARBON DIOXIDE RELEASE DURING INCUBATION OF LOW FLAT AND HIGH FLAT SOILS.

Experiment 6

Effects of temperature (30°C & 50°C) and pH (4.1 to 7.2) on levels of mineral-N (NH_4-, NO_2- and NO_3-N) and ammonia volatilization during incubation (6 weeks, 50% mwhc) of LOW FLAT soil without and with addition of ammonium sulphate (50 ppm N)

Experiment 7

Effects of temperature (30°, 40°, 50° & 60°C) and moisture content (50% and 133% mwhc) on levels of mineral-N (NH_4- and NO_3-N) and carbon dioxide release during incubation (2, 4 and 8 weeks) of HIGH FLAT soil.

CHAPTER 5

EFFECTS OF TEMPERATURE, pH AND MOISTURE CONTENT ON LEVELS OF MINERAL-N (NH_4-, NO_2-, and NO_3-N), AMMONIA VOLATILIZATION AND CARBON DIOXIDE RELEASE DURING INCUBATION OF LOW FLAT AND HIGH FLAT SOILS

This chapter describes experiments dealing with the combined effects of varying temperature and pH (Expt.6) and temperature and moisture contents (Expt.7). In Expt. 6 NH_4-, NO_2- and NO_3-N levels in the soils and NH_3 volatilization were determined. In Expt. 7 NH_4- and NO_3-N levels in the soils and CO_2 release were determined.

Experiment 6

Effects of temperature (30°C & 50°C) and pH (4.1 to 7.2) on levels of mineral-N (NH_4-, NO_2-, and NO_3-N) and ammonia volatilization during incubation (6 weeks, 50% mwhc) of LOW FLAT soil without and with addition of ammonium sulphate (50 ppm N)

METHODS

The LOW FLAT (LF) soils which had been pre-neutralised with varying levels of calcium carbonate (as described in

chapter 4) to give soils of pH 4.1, 4.7, 5.7 and 7.2 were used. The experiment was set up 3 months after air-dry storage of these soils. Sufficient incubation tubes were set up with soils of each pH to allow for duplicate determinations of NH_4-, NO_2- and NO_3-N and ammonia volatilization after 6 weeks of incubation at 50% mwhc and at incubation temperatures of 30°C and 50°C. Other sets of soils of each pH were treated with finely ground ammonium sulphate (50 ppm N on the dry soil basis) and sufficient tubes of these were prepared to allow for the same incubation conditions as with the control soils. The extractant used for determining NH_4-, NO_2- and NO_3-N was normal potassium chloride (N KCl) after a preliminary shaking with water to determine pH. Initial (zero incubation) pH and levels of NH_4- and NO_3-N were also determined.

RESULTS

Results are shown in Table 13 (A and B). Table 13 A shows levels of NH_4-, NO_3-, and min-N initially and after 6 weeks of incubation and also accumulation values for each of these constituents. Table 13 B shows levels of NO_2-N and ammonia-N volatilization. Initial and final pH values are shown in Table 13 A and final pH values in Table 13 B. Figure 8 shows initial and final min-N and NO_3-N levels in the soils and also levels of volatilized ammonia after 6 weeks of incubation.

TABLE 13 (A and B)

EFFECTS OF TEMPERATURE(30° & 50°C) and pH(4.1 to 7.2)ON LEVELS OF MINERAL-N(NH_4-, NO_2- and NO_3-N) DURING INCUBATION (6 weeks, 50% mwhc) OF LOW FLAT SOIL WITHOUT AND WITH ADDITION OF AMMONIUM SULPHATE (50 ppm N)

(Results are given in ppm on dry soil basis)

13 A. Results for NH_4-, NO_3-, and min-N

pH level	Temp.	Added N(+)	NH_4-N		NO_3-N		min-N		pH	
			0 Wk.	6 wk.	0.wk.	6 wk.	0.wk.	6wk.	0 wk.	6 Wk
4.1	30°C	level nil N	34.3	35.7	15.4	17.5	49.7	53.2	4.0	4.1
	30°C	level + N	88.9	93.8	23.1	14.0	112.0	107.8	4.0	4.1
	30°C	accum.nil N		1.4		2.1		3.5		4.1
	30°C	accum. + N		4.9		-9.1		-4.2		4.1
	50°C	level nil N	34.3	81.0	15.4	5.8	49.7	86.8	4.0	4.3
	50°C	level + N	88.9	118.3	23.1	18.9	112.0	137.2	4.0	4.2
	50°C	accum.nil N		46.7		-9.6		37.1		4.3
	50°C	accum. + N		29.4		-4.2		25.2		4.2
4.7	30°C	level nil N	25.6	19.6	18.2	30.1	43.8	49.7	4.7	4.7
	30°C	level + N	84.7	76.3	18.9	28.0	103.6	104.3	4.6	4.6
	30°C	accum.nil N		-6.0		11.9		5.9		4.7
	30°C	accum. + N		-8.4		9.1		0.7		4.6
	50°C	level nil N	25.6	52.5	18.2	27.3	43.8	79.8	4.7	4.8
	50°C	level + N	84.7	111.0	18.9	18.2	103.6	129.2	4.6	4.8
	50°C	accum.nil N		26.9		9.1		36.0		4.8
	50°C	accum. + N		26.3		-0.7		25.6		4.8
5.7	30°C	level nil N	28.7	14.7	17.9	35.7	46.6	50.4	5.6	5.5
	30°C	level + N	76.3	41.6	20.3	51.5	96.6	93.1	5.6	5.2
	30°C	accum.nil N		-14.0		17.8		3.8		5.5
	30°C	accum. + N		-34.7		31.2		-3.5		5.2
	50°C	level nil N	28.7	43.4	17.9	29.4	46.6	72.8	5.6	6.1
	50°C	level + N	76.3	98.5	20.3	13.0	96.6	111.5	5.6	5.6
	50°C	accum.nil N		14.7		11.5		26.2		6.1
	50°C	accum. + N		22.2		-7.3		14.9		5.6
7.2	30°C	level nil N	27.3	3.0	12.3	41.1	39.6	44.1	7.2	7.4
	30°C	level + N	77.0	33.5	16.5	50.5	93.5	84.0	7.3	7.2
	30°C	accum.nil N		-24.3		28.8		4.5		7.4
	30°C	accum. + N		-43.5		34.0		-9.5		7.2
	50°C	level nil N	27.3	39.9	12.3	11.1	39.6	51.0	7.2	7.6
	50°C	level + N	77.0	59.4	16.5	12.4	93.5	71.8	7.3	7.6
	50°C	accum.nil N		12.6		-1.2		11.4		7.6
	50°C	accum. + N		-17.6		-4.1		-21.7		7.6

L.S.D. at $P<0.05$ NH_4-N 3.3, NO_3-N 1.7, min-N 3.0 ppm

accum. = accumulated mineral-N values.

TABLE 13 (A.and B)

EFFECTS OF TEMPERATURE (30° & 50°C) and pH (4.1 to 7.2) ON LEVELS OF MINERAL-N (NH_4-, NO_2-, and NO_3-N) AND AMMONIA VOLATILIZATION DURING INCUBATION (6 weeks, 50%mwhc) OF LOW FLAT SOIL WITHOUT AND WITH ADDITION OF AMMONIUM SULPHATE (50 ppm N)

(Results are given in ppm on dry soil basis)

13 B. Results for NO_2- and NH_3-N volatilization after 6 weeks

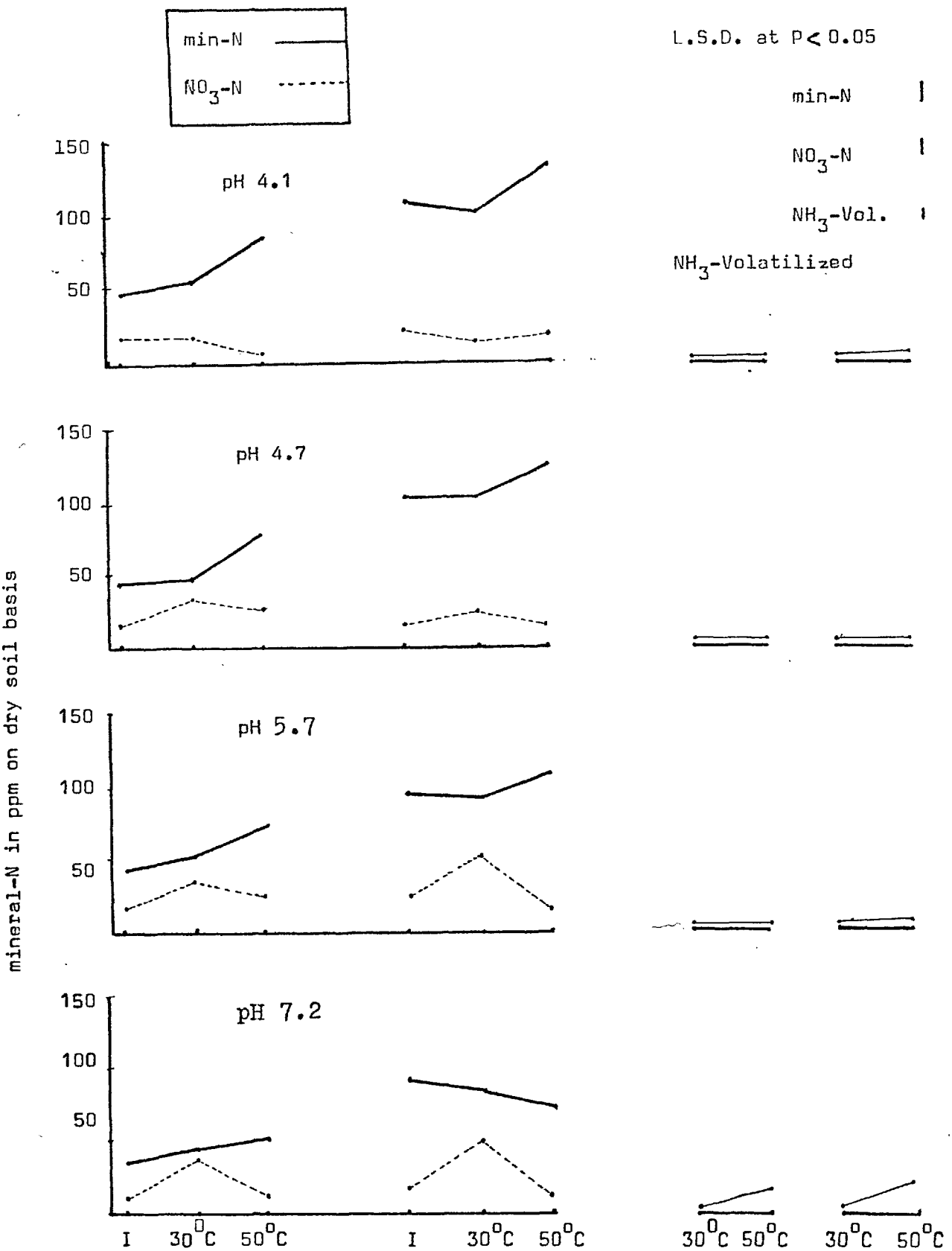
pH level	Temp.	Added N(+)	NO_2-N	NH_3-N Vol.	pH
4.1	30°C	nil N	0.0*	0.43	4.1
	30°C	+ N	0.0	0.33	4.1
	50°C	nil N	0.0	0.35	4.3
	50°C	+ N	0.0	1.43	4.2
4.7	30°C	nil N	0.0	0.81	4.7
	30°C	+ N	0.0	0.21	4.6
	50°C	nil N	0.0	2.1	4.8
	50°C	+ N	0.0	3.6	4.8
5.7	30°C	nil N	0.05	0.3	5.5
	30°C	+ N	0.04	0.6	5.2
	50°C	nil N	0.073	4.1	6.1
	50°C	+ N	0.140	4.6	5.6
7.2	30°C	nil N	0.245	0.7	7.4
	30°C	+ N	0.125	2.1	7.2
	50°C	nil N	0.150	15.6	7.6
	50°C	+ N	0.170	18.3	7.6

L.S.D. at $P < 0.05$ for NO_2-N 0.02 and for NH_3 vol.0.07 ppm

* $<$0.02 ppm N

FIGURE 8

EFFECTS OF TEMPERATURE (30 & 50°C) AND pH (4.1 to 7.2) ON LEVELS OF MINERAL-N, NITRATE-N AND AMMONIA VOLATILIZATION DURING INCUBATION (6 weeks, 50% mwhc) OF LOW FLAT SOIL WITHOUT AND WITH ADDITION OF AMMONIUM SULPHATE (50 ppm N)



I represents initial value

Soil not treated with ammonium sulphate

There was only slight accumulation of min-N (3.5 - 5.9 ppm N) during incubation at 30°C, with no significant difference due to pH. There was greater mineral-N accumulation at all pH levels at 50°C than at 30°C. Maximum mineral-N accumulation at 50°C occurred at pH 4.1 and 4.7, and mineral-N accumulation decreased with further increasing pH.

Nitrate accumulation at 30°C was just significant at pH 4.1 and increased with pH. At 50°C significant amounts of nitrate accumulated to about the same extent at pH 4.7 and 5.7. At pH 7.2 there was no change in nitrate level, whilst at pH 4.1 there was significant disappearance of some of the nitrate present initially.

At 30°C there was significant though slight loss of ammonia by volatilization at all pH levels. At 50°C there was slight ammonia volatilization at pH 4.1, and this evolution increased with pH up to a maximum of 15.6 ppm at pH 7.2.

At pH 4.1 and 4.7 no nitrite accumulated (the lower limit of detection of NO_2-N was 0.02 ppm N) at either temperatures. At pH 5.7 and 7.2 there was significant nitrite accumulation at both temperatures, although the magnitude of the levels attained were very low (the maximum level was 0.245 ppm NO_2-N).

Soil treated with ammonium sulphate (50 ppm N)

After incubation at 30°C mineral-N was unaffected at pH 4.7, showed a slight but significant decrease at pH 4.1 and 5.7 and somewhat greater decrease at pH 7.2. At 50°C maximum mineral-N accumulation occurred at pH 4.1 and 4.7. At pH 5.7 mineral-N accumulation was somewhat less, whilst at pH 7.2 there was a considerable decrease in mineral-N content due to incubation.

At 30°C there was some loss of nitrate at pH 4.1, but at pH 4.7 there was some nitrate accumulation and the extent of accumulation increased with pH up to 7.2. At 50°C there was no change in nitrate at pH 4.7, whilst at the other pH levels there was small but significant losses of nitrate. Where nitrate accumulated it occurred to about the same extent without and with added N at pH 4.7, but to a somewhat greater extent with added N at the higher pH levels.

There was significant though slight loss of ammonia by volatilization at all pH levels at 30°C, with loss at pH 7.2 being somewhat greater than that at lower pH levels. At 50°C volatilization losses of ammonia were greater at all pH levels than at 30°C. Loss at 50°C increased with pH to a maximum of 18.3 ppm NH_3-N at pH 7.2.

There was no accumulation of nitrite at pH 4.1 or 4.7 at either temperatures. There was significant though

very small accumulation of nitrite at both temperatures and at pH 5.7 and 7.2.

Changes in pH due to incubation ranged from nil to 0.5 units. In the soils of nominal pH 4.1 and 4.7 there was either no change or slight increases of pH ($\not>$ 0.3 units) due to incubation. In the soils of nominal pH 5.7 and 7.2 there were either increases or decreases in pH of up to 0.5 units.

DISCUSSION

The lack of any effect of increasing the soil pH on nitrogen mineralization during incubation at 30°C indicates that at this temperature the mere raising of soil pH is in itself insufficient to bring about much mineralization of organic nitrogen compounds. This differs from temperate-region soils where raising the soil pH has a marked affect in increasing subsequent mineralization of nitrogen during incubation (232). On the other hand, with incubation at 50°C there was a considerable increase in mineralization of nitrogen at all, including the original pH levels. This is in agreement with what happens in typical English soil.

At both incubation temperatures there was a distinct trend for addition of ammonium sulphate to decrease the

extent of mineralization of nitrogen at all pH levels. This was so even when allowances were made for the losses of ammonia by volatilization. This indicates a toxic effect of added ammonium sulphate on those organisms responsible for mineralization of organic nitrogen compounds.

Apart from some immobilization of nitrate in the soil of pH 4.1 with added ammonium sulphate, the increase in nitrate accumulation during incubation at 30°C due to increasing pH whether or not ammonium sulphate was added is similar to that occurring in temperate-region soils. However, the occurrence of significant nitrate accumulation even at pH 4.7 is unusual. On the other hand, the accumulation of nitrate at 50°C is unlike anything reported for temperate-region soils, especially since this occurred only in the two soils of pH 4.7 and 5.7. Here again the occurrence of heterotrophic nitrification is indicated.

The increasing ammonia volatilization with increasing temperature and pH which occurred where ammonium sulphate was applied is similar to that described for temperate-region soils (233). However, the occurrence of significant ammonia volatilization even at the lowest pH (pH 4.1) at 50°C indicates that this may have some affect on the nitrogen balance in these soils.

The significant occurrence of nitrite in the soils of pH 5.7 and 7.2, although of low magnitude after 6 weeks

incubation is interesting. This is particularly so at pH 5.7, where it would be thought that the reaction

$$NO_2^- + NH_4^+ \longrightarrow N_2 + 2H_2O$$

would result in complete disappearance of nitrite, especially since there was always adequate NH_4-N present during the incubation period.

Experiment 7

Effects of temperature (30°, 40°, 50° and 60°C) and moisture content (50% and 133% mwhc) on levels of mineral-N (NH_4- and NO_3-N) and carbon dioxide release during incubation (2, 4 and 8 weeks) of HIGH FLAT soil

METHODS

The original HIGH FLAT (HF) soil (pH 4.2) was set up for incubation in the usual way at four temperatures (30°, 40°, 50° and 60°C) and at two moisture contents (50% and 133% mwhc) at each temperature. After 2, 4 and 8 weeks of incubation analyses were made for soil mineral-N levels and carbon dioxide release, and pH was also determined. The Morgan reagent was used for extracting mineral-N.

RESULTS

Results for mineral-N levels are shown in Table 14 and for mineral-N accumulation values in Table 15. Mineral-N levels are also shown diagramatically in Figure 9. Carbon dioxide release values are shown in Table 16 (for release during the 0-2, 2-4, and 4-8 week periods and also cumulative values). Figure 10 shows cumulative release of carbon dioxide.

Mineral-N levels

After 2 weeks of incubation mineral-N levels increased to about the same extent at 30°, 40°, and 50°C at 50% and 133% mwhc. At 60°C mineral-N levels were considerably greater than at the lower temperatures, but only at 50% mwhc.

After 4 weeks at 50% mwhc mineral-N levels increased, usually significantly, with increasing temperature. At 133% mwhc mineral-N levels were significantly greater at 60°C than at the lower temperatures. At 40°, 50° and 60°C mineral-N levels were greater at 50% mwhc than at 133% mwhc.

After 8 weeks at both moisture contents there was little further change in mineral-N levels at 30° and 40°C, but a fair increase at 50° and 60°C. These increases were particularly marked at 133% mwhc, so that by the end of incubation mineral-N levels at all temperatures were of the the same order at both moisture contents. In contrast,

TABLE 14

EFFECTS OF TEMPERATURE (30°,40°,50° & 60°C) AND MOISTURE CONTENT (50% & 133% mwhc) ON LEVELS OF MINERAL-N (NH_4- and NO_3-N) DURING INCUBATION (2,4 & 8 weeks) OF HIGH FLAT SOIL

(Results are given in ppm on dry soil basis)

		NH_4-N	NO_3-N	min-N	pH
Initial values		9.8	7.4	17.2	4.2
After 2 weeks incubation					
%mwhc	Temp(°C)	NH_4-N	NO_3-N	min-N	pH
50	30	28.0	5.3	33.3	4.4
50	40	30.5	5.6	36.1	4.5
50	50	22.0	10.0	32.0	4.3
50	60	58.5	9.8	68.3	4.6
133	30	23.5	1.9	25.4	5.6
133	40	24.9	1.4	26.3	5.1
133	50	24.2	6.7	30.9	5.1
133	60	37.5	3.2	40.7	4.9
After 4 weeks incubation					
50	30	30.8	11.6	42.4	4.3
50	40	37.6	20.9	58.5	4.4
50	50	55.0	10.9	65.9	4.5
50	60	81.2	7.4	88.6	4.5
133	30	33.3	3.5	36.8	5.9
133	40	35.7	4.2	39.9	5.4
133	50	39.0	2.5	41.5	5.0
133	60	55.5	4.0	59.5	4.7
After 8 weeks incubation					
50	30	27.3	22.4	49.7	4.1
50	40	42.4	9.1	51.5	4.5
50	50	73.5	16.0	89.5	4.6
50	60	106.0	17.5	123.5	4.3
133	30	42.9	4.6	47.5	6.0
133	40	30.5	5.2	35.7	5.0
133	50	81.9	13.3	95.2	5.2
133	60	119.7	1.1	120.8	5.0

L.S.D. at $P < 0.05$ NH_4-N 14.0, NO_3-N 4.0, min-N 16.1 ppm

TABLE 15

MINERAL-N ACCUMULATION VALUES FOR EXPERIMENT 7 AFTER EACH INCUBATION PERIOD (See Table 14)

(Results are given in ppm on dry soil basis)

% mwhc	Temp.(°C)	NH_4-N	NO_3-N	min-N	pH
		After 2 weeks incubation			
50	30	18.2	-2.1	16.1	4.4
50	40	20.7	-1.8	18.9	4.5
50	50	12.2	2.6	14.8	4.3
50	60	48.7	2.4	51.1	4.6
133	30	13.7	-5.5	8.2	5.6
133	40	15.1	-6.0	9.1	5.1
133	50	14.4	-0.7	13.7	5.1
133	60	27.7	-4.2	23.5	4.9
		After 4 weeks incubation			
50	30	21.0	4.2	25.2	4.3
50	40	27.8	13.5	41.3	4.4
50	50	45.2	3.5	48.7	4.5
50	60	71.4	0.0	71.4	4.5
133	30	23.5	-3.9	19.6	5.9
133	40	25.9	-3.2	22.7	5.4
133	50	29.2	-4.9	24.3	5.0
133	60	45.7	-3.4	42.3	4.7
		After 8 weeks incubation			
50	30	17.5	15.0	32.5	4.1
50	40	32.6	1.7	34.3	4.5
50	50	63.7	8.6	72.3	4.6
50	60	96.2	10.1	106.3	4.3
133	30	33.1	-2.8	30.3	6.0
133	40	20.7	-2.2	18.5	5.0
133	50	72.1	5.9	78.0	5.2
133	60	109.9	-6.3	103.6	5.0

L.S.D. at $P<0.05$ NH_4-N 14.0, NO_3-N 4.0, min-N 16.1 ppm.

FIGURE 9

EFFECTS OF TEMPERATURE (30^{0},40^{0}, 50^{0}, & 60^{0}C) AND MOISTURE CONTENT (50 & 133%mwhc) ON LEVELS OF MINERAL-N (NH_4-N and NO_3-N) AND CARBON DIOXIDE RELEASE DURING INCUBATION (2,4 & 8 weeks) OF HIGH FLAT SOIL

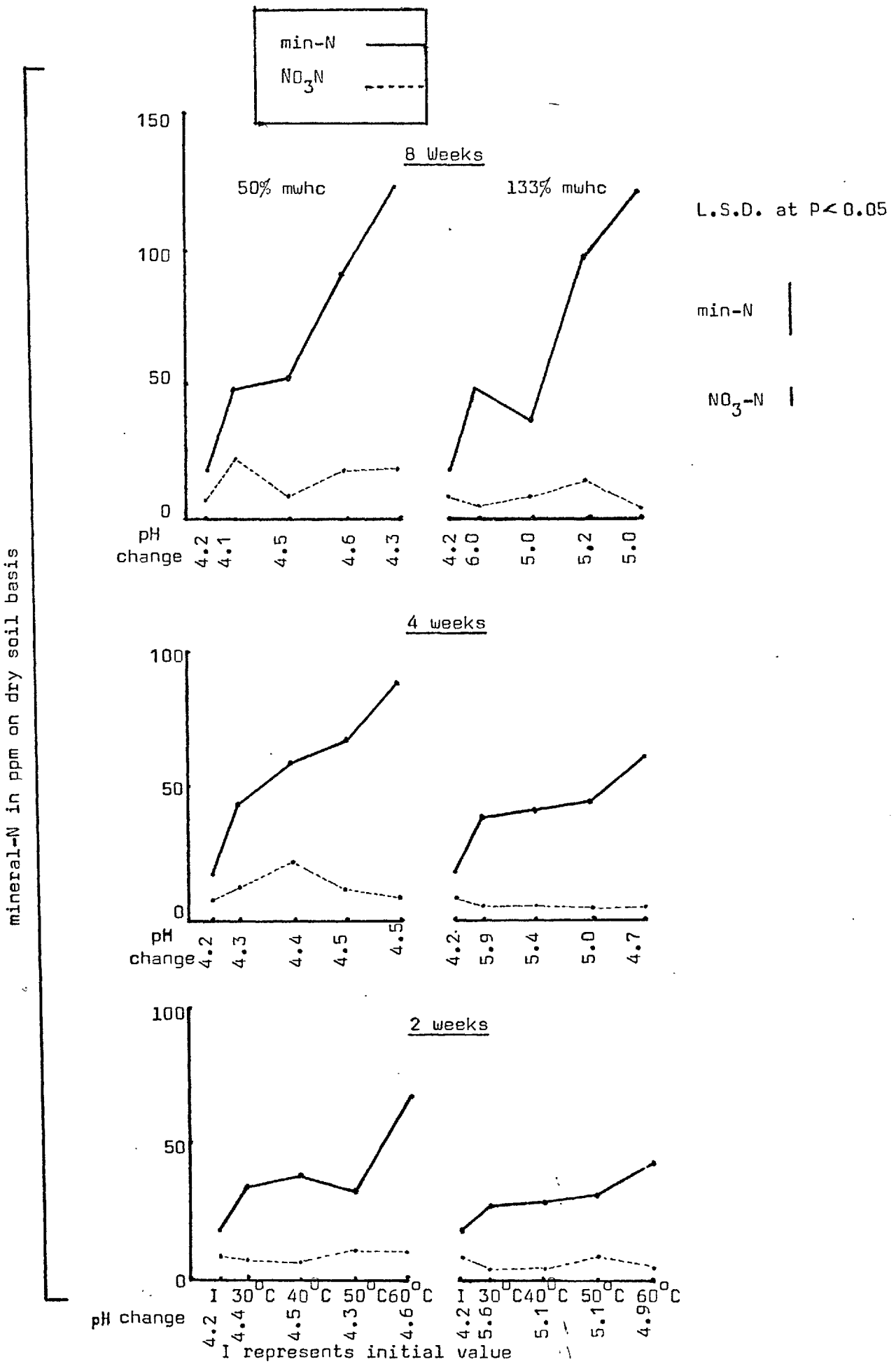


TABLE 16

EFFECTS OF TEMPERATURE (30°, 40°, 50° & 60°C) AND MOISTURE CONTENT (50% & 133% mwhc) ON CUMULATIVE CARBON DIOXIDE RELEASE DURING INCUBATION (2, 4 & 8 weeks) OF HIGH FLAT SOIL

(Results are given in ppm on dry soil basis)

Carbon dioxide release during each period

% mwhc	Temp(°C)	0-2 wk.	2-4 wk.	4-8 wk.	Total
50	30	925	775	763	2463
50	40	1163	775	1350	3288
50	50	1238	1000	2300	4538
50	60	2025	1213	2188	5426
133	30	988	1025	725	2738
133	40	1025	1063	1363	3451
133	50	1113	925	1151	3189
133	60	1488	1225	2013	4726

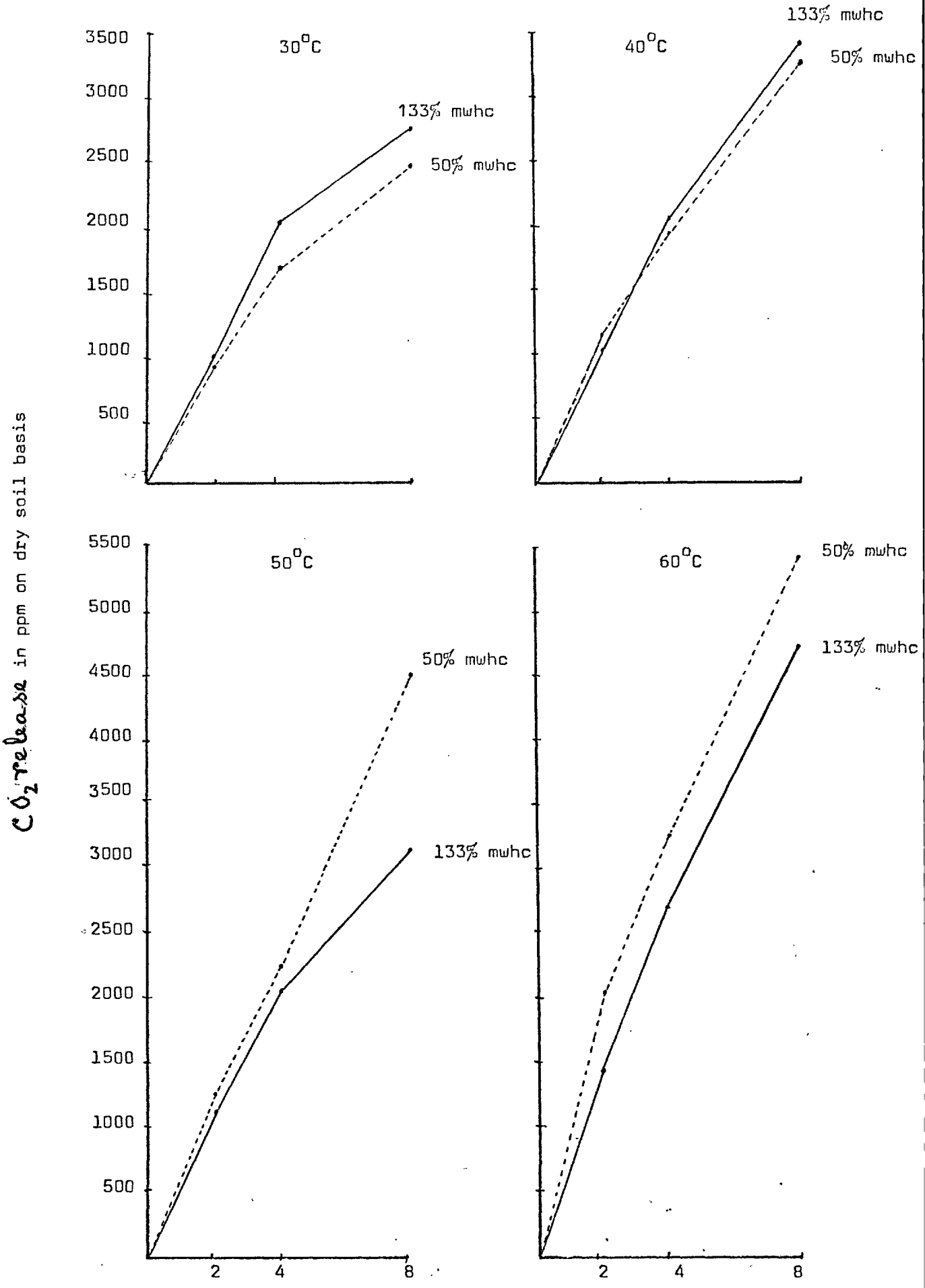
Cumulative Carbon dioxide release

% mwhc	Temp(°C)	2 wk.	4 wk.	8 wk.	
50	30	925	1700	2463	
50	40	1163	1938	3288	
50	50	1238	2238	4538	
50	60	2025	3238	5426	
133	30	988	2013	2738	
133	40	1025	2088	3451	
133	50	1113	2038	3189	
133	60	1488	2713	4726	

L.S.D. at $P < 0.05$ 210 ppm

FIGURE 10

EFFECTS OF TEMPERATURE (30°, 40°, 50° & 60°C) AND MOISTURE CONTENTS (50% and 133% mwhc) ON CUMULATIVE CARBON DIOXIDE RELEASE DURING INCUBATION (2,4 & 8 WEEKS)OF HIGH FLAT SOIL



at 30° and 40°C there were relatively small increases in mineral-N after the first 2 weeks of incubation.

Nitrate levels

After 2 weeks of incubation at 50% mwhc there were no significant changes in nitrate at any temperatures. At 133% mwhc there were small but significant losses of nitrate at all temperatures except 50°C.

After 4 weeks at 50% mwhc there were significant increases in nitrate at 30° and 40°C. At 133% mwhc there was loss of nitrate only at 50°C.

After 8 weeks at 50% mwhc there were further increases in nitrate at 30°, 50°, and 60°C, whilst at 40°C the nitrate which had accumulated during the first 4 weeks disappeared during the next 4 weeks. At 133% mwhc there was a significant increase in nitrate at 50°C, but not at the other temperatures.

pH

At 50% mwhc there were increases in pH of up to 0.4 units due to incubation. At 133% mwhc pH increased due to incubation by 0.5 - 1.8 units and most of these increases occurred during the first 2 weeks. The greatest increase occurred at 30°C.

Carbon dioxide release

During the 0 - 2 week period carbon dioxide release (Table 16, Figure 10) increased with temperature at both

moisture contents; there were no significant differences between the two moisture contents at 30°, 40° and 50°C, whilst at 60°C carbon dioxide release was higher at the lower moisture contents.

During 0 - 4 weeks at 50% mwhc there was a moderate increase in carbon dioxide release with increasing temperature up to 50°C, but a much greater increase at 60°C. At 133% mwhc there was little difference due to temperature at 30°, 40° and 50°C, whilst release at 60°C was somewhat higher. Carbon dioxide release at 30° and 40°C was lower at 50% mwhc than at 133% mwhc, whilst at 50° and 60°C the reverse was true.

During 0 - 8 weeks carbon dioxide release increased with temperature at 50% mwhc. At 133% mwhc carbon dioxide release increased with temperature up to 40°C, decreased up to 50°C, and showed its highest level at 60°C. At 30° and 40°C carbon dioxide release was less at 50% mwhc than at 133% mwhc, whilst the reverse held at 50° and 60°C.

DISCUSSION

At 30° and 40°C there was a general trend for mineral-N accumulation to be greater at 50% mwhc than at 133% mwhc during all stages of incubation. However, although there was similar trend with up to 4 weeks incubation at 50° and 60°C, during the 4 - 8 week period at these two higher

temperatures min-N accumulation increased to such an extent that by the end of incubation mineral-N levels were as high at 133% mwhc than at 50% mwhc. This implies that over short periods of water-logging at higher temperatures nitrogen mineralization is likely to be less than at aerobic moisture levels but with prolonged periods of water-logging it is likely to be as high as at aerobic moisture content.

The significant losses of nitrate which occurred at all temperatures during the 0 - 4 weeks period were probably due to denitrification and/or immobilization. With incubation up to 8 weeks there were no further changes in nitrate levels at 30°, 40° and 60°C. At 50°C, however, there was a small but significant increase in nitrate. This is particularly interesting in that any one of the factors of low pH, high temperature (50°C) or water-logging would normally prevent autotrophic nitrification. This again indicates the possible occurrence of heterotrophic nitrification. There is no clear reason why this should happen at 50°C but not at 30°, 40° and 60°C. If a heterotrophic process is responsible for this nitrate accumulation at 50°C, it may be that this is near the optimum temperature for this heterotrophic organism to function under conditions of low pH and water-logging. It is worth noting that it was at 50°C under water-logging where carbon dioxide release during 4 - 8 week period was significantly lower than at 40°C (Table 16); this was the only case where the normal trend

for carbon dioxide release to increase with temperature did not apply.

The main difference in carbon dioxide release between aerobic and water-logged incubation occurred at 50° and 60°C, where carbon dioxide release was greater under the former condition. This implies that soil organic matter is decomposed more rapidly under high temperature and at aerobic than under anaerobic conditions. At 30°C and 40°C the reverse tended to apply. At 30°C this was probably due to the higher pH levels reached under water-logging, 1.2 to 1.9 units higher than in the aerobic soil, which in turn was related to the higher ammonia accumulation under water-logging.

Under aerobic conditions there was little change in pH between 30° and 60°C incubation conditions in spite of the considerably higher accumulation of ammonia at the higher temperatures. Under anaerobic conditions pH was actually less at 60°C than at 30°C in spite of considerably higher accumulation at 60°C. This implies that at both moisture contents, but particularly under water-logging, organic acids were being produced to a much greater extent at 60°C than at 30°C and that these acids were preventing pH from increasing. It may be that in this soil at 60°C bacteria are inoperative whilst fungi and actinomycetes can still function. The latter two types of organism are effective in converting complex organic

compounds to organic acids but are relatively ineffective in converting organic acids to carbon dioxide.

S U M M A R Y

Experiment 6

NH_4-, NO_2- and NO_3-N and ammonia volatilization were determined during incubation (6 weeks) at 30° and 50°C of original LF soil (pH 4.1) and the same soil which had been treated with varying amounts of calcium carbonate to give pH levels of 4.7, 5.7 and 7.2. The experiment was done without and with addition of ammonium sulphate (50 ppm N on dry soil basis) just before incubation.

In the absence of added ammonium sulphate there was little mineralization of nitrogen at any pH at 30°C; at 50°C there was a fair amount of nitrogen mineralization, but the extent of this decreased with increasing pH. At 30°C nitrate accumulation increased with pH; at 50°C nitrate accumulated only at pH 4.7 and 5.7 and disappeared at pH 4.1.

Where ammonium sulphate was added there was a slight disappearance of mineral-N due to incubation at 30°C at all pH levels except 4.7; at 50°C there was a fair accumulation

of mineral-N at pH 4.1 and 4.7, somewhat lower accumulation at pH 5.7 and a fair disappearance of mineral-N at pH 7.2. At 30°C nitrate disappeared at pH 4.1, whilst at the higher pH levels nitrate accumulation increased with pH; at 50°C nitrate tended to disappear at all pH levels.

Very small, but significant amounts, of nitrite accumulated at 30° and 50°C but only at pH 5.7 and 7.2. There was small but significant ammonia volatilization at all pH levels at both temperatures irrespective of addition of ammonium sulphate. Ammonia volatilization reached fairly high levels only at 50°C and then only in the soil of pH 7.2; the levels were similar with and without addition of ammonium sulphate.

Experiment 7

NH_4- and NO_3-N and carbon dioxide release were determined during incubation (2, 4 and 8 weeks) at temperatures 30°, 40°, 50° and 60°C and moisture contents of 50% and 133% mwhc in the original HF soil.

At 30° and 40°C mineral-N accumulation was greater at 50% than at 133% mwhc during all stages of incubation. At 50° and 60°C mineral-N accumulation was less at 133% mwhc than at 50% mwhc but only early in incubation; after 8 weeks of incubation mineral-N levels were of the same order at both moisture contents.

At 133% mwhc there was significant loss of nitrate at all temperatures early in incubation; however there was significant gain in nitrate at 50°C during the 4 - 8 weeks incubation period. At 50% mwhc there was accumulation of nitrate at 30°, 50° and 60°C, but only during the last 4 weeks of incubation; at 40°C nitrate which accumulated during the first 4 weeks disappeared during the last 4 weeks of incubation.

Carbon dioxide release at 30° and 40°C tended to be greater at 133% than at 50% mwhc, whereas at 50° and 60°C the reverse effect occurred.

CHAPTER 6

EFFECTS OF AEROBIC INCUBATION OF HIGH FLAT (HF) SOIL TREATED WITH VARIOUS ORGANIC MATERIALS ON MINERAL-N LEVELS, CARBON DIOXIDE RELEASE AND SOME EXTRACTABLE METALLIC ELEMENTS

Experiment 8

Effects of 0.5% straw, green grass, sucrose, and cellulose+ sucrose (1:1) on levels of mineral-N, carbon dioxide release and extractable iron, manganese and copper during incubation (2, 6 and 13 weeks at 30°C and 50% mwhc) of HIGH FLAT soil (200 ppm N as ammonium nitrate added to all including control)

CHAPTER 6

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Experiment 8

Effects of 0.5% straw, green grass, sucrose, and cellulose+ sucrose (1:1) on levels of mineral-N, carbon dioxide release and extractable iron, manganese and copper during incubation (2, 6 and 13 weeks at 30°C and 50% mwhc) of HIGH FLAT (HF) soil (200 ppm N as ammonium nitrate added to all including control)

METHODS

A bulk of the original HIGH FLAT (HF) soil was mixed with finely ground ammonium nitrate to give 200 ppm N on the dry soil basis. Suitable portions of this soil were then mixed with 0.5% by weight of dried ground straw (42.5%C, 0.525%N), green grass (38.0%C, 0.90%N), sucrose (42.1%C), and cellulose (44.4%C) + sucrose (1:1 mixture). Sufficient 10g. replicates were set up for incubation to allow for analysis in duplicates initially and after 2, 6 and 13 weeks of incubation. Water was added to 50% mwhc and incubation temperature was 30°C. The barium peroxide method was used for aeration and absorption and measurement of carbon dioxide

release. After each period of incubation distilled water was added to measure pH and the soil was then extracted with Morgan reagent and the extracts analysed for NH_4-, NO_3-, Fe, Mn and Cu. The residual soil was then extracted with 0.1N EDTA-Na (pH 4) and the extract analysed for Fe, Mn and Cu.

RESULTS

Table 17 and Figure 11 show results for levels of mineral-N (NH_4- and NO_3-N) and pH initially and after each incubation period. Table 18 shows accumulation values obtained by subtracting initial levels of NH_4-, NO_3- and min-N from those obtained after each period of incubation. Figure 12 shows results for the extent of immobilization of NH_4-, NO_3-, and min-N during incubation, allowance being made for the amounts of NH_4- and NO_3-N present initially in the soil and organic materials, with the control being taken as zero. Table 19 shows results for Morgan-, EDTA-Na and total (Morgan+EDTA-Na)- extractable Fe, Mn and Cu. Table 20A shows carbon dioxide release during various periods of incubation; Table 20B and Figure 13 show cumulative carbon dioxide release.

Nitrogen levels

The control soil (not receiving organic material but receiving 200 ppm N) showed a slight increase in min-N up

TABLE 17

EFFECTS OF 0.5% STRAW, GREEN GRASS, SUCROSE AND CELLULOSE+SUCROSE(1:1 ON LEVELS OF MINERAL-N DURING INCUBATION (2,6 & 13 weeks at 30°C and 50% mwhc) OF HIGH FLAT SOIL (200 ppm N from ammonium nitrate added to all including control)

(Results are in ppm on dry soil basis)

	Initial Values				After 2 weeks incubation			
Treatment	NH_4-N	NO_3-N	min-N	pH	NH_4-N	NO_3-N	min-N	pH
Control	84	109	193	3.9	102	95	197	4.1
Straw	112	111	223	3.9	93	100	193	4.1
Grass	122	106	228	3.9	101	91	192	4.2
Sucrose	104	112	216	3.9	49	11	60	5.2
Cellulose+ Sucrose	97	104	201	3.9	77	71	148	4.2
	After 6 weeks incubation				After 13 weeks incubation			
Control	103	97	200	4.2	107	84	191	4.2
Straw	101	83	184	4.0	101	85	186	4.2
Grass	111	74	185	4.2	119	73	192	4.4
Sucrose	79	7	86	5.4	91	10	101	5.1
Cellulose+ Sucrose	83	43	126	4.5	74	8	82	5.0

L.S.D. at $P<0.05$ NH_4-N 6.0, NO_3-N 7.0, min-N 8.0 ppm

FIGURE 11

EFFECTS OF 0.5% STRAW, GREEN GRASS, SUCROSE, & CELLULOSE+SUCROSE (1:1) ON LEVELS OF MINERAL-N (NH_4-and NO_3-N) DURING INCUBATION (2,6 & 13 weeks, 30°C & 50% mwhc) OF HIGH FLAT SOIL (200ppm N from ammonium nitrate added to all including control)

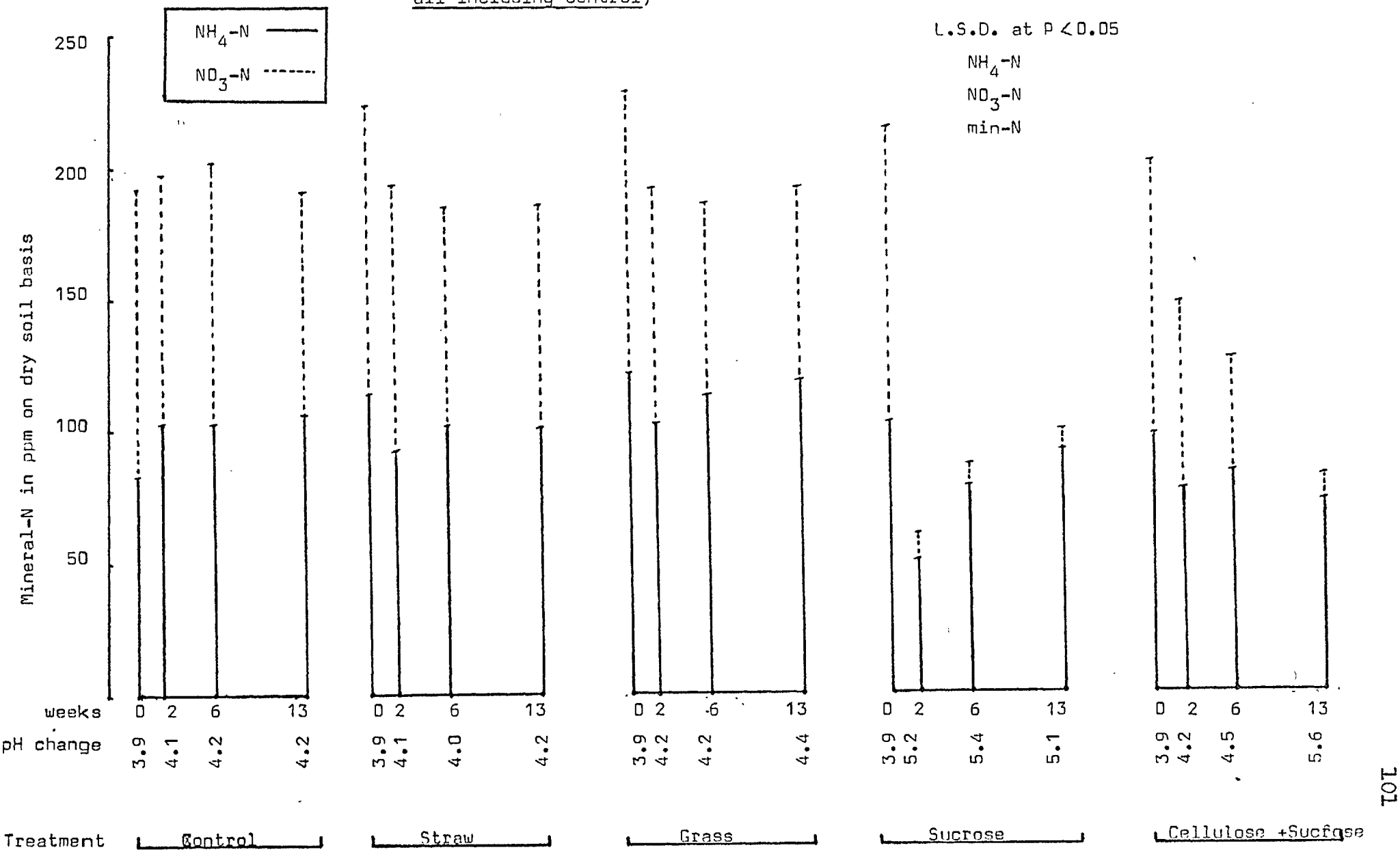


TABLE 18

MINERAL-N ACCUMULATION VALUES FOR EXPERIMENT 8 AFTER EACH INCUBATION PERIOD (See Table 17)

(Results are given in ppm on dry soil basis)

Treatment	NH_4-N	NO_3-N	min-N	pH
After 2 weeks incubation				
Control	18	-14	4	4.1
Straw	-19	-11	-30	4.1
Grass	-21	-15	-36	4.2
Sucrose	-55	-101	-156	5.2
Cellulose+ Sucrose	-20	-33	-53	4.2
After 6 weeks incubation				
Control	19	-12	7	4.2
Straw	-11	-28	-39	4.0
Grass	-11	-32	-43	4.2
Sucrose	-25	-105	-130	5.4
Cellulose+ Sucrose	-14	-61	-75	4.5
After 13 weeks incubation				
Control	23	-25	-2	4.2
Straw	-11	-26	-37	4.2
Grass	-3	-33	-36	4.4
Sucrose	-13	-102	-115	5.1
Cellulose+ Sucrose	-23	-96	-119	5.0

L.S.D. at $P < 0.05$ NH_4-N 6.0, NO_3-N 7.0, min-N 8.0 ppm

FIGURE 12

N IMMOBILIZATION DURING 13 WEEKS OF INCUBATION OF HF SOIL TREATED WITH VARIOUS ORGANIC MATERIALS AND NH_4 NO_3 (200 ppm N)

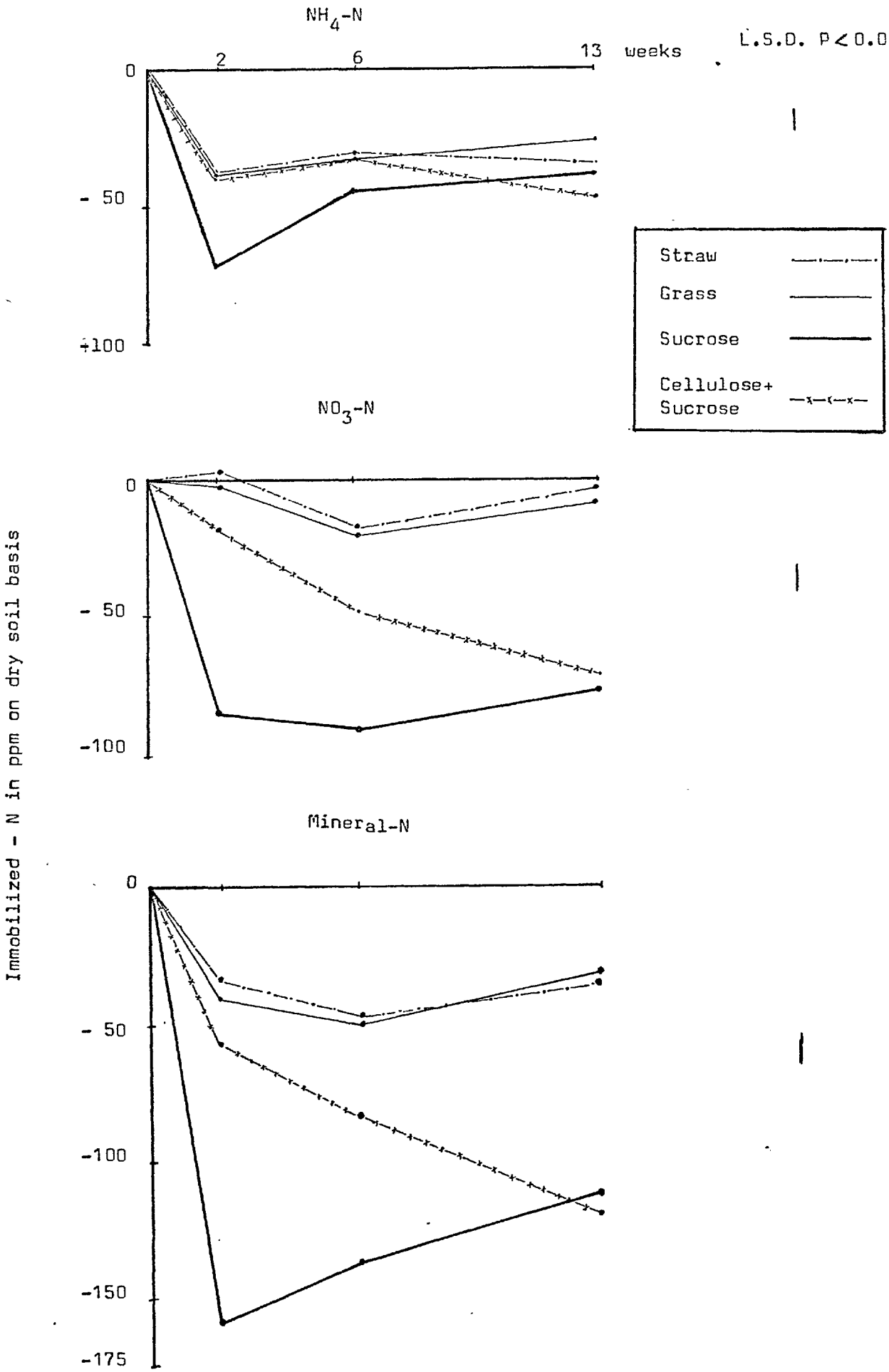


TABLE 19

IRON (Fe), MANGANESE (Mn), AND COPPER (Cu) EXTRACTED BY MORGAN AND EDTA REAGENTS AFTER 0, 2, 6 & 13 WEEKS OF INCUBATION OF HIGH FLAT SOIL TREATED WITH 0.5% STRAW, GREEN GRASS, SUCROSE, AND CELLULOSE+SUCROSE (1:1)

(Results are given in ppm on dry soil basis)

Total = Morgan + EDTA Extractants

Iron (Fe)

Treatment	Reagent	Weeks 0	2	6	13
Control	Morgan	9.0	7.2	0.9	5
	EDTA	27.0	23.0	20.0	14
	Total	36.0	30.2	20.9	19
Straw	Morgan	8.9	7.6	1.0	5
	EDTA	27.0	22.8	20.0	14
	Total	35.9	30.4	21.0	19
Grass	Morgan	8.8	7.6	1.3	5
	EDTA	27.0	24.0	22.3	15
	Total	35.8	31.6	23.6	20
Sucrose	Morgan	9.0	200.0	50.0	10
	EDTA	26.0	175.0	103.0	30
	Total	35.0	375.0	153.0	40
Cellulose+ Sucrose	Morgan	9.8	9.2	1.0	6
	EDTA	33.6	130.2	22.5	17
	Total	43.4	139.4	23.5	23

Manganese (Mn)

Treatment	Reagent	Weeks 0	2	6	13
Control	Morgan	8.8	10.3	14.0	13
	EDTA	2.8	3.6	3.9	5
	Total	11.6	13.9	17.9	18
Straw	Morgan	8.5	11.0	16.0	15
	EDTA	3.0	3.8	5.0	6
	Total	11.5	14.8	21.0	21
Grass	Morgan	8.5	14.2	19.6	19
	EDTA	3.2	5.0	6.0	7
	Total	11.7	19.2	25.6	26
Sucrose	Morgan	8.1	40.5	105.0	4
	EDTA	3.0	16.4	15.5	10
	Total	11.1	56.9	120.5	14
Cellulose+ Sucrose	Morgan	7.6	13.3	200.0	2
	EDTA	3.0	4.8	5.5	8
	Total	10.6	18.1	205.5	10

Copper (Cu)

Treatment	Reagent	0	2	6	13
Control	Morgan	0.7	0.8	0.0	0.4
	EDTA	1.8	2.0	1.6	1.0
	Total	2.5	2.8	1.6	1.4
Straw	Morgan	0.8	0.9	0.0	0.6
	EDTA	2.0	2.3	1.8	1.2
	Total	2.8	3.2	1.8	1.8
Grass	Morgan	1.0	1.0	0.0	0.6
	EDTA	2.1	2.3	1.9	1.4
	Total	3.1	3.3	1.9	2.0
Sucrose	Morgan	0.8	0.7	0.0	0.6
	EDTA	2.0	1.6	1.7	1.6
	Total	2.8	2.3	1.7	2.2
Cellulose+ Sucrose	Morgan	0.8	0.9	0.0	0.5
	EDTA	2.2	2.4	2.0	1.5
	Total	3.0	3.3	2.0	2.0

L.S.D. at $P < 0.05$

Iron (Fe) 4.6 ppm

Manganese(Mn) 1.6 ppm

Copper (Cu) 0.15 ppm

TABLE 20 A

CARBON DIOXIDE RELEASE AFTER EACH INCUBATION PERIOD FROM HF SOIL TREATED WITH 0.5% STRAW, GREEN GRASS, SUCROSE, SUCROSE+CELLULOSE (1:1)

(Results are in ppm CO_2 on dry soil basis)

Treatment	3 days	1 Wk.	2 Wk.	4 Wk.	6 Wk.	9 Wk.	13 Wk.
Control	800	713	863	963	1025	750	888
Straw	938	1025	1188	1238	1388	1363	1463
Grass	1363	1313	1250	1600	1650	1725	1475
Sucrose	1775	2013	1713	1850	1763	1725	1563
Cellulose+ Sucrose	1613	1250	1225	1375	1575	1688	1800

TABLE 20 B

CUMULATIVE CARBON DIOXIDE RELEASE AFTER 3 DAYS AND 1,2,6 AND 13 WEEKS INCUBATION OF HF SOIL TREATED WITH 0.5% STRAW, GREEN GRASS, SUCROSE, SUCROSE+CELLULOSE (1:1)

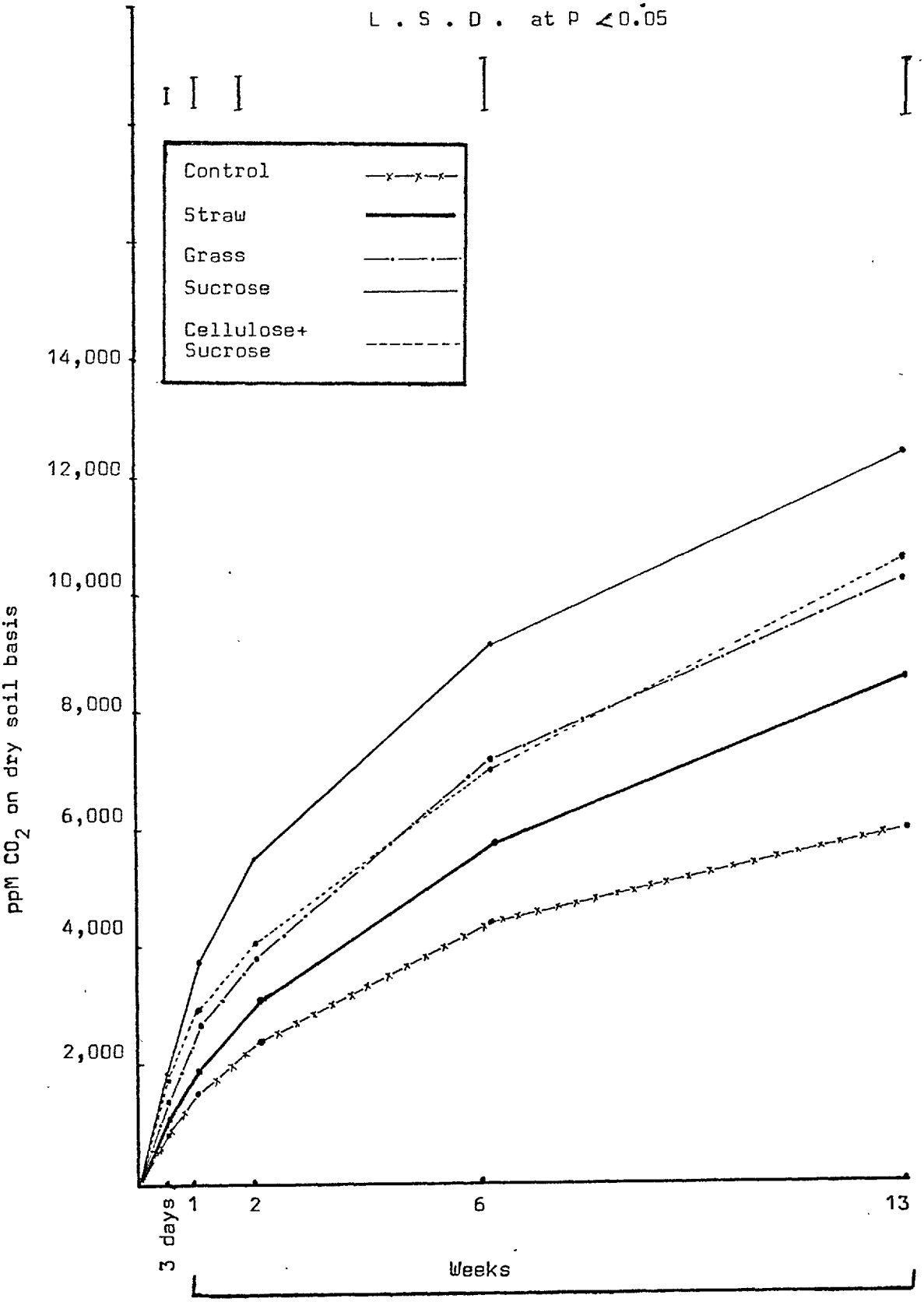
(Results are in ppm CO_2 on dry soil basis)

Treatment	3 Days	1 Wk.	2 Wk.	6 Wk.	13 Wk.	% added C → CO_2^*
Control	800	1513	2376	4364	6002	
Straw	938	1963	3151	5777	8603	33.3
Grass	1363	2676	3926	7176	10376	63.1
Sucrose	1775	3788	5501	9114	12402	82.6
Cellulose+ Sucrose	1613	2863	4088	7038	10526	55.2
L.S.D. at P<0.05	253	398	411	754	797	

* Percentage recovery of added organic carbon as carbon dioxide.

FIGURE 13

CUMULATIVE CARBON DIOXIDE RELEASE AFTER 3 DAYS AND 1,2,6 AND 13 WEEKS INCUBATION OF HF SOIL TREATED WITH 0.5% STRAW, GREEN GRASS, SUCROSE AND CELLULOSE + SUCROSE (1:1)



to 6 weeks of incubation followed by a slight decrease up to 13 weeks; however, these changes were not significant. There was, however, significant increase in NH_4-N after all periods, whilst nitrate decreased significantly with time of incubation.

Straw and grass treatments gave similar results with respect to nitrogen changes throughout incubation. After 2 weeks there was a net decrease in min-N and this was accounted for entirely by a decrease in NH_4-N. After 6 weeks there was a further small decrease in min-N and this was accounted for by a fair decrease in nitrate accompanied by a small increase in NH_4-N. After 13 weeks there were no further significant changes in NH_4- or NO_3-N levels due to straw or grass.

The sucrose treatment caused considerably greater decrease in min-N levels than did straw and grass after all periods of incubation. The greatest decrease in min-N due to sucrose occurred after 2 weeks and there was some increase in min-N levels with further incubation. After 2 weeks the decrease in min-N was accounted for to about the same extent by decreases in NH_4-N and NO_3-N. However, after 6 and 13 weeks the decrease in min-N were accounted for to a greater extent by decreases in NO_3-N than in NH_4-N.

The cellulose+sucrose treatment caused less decrease in min-N than did the sucrose treatment up to 2 weeks, in

particular, and also up to 6 weeks, but after 13 weeks the decreases were similar with both treatments. The cellulose+sucrose treatment caused a progressive decrease in min-N with time of incubation up to 13 weeks. The decrease in min-N up to 2 weeks was accounted for by a greater decrease in NH_4-N than in NO_3-N, whilst after 6 and 13 weeks the reverse applied.

Extractable Fe, Mn and Cu

Morgan-extractable iron (Morgan-Fe), EDTA-Fe and total Fe were increased during incubation only in the sucrose-treated soil. The increases were particularly great after 2 weeks but were also higher after 6 and 13 weeks than in the control. The Cellulose+sucrose treatment was the only treatment which increased EDTA and total Fe and then only after 2 weeks.

Morgan and total Mn were increased by grass, sucrose, and cellulose+sucrose treatments after 2 and 6 weeks. The increase persisted up to 13 weeks for grass, but in the case of sucrose and cellulose+sucrose Morgan and total Mn were decreased by 13 weeks. EDTA-Mn was increased by sucrose after all periods and by cellulose+sucrose only after 6 and 13 weeks.

Morgan-Cu was increased slightly, but significantly by all treatments, but only after 13 weeks. EDTA- and total Cu were increased slightly but significantly by all

treatments after most periods of incubation; the only exceptions were decreases in EDTA- and total Cu but then only after 2 weeks.

Carbon dioxide release

Table 20 B and Fig.13 show that the highest release of carbon dioxide occurred from the sucrose treated soil after all periods of incubation. Carbon dioxide release then decreased in the order of treatments cellulose+sucrose, grass, straw, and control. There were much greater differences in the rate of carbon dioxide release early in incubation, particularly during the 1st week, due to the type of organic material added. However, during the later periods of incubation the differences decreased, and during the 6 - 13 week period there were virtually no differences in the rates of release of carbon dioxide in the organic matter treated soils. However, the rate of release from the control soil during this period was still less than from the treated soils.

The percentage recovery of added organic carbon as carbon dioxide (Table 20 B last column) increased in the order straw, cellulose+sucrose, grass and sucrose.

DISCUSSION

In discussing the results it must be realized that mineralization (mobilization) of the organic N of soil,

grass and straw can occur concurrently with immobilization of inorganic N(NH_4- and/or NO_3-N) due to the nitrogen requirements of the microorganisms attacking the added organic materials and native organic N. Thus at any stage of incubation the mineral-N level due to a particular treatment represents the net effect of mineralization over immobilization or vice-versa.

The very high immobilization of N (80% of that present initially) after 2 weeks due to sucrose compared with the relatively small immobilization due to addition of other organic materials is a reflection of the ease with which sucrose is attacked by soil microorganisms. With incubation beyond 2 weeks the extent of net immobilization due to sucrose decreased because mineralization rate exceeded immobilization rate. However, the rate of mineralization during the 2 to 13 weeks period was relatively low, so that by the end of incubation 56% of the mineral-N present initially was still immobilized.

Grass and straw were decomposed relatively slowly compared with sucrose as shown by the fact that maximum net immobilization occurred only after 6 weeks. However, with incubation beyond 6 weeks the rate of immobilization of N was very similar to that with the sucrose treatment. This is shown by the approximately parallel decrease in immobilized-N during the 6 to 13 week period with these three treatments.

The cellulose+sucrose treatment caused a continuous increase in net N immobilization over the 13 weeks. Presumably the sucrose in the mixed treatment decomposed rapidly whilst cellulose decomposed only slowly so that even by 13 weeks there was still the highest net immobilization of N.

Both grass and straw tended to decompose relatively slowly and to about the same extent in this soil, in spite of the higher N content of the grass (0.90%N) compared with that of the straw (0.525%N). It is possible that this soil does not have a very active population of micro-organisms suitable for decomposing organic nitrogenous materials. On the other hand, it is clear that an active population of carbohydrate-decomposing microorganisms are present as shown by the relatively great extent of immobilization where sucrose or sucrose+cellulose were added. The lack of any differences in nitrogen changes during incubation between grass and straw in this acid soil (pH 4.2) differs considerably from results obtained with an English soil of similar texture but having a pH of 6.4 (232). In the latter soil the results for straw decomposition were similar to those obtained with the Pakistan soil, namely, maximum immobilization of N occurring during the early period of incubation, but little net decrease in immobilization even after prolonged incubation. However, with the grass treatment the English soil showed less net immobilization of N and in addition by a further 9 weeks of incubation

there was a net gain in N indicating that mineralization had exceeded immobilization. This indicates that the English soil has a suitable micro-organic population for decomposing both the organic N and carbohydrate constituents of the grass.

With those treatments (straw, grass and sucrose) where mineralization occurred during the later periods of incubation, the min-N accumulated entirely as NH_4-N. In the control soil there was a decrease in nitrate and increase in NH_4-N over 13 weeks of incubation. The lack of any nitrate accumulation indicates that nitrification was inhibited even in the control soil. It appears that nitrification (whether this was autotrophic or heterotrophic) was inhibited by the addition of ammonium nitrate in the control soil. In the organic matter-treated soils the presence of ammonium nitrate and/or added organic materials and their residues appeared to have inhibited nitrification. These results are quite different from those obtained by Quraishi (244) using an English soil of similar texture to the HF soil but having a pH 6.5. He found during the later (mineralization) phase of incubation of soil treated with grass and straw that min-N accumulated entirely as nitrate.

The rate and extent of carbon dioxide release during incubation is a measure of the decomposition of added and native organic carbonaceous materials. It was seen that

the greatest release of carbon dioxide after all periods of incubation occurred where sucrose was added. This was accompanied by the greatest extent of immobilization of mineral-N with up to 6 weeks of incubation. However, after 13 weeks incubation net N immobilization due to sucrose was no different from that due to cellulose+sucrose treatment. Although grass decomposed more rapidly and to a greater extent than did straw the pattern of immobilization of nitrogen was similar with both materials after all incubation periods. On the other hand, whereas grass and cellulose+ sucrose showed a similar pattern of decomposition, their pattern of N immobilization was quite different.

Sucrose and cellulose+sucrose were the only treatments which had any considerable effects on Morgan and EDTA extractable Fe and Mn. It is well known that insoluble Fe (III) and Mn (IV) compounds in soil are subject to microbial conversion to soluble Fe (II) and Mn(II) forms and that these conversions are favoured by low pH and the presence of decomposable organic materials (I). Morgan extractable forms of Fe and Mn are essentially exchangeable Fe(II) and Mn(II) forms of these elements; EDTA extractable forms are the complex (including chelated) forms of Fe(II&III) and Mn (II&IV). The maximum extractable levels of Fe occurred after 2 weeks and Mn after 6 weeks of incubation where sucrose or cellulose+sucrose were added. This indicates that the main reduction of Mn occurred mainly after most of the Fe

had been reduced. Whereas the increased extractable Fe was accounted for by both exchangeable and complexed forms, the increased extractable Mn was accounted for mainly as the exchangeable form. Both of the extractable forms of Fe and Mn decreased to levels little different from the control soil by the end of incubation.

SUMMARY

A study was made of the effects of adding 0.5% straw, grass, sucrose, and cellulose+sucrose (1:1) on levels of min-N, carbon dioxide release and extractable iron, manganese and copper during incubation (2, 6 and 13 weeks at 30°C and 50% mwhc) of HF soil (200 ppm N as ammonium nitrate added to all including control).

Sucrose caused the most rapid and greatest decrease in min-N, whilst grass and straw caused the least decrease during incubation. Cellulose+sucrose immobilization increased up to 13 weeks. After the immobilization phase there was relatively small mineralization of nitrogen with any of the treatments, and the mineralized-N accumulated entirely as NH_4-N.

Carbon dioxide release during incubation was increased to the greatest extent by the sucrose treatment and to the least extent by straw. Grass and cellulose+sucrose gave intermediate results. The recovery of added organic carbon

as carbon dioxide was 33.3% for straw, 55.2% for cellulose+ sucrose, 63.1% for grass and 82.6% for sucrose.

Sucrose and cellulose+sucrose increased Morgan and EDTA extractable Fe and Mn to a considerable extent after 2 and 6 weeks of incubation but had little effect after 13 weeks of incubation. Straw and grass had relatively little effect on extractable Fe and Mn. All added organic materials had small but significant effects on extractability of copper (Cu).

CHAPTER 7

MINERAL-N LEVELS DURING INCUBATION OF HIGH FLAT (HF) SOIL TREATED WITH UREA AND $(NH_4)_2SO_4$

Experiment 9

Mineral-N levels during incubation (30°C, 50% mwhc) for 1, 3 and 6 weeks of original HIGH FLAT soil treated with varying levels of urea and ammonium sulphate.

Experiment 10

Effects of addition of calcium carbonate and urea on mineral-N levels during incubation (at 30°C, 50% mwhc) for 3 weeks of HIGH FLAT (HF) soil which had been allowed to react with straw+urea and compost for 6 months

CHAPTER 7

MINERAL-N LEVELS DURING INCUBATION OF HIGH FLAT (HF) SOIL TREATED WITH UREA AND $(NH_4)_2SO_4$

Experiment 9

Mineral-N levels during incubation (30°C, 50% mwhc) for 1, 3 and 6 weeks of original HIGH FLAT soil treated with varying levels of urea and ammonium sulphate

METHODS

The original HIGH FLAT (HF) soil was set up for incubation (30°C, 50% mwhc) after addition of finely ground urea and ammonium sulphate to supply 50, 100 and 200 ppm N on dry soil basis. A control, receiving no N was also set up. Analyses were made for NH_4-N and NO_3-N initially and after each incubation period with Morgan reagent.

RESULTS

Table 21 and Figure 14 show mineral-N levels during incubation. Table 22 shows accumulation values (a) due to soil and added N sources and (b) due to added N sources.

TABLE 21

MINERAL-N LEVELS DURING INCUBATION (30°C, 50% mwhc) FOR 1,3,& 6 WEEKS OF ORIGINAL HIGH FLAT SOIL TREATED WITH VARYING LEVELS OF UREA AND AMMONIUM SULPHATE ($(NH_4)_2SO_4$)

(Results are in ppm on dry soil basis)

Treatment	Level of N in ppm	NH_4-N	NO_3-N	min-N	pH
Initial Values					
Control	Nil	14	7	21	4.2
Urea	50	14	7	21	4.2
"	100	14	12	26	4.2
"	200	14	13	27	4.2
$(NH_4)_2SO_4$	50	48	11	59	4.1
"	100	81	10	91	4.1
"	200	178	11	189	4.0
After 1 Week incubation					
Control	Nil	26	2	28	4.3
Urea	50	50	20	70	4.7
"	100	88	21	109	5.0
"	200	143	20	163	5.6
$(NH_4)_2SO_4$	50	62	10	72	4.4
"	100	93	6	99	4.4
"	200	181	5	186	4.2
After 3 weeksincubation					
Control	Nil	36	20	56	4.4
Urea	50	59	22	81	4.6
"	100	84	46	130	5.0
"	200	165	32	197	5.3
$(NH_4)_2SO_4$	50	96	-9	87	4.4
"	100	115	14	129	4.4
"	200	196	-4	192	4.3
After 6 weeks incubation					
Control	Nil	23	22	45	4.0
Urea	50	62	13	75	4.5
"	100	82	41	123	4.5
"	200	148	18	166	5.0
$(NH_4)_2SO_4$	50	72	8	80	4.3
"	100	104	13	117	4.3
"	200	186	6	192	4.3

L.S.D. at $P < 0.05$ NH_4-N 8.5, NO_3-N 7.4, min-N 9.0 ppm

FIGURE 14

MINERAL-N LEVELS DURING INCUBATION (30°C, 50 mwhc) FOR 1,3 & 6 WEEKS OF OF ORIGINAL HIGH FLAT SOIL TREATED WITH VARYING LEVELS OF UREA AND AMMONIUM SULPHATE ($(NH_4)_2SO_4$)

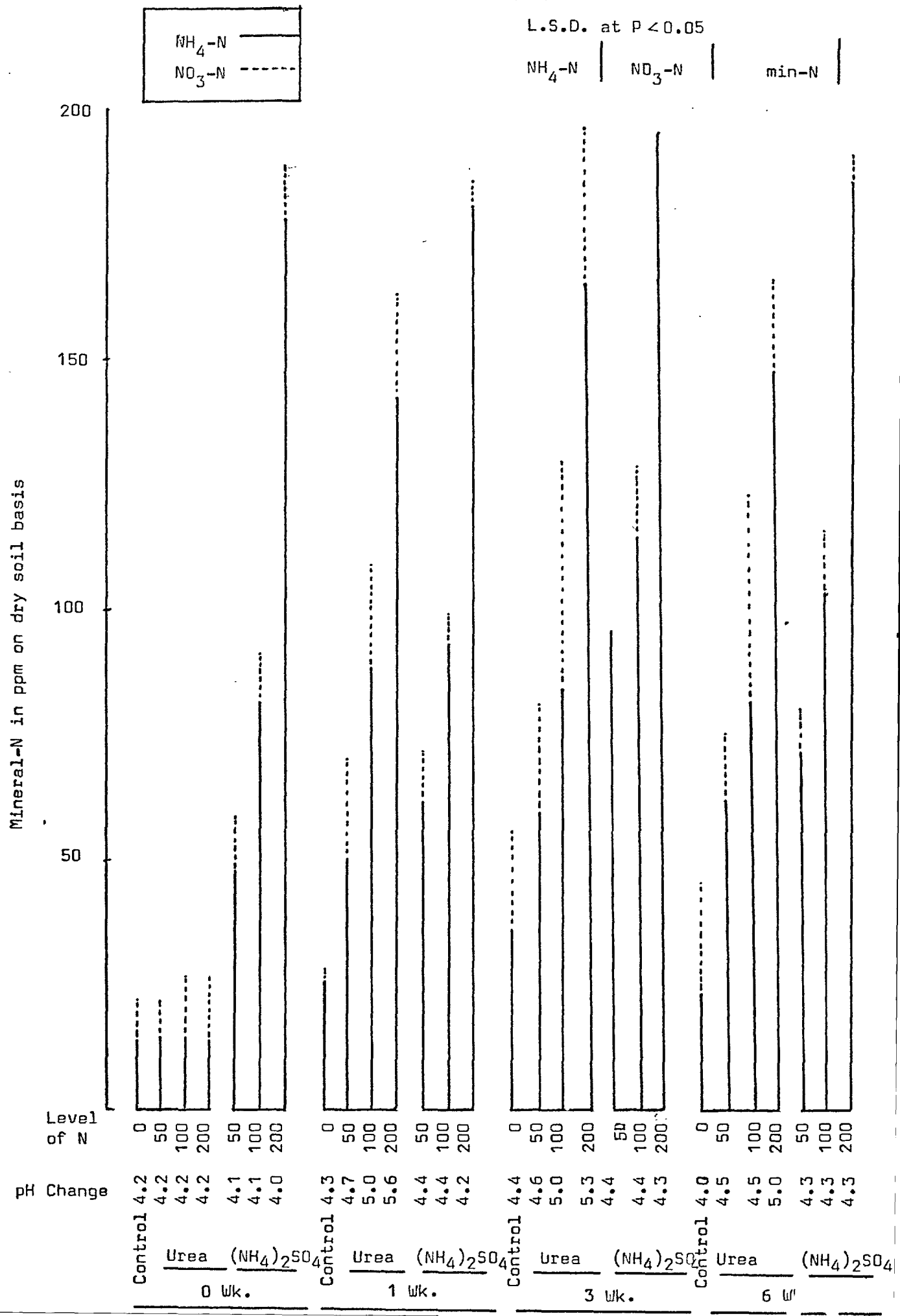


TABLE 22

ACCUMULATION OF MINERAL-N VALUES OF EXPERIMENT 9 (See Table 21)

(Results are in ppm on dry soil basis)

		Accumulation due to soil and added N sources			Accumulation due to added N sources			
		After 1 week incubation			After 1 week incubation			
Treatment	Level of N in ppm	NH_4-N	NO_3-N	min-N	NH_4-N	NO_3-N	min-N	pH
Control	Nil	12	-5	7				4.3
Urea	50	36	13	49	24	18	42	4.7
"	100	74	9	83	62	14	76	5.0
"	200	129	7	136	117	12	129	5.6
$(NH_4)_2SO_4$	50	14	-1	13	2	4	6	4.4
"	100	12	-4	8	0	1	1	4.4
"	200	3	-6	-3	-9	-1	-10	4.2
		After 3 weeks incubation			After 3 weeks incubation			
Control	Nil	22	13	35				4.4
Urea	50	45	15	60	23	2	25	4.6
"	100	70	34	104	48	21	69	5.0
"	200	151	19	170	129	6	135	5.3
$(NH_4)_2SO_4$	50	48	-20	28	26	-33	-7	4.4
"	100	34	4	38	12	-9	3	4.4
"	200	18	-15	3	-4	-28	-32	4.3
		After 6 weeks incubation			After 6 weeks incubation			
Control	Nil	9	15	24				4.0
Urea	50	48	6	54	39	-9	30	4.5
"	100	68	29	97	59	14	73	4.5
"	200	134	5	139	125	-10	115	5.0
$(NH_4)_2SO_4$	50	24	-3	21	15	-18	-3	4.3
"	100	23	3	26	14	-12	2	4.3
"	200	8	-5	3	-1	-20	-21	4.3

L.S.D. at $P < 0.05$ NH_4-N 8.5, NO_3-N 7.4, min-N 9.0 ppm

Mineral-N

All the mineralization of urea which occurred did so during the first week of incubation; with further incubation up to 6 weeks there was usually a slight decline in mineral-N derived from urea at all application levels. There was a general decline in the extent of accumulation of mineral-N with increasing level of ammonium sulphate. This decline appeared to be due to loss of applied NH_4-N and was particularly high with the 200 ppm N application.

Nitrate-N

With 100 ppm added urea-N there were significant increases in nitrate derived from urea after all periods of incubation. With 50 and 200 ppm urea-N applications, nitrate accumulation occurred during the first week of incubation, but nitrate disappeared with further incubation. There was no nitrate accumulation from ammonium sulphate with any applied level or after any period of incubation. With incubation after 1 week there was apparent loss of nitrate derived from ammonium sulphate, the smallest loss occurring where 100 ppm NH_4-N was added.

There were increases in pH due to urea applications after all periods of incubation, with the maximum increases after 1 week and a tendency for pH to decrease with further incubation. However, even after 6 weeks pH was higher in the treated soils than in the control soil.

DISCUSSION

The apparent recovery of urea-N as mineral-N ranged from 50% to 84%. It appears that either urea-N was not completely mineralized, but this seems unlikely, or that the urea-N itself or the NH_4- or NO_3 formed from it were immobilized. Where mineral-N was immobilized this occurred only during the 3 to 6 week incubation period. The form of mineral nitrogen which was immobilized varied with the rate of urea application. Where 200 ppm urea-N were applied both NH_4- and NO_3-N appeared to be the sources of immobilized N. Where 100 ppm urea-N was applied there was no significant immobilization of either NH_4- or NO_3-N. Where 50 ppm urea-N was applied there was significant immobilization only of nitrate.

Where 50 or 200 ppm NH_4-N from ammonium sulphate was applied nitrate was immobilized during the 1 to 3 week period but accumulated again up to 6 weeks.

There was no accumulation of nitrate where ammonium sulphate was added at any level. On the other hand, when urea was added significant accumulation of nitrate occurred after 1 week from all levels of urea. This accumulation persisted to the end of incubation where 100 ppm urea-N was applied, but disappeared after 1 week with the 50 and 200 ppm urea-N treatments. The increase in soil pH (by 0.4 to 1.3 units) due to the urea treatments may have stimulated

autotrophic nitrification. However, the possibility of heterotrophic nitrification occurring as well cannot be excluded, since during the first week of incubation all the nitrate apparently came from urea with all levels of applied urea. In addition with the 100 ppm urea-N treatment approximately two thirds of the nitrate formed appeared to have been derived from urea. The persistance of nitrate accumulation due to 100 ppm urea-N in contrast to loss of nitrate after 1 week where the two other levels of urea were applied must be due to immobilization of nitrate in the latter two treatments.

Experiment 10

Effects of addition of calcium carbonate and urea on mineral-N levels during incubation (at 30°C, 50% mwhc) for 3 weeks of HIGH FLAT (HF) soil which had been allowed to react with straw+urea and compost for 6 months

In all the experiments described previously nitrogen changes during incubation have been studied using the original soils treated with various sources of nitrogen and different forms of organic materials just before incubation. The purpose of this experiment was to determine to what extent nitrogen changes in the soils were affected by treating

the soils with straw and compost and allowing these to decompose for 6 months before setting up the soil for incubation. The treatments given just before incubation were addition of 0.2% calcium carbonate or 100 ppm urea-N separately and together.

METHODS

500g. portions of HF soil were mixed with (i) 1% by weight of finely ground compost (40% organic carbon, 2.52% total N and (ii) 1% finely ground straw (42.5% organic carbon, 0.525% total N) and 100 ppm nitrogen as finely ground urea (the urea-N was added to ensure that mineral-N did not limit decomposition of straw). The treated soils and also 500g of original soil were placed in pots and wetted to approximatel 50% mwhc. The pots were held at room temperature (18 -22°C) for 6 months and water was added when necessary to maintain moisture content between 40% and 50% mwhc. The soils were then air-dried and passed through 1 mm sieve. The three soils (control, straw-, and compost-treated) were set up for incubation (30°C, 50% mwhc) for 3 weeks with the following treatments being given just before incubation (i) nothing added, (ii) 0.2 % calcium carbonate added, (iii) 100 ppm urea-N added and (iv) 0.2% calcium carbonate and 100 ppm urea-N added. Mineral-N levels were determined in Morgan extracts.

RESULTS

6 months pre-incubation period

Mineral-N values at the beginning and end of the 6 months period after treatment with straw+urea and compost, and total nitrogen and Walkley-Black carbon values at the end of 6 months are shown in Table 23.

In the control soil ammonium-N increased and nitrate-N decreased over the 6 months and mineral-N was hardly affected. In the straw+urea-treated soil mineral-N increased by 50.4 ppm, with ammonia accumulation being somewhat higher than nitrate accumulation. In the compost treated soil mineral-N increased by 30.1 ppm, with ammonia accumulation being twice that of nitrate. There was virtually no change in pH in any of the soils over 6 months. Total nitrogen content at the end of 6 months was increased to a greater extent by the compost than by the straw+urea treatment. Walkley-Black carbon content was increased slightly by straw+urea and to a moderate extent by compost.

Incubation period

Table 24 and Figure 15 show levels of mineral-N obtained during incubation with the treatments described above (calcium carbonate and urea alone and in combination). Table 25 shows the corresponding accumulation values.

TABLE 23

MINERAL-N VALUES AT THE BEGINNING AND END OF THE 6 MONTHS PERIOD AFTER TREATMENT WITH STRAW+UREA AND COMPOST AND TOTAL NITROGEN AND WALKLEY-BLACK CARBON VALUES AT THE END OF 6 MONTHS

(Results are in ppm N on dry soil basis)

Treatment	NH_4-N		NO_3-N		min-N		pH	
	0	6 months	0	6 months	0	6 months	0	6 months
Control	9.3	13.3	8.4	2.8	17.7	16.1	4.2	4.1
Straw+ Urea	15.4	46.4	7.7	27.1	23.1	73.5	4.1	4.1
Compost	15.4	35.7	21.7	31.5	37.1	67.2	4.1	4.1

TOTAL N, AND WALKLEY-BLACK C AND pH AFTER 6 MONTHS

	Total N (%)	Walkley-Black C(%)	pH
Control	0.073	0.92	4.1
Straw+Urea	0.079	0.93	4.1
Compost	0.086	0.99	4.1

TABLE 24

EFFECTS OF ADDITION OF CALCIUM CARBONATE (0.2%) AND UREA (100 ppm N) ON MINERAL-N LEVELS DURING INCUBATION (at 30°C, 50% mwhc) FOR 3 WEEKS OF HIGH FLAT (HF) SOIL WHICH HAD BEEN ALLOWED TO REACT WITH STRAW AND COMPOST FOR 6 MONTHS

(Results are in ppm N on dry soil basis)

WITHOUT ADDED CALCIUM CARBONATE ($CaCO_3$)

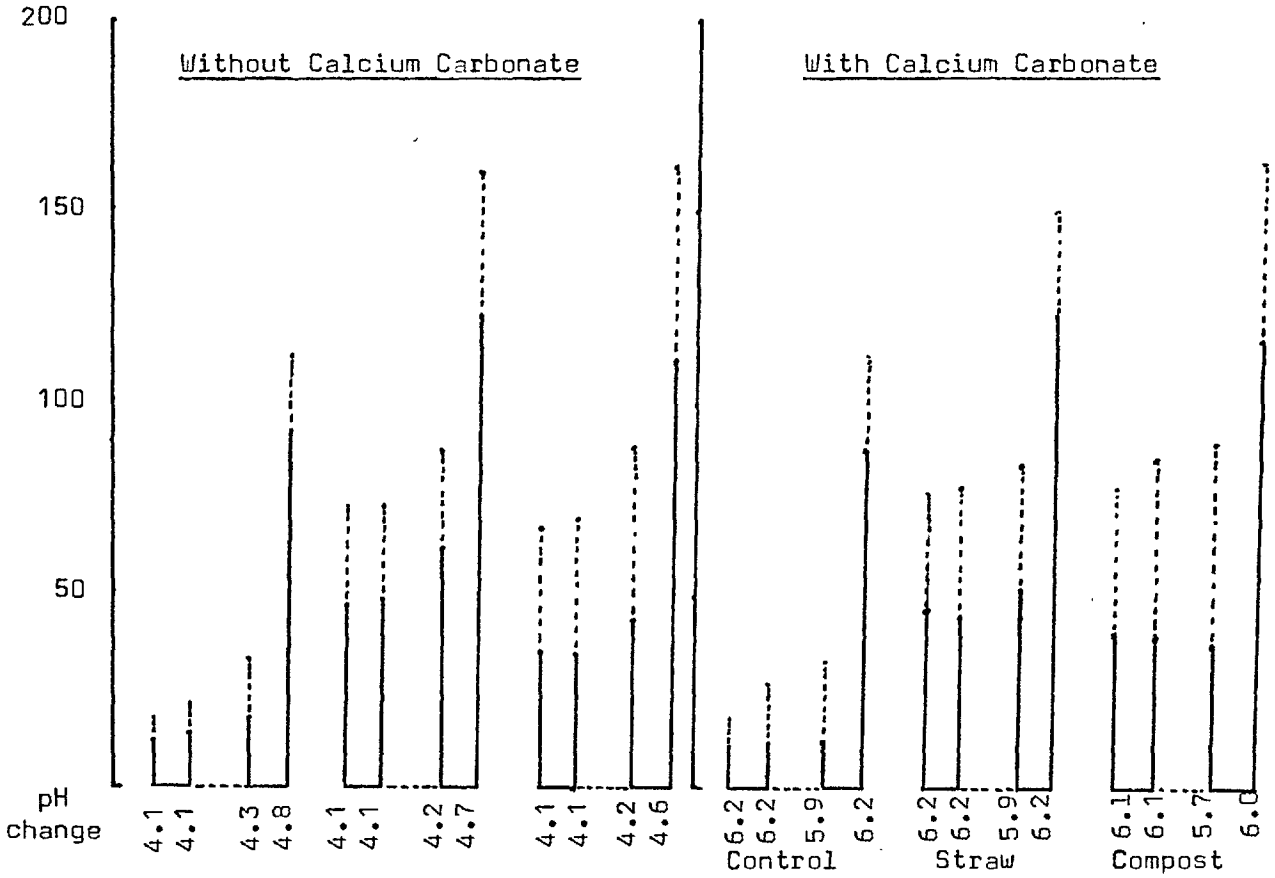
After 0 week incubation

Treatment	NH_4-N		NO_3-N		min-N		pH	
	Nil N	Added N	Nil N	Added N	Nil N	Added N	Nil N	Added N
Control	13.3	14.7	2.8	6.3	16.1	21.0	4.1	4.1
Straw	46.4	47.6	27.1	25.9	73.5	73.5	4.1	4.1
Compost	35.7	35.0	31.5	34.3	67.2	69.3	4.1	4.1
After 3 weeks incubation								
Control	18.2	91.7	14.7	20.3	32.9	112.0	4.3	4.8
Straw	60.2	121.8	27.3	37.8	87.5	159.6	4.2	4.7
Compost	42.7	109.8	46.9	50.5	89.6	160.3	4.2	4.6
WITH ADDED CALCIUM CARBONATE								
After 0 week incubation								
Control	11.2	11.2	8.4	16.1	19.6	27.3	6.2	6.2
Straw	46.2	45.5	30.8	32.2	77.0	77.7	6.2	6.2
Compost	39.9	39.2	39.2	46.2	79.1	85.4	6.1	6.1
After 3 weeks incubation								
Control	14.0	88.9	18.2	24.5	32.2	113.4	5.9	6.2
Straw	51.8	122.5	32.9	27.3	84.7	149.8	5.9	6.2
Compost	39.2	115.0	51.1	48.1	90.3	163.1	5.7	6.0
WITHOUT ADDED UREA								
After 0 week incubation								
Treatment	Nil $CaCO_3$	Added $CaCO_3$	Nil $CaCO_3$	Added $CaCO_3$	Nil $CaCO_3$	Added $CaCO_3$	Nil $CaCO_3$	Added $CaCO_3$
Control	13.3	11.2	2.8	8.4	16.1	19.6	4.1	6.2
Straw	46.4	46.2	27.1	30.8	73.5	77.0	4.1	6.2
Compost	35.7	39.9	31.5	39.2	67.2	79.1	4.1	6.1
After 3 weeks incubation								
Control	18.2	14.0	14.7	18.2	32.9	32.2	4.3	5.9
Straw	60.2	51.8	27.3	32.9	87.5	84.7	4.2	5.9
Compost	42.7	39.2	46.9	51.1	89.6	90.3	4.2	5.7

L.S.D. at $P < 0.05$ NH_4-N 1.6, NO_3-N 1.4, min-N 2.8 ppm

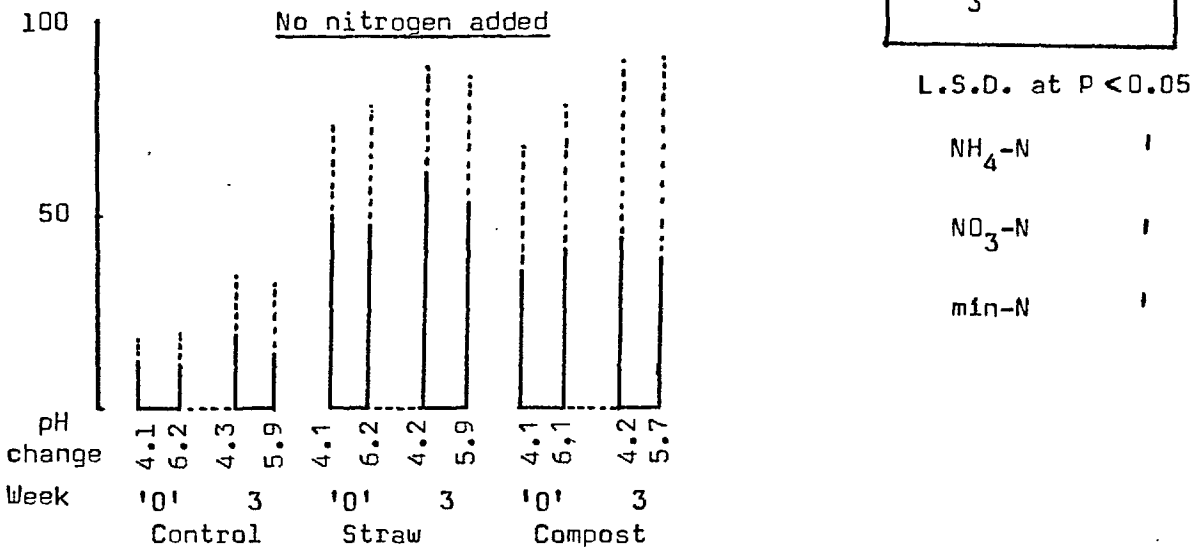
FIGURE 15

EFFECTS OF ADDITION OF CALCIUM CARBONATE (0.2%) AND UREA (100 ppm N) ON MINERAL-N LEVELS DURING INCUBATION (at 30°C, 50% mwhc) FOR 3 WEEKS OF HIGH FLAT (HF) SOIL (WHICH HAVE BEEN ALLOWED TO REACT WITH STRAW AND COMPOST FOR 6 MONTHS)



For each pair of mineral-N values the left hand one represent values with "No N" and the right hand represents values with added "N".

Mineral-N in ppm N on dry soil basis



For each pair of mineral-N values the left hand one represents values without calcium carbonate and the right hand one represents values with added calcium carbonate.

TABLE 25

MINERAL-N ACCUMULATION VALUES FOR EXPERIMENT 10 AFTER 3 WEEKS OF INCUBATION (See Table 24)

(Results are in ppm N on dry soil basis)

Without added calcium carbonate

Treatment	NH_4-N		NO_3-N		min-N		pH	
	Nil N	Added N	Nil N	Added N	Nil N	Added N	Nil N	Added N
Control	4.9	77.0	11.9	14.0	16.8	91.0	4.3	4.8
Straw	13.8	74.2	0.2	11.9	14.0	86.1	4.2	4.7
Compost	7.0	74.8	15.4	16.2	22.4	91.0	4.2	4.6
With added calcium carbonate								
Control	2.8	77.7	9.4	8.4	12.2	86.1	5.9	6.2
Straw	5.6	77.0	2.1	-4.9	7.7	72.1	5.9	6.2
Compost	-0.7	75.8	11.9	1.9	11.2	77.7	5.7	6.0
Without added urea								
	Nil $CaCO_3$	Added $CaCO_3$	Nil $CaCO_3$	Added $CaCO_3$	Nil $CaCO_3$	Added $CaCO_3$	Nil $CaCO_3$	Added $CaCO_3$
Control	4.9	2.8	11.9	9.4	16.8	12.2	4.3	5.9
Straw	13.8	5.6	0.2	2.1	14.0	7.7	4.2	5.9
Compost	7.0	-0.7	15.4	11.9	22.4	11.2	4.2	5.7

L.S.D. at $P < 0.05$ NH_4-N 1.6, NO_3-N 1.4, min-N 2.8 ppm

Without added calcium carbonate

Where no urea was added mineral-N accumulation during incubation was higher in the compost- than in the straw-treated and control soils. Mineral-N accumulated mainly as ammonium-N in the straw-treated soil and as nitrate-N in the two other soils. Where urea was added there were significant but small differences due to the pre-incubation treatment. The straw treatment decreased whilst the compost treatment had no effect on mineral-N accumulation. With all treatments mineral-N accumulated mainly as ammonium-N.

With added calcium carbonate

Where no urea was added the straw treatment but not the compost decreased mineral-N and nitrate-N accumulation. Where urea was added there were again significant but small differences due to pre-incubation treatment. The straw treatment decreased mineral-N and nitrate-N accumulation more than did compost and the straw resulted in a net loss of nitrate-N.

Without added urea

Without added calcium carbonate mineral-N accumulation was higher with all pre-incubation treatments than with calcium carbonate particularly in the compost-treated soil.

The straw treatment decreased whilst the compost treatment increased nitrate-N accumulation with or without added calcium carbonate. The straw treatment increased ammonium-N accumulation with or without added calcium carbonate; the compost treatment increased ammonium-N accumulation without added calcium carbonate but decreased it with added calcium carbonate.

DISCUSSION

During the 6 months pre-incubation period it was calculated (Table 23) that about 80% of the compost-carbon and about 97% of the straw-carbon had disappeared from the soil presumably as carbon dioxide. This testifies to the high activity of heterotrophic organisms in decomposing most or virtually all of the added carbonaceous materials, in spite of the relatively low pH (4.1) of this soil. This is in agreement with the well-known observation that organic manures added to such tropical soils disappear quite rapidly and show no tendency to accumulate. On the other hand, in acid British forest soils the leaf fall tends to undergo only partial decomposition and to accumulate so as to increase the humus content of the soil (mor humus).

The main purpose of this experiment was to determine whether treatment of the HF soil with organic materials for some months before hand would have any effect on subsequent changes involving added urea with and without calcium

carbonate addition. Even though much or all of the organic materials added during the pre-incubation period had disappeared after 6 months, it seems reasonable to assume that the microbiological pattern of the soil had been altered as a result of the straw and compost decomposition. However, it was found that the mineralization rate of urea was little affected by the pre-incubation straw and compost treatments where no calcium carbonate was added before incubation. Where calcium carbonate was added to raise the soil pH to about 6 both pre-incubation treatments tended to decrease the extent of mineralization of urea, but only to a limited extent. The generally higher accumulation of nitrate without than with added calcium carbonate where urea was added indicates that urea inhibited heterotrophic nitrification. If the autotrophic nitrification process was operative in this soil we would expect that raising the pH by calcium carbonate addition would increase nitrification rate, but the reverse occurred. The addition of urea with calcium carbonate raised the pH even further but resulted in even less nitrate-N accumulation in the straw- and compost-treated soils.

S U M M A R Y

Experiment 9

Mineral-N levels were determined after adding 50, 100 and 200 ppm N as urea and ammonium sulphate to the

HIGH FLAT (HF) soil before incubation (30°C, 50% mwhc) for 1, 3 and 6 weeks.

Maximum mineralization of urea occurred during the 1st week of incubation. There was loss of mineral-N with time of incubation, probably by immobilization, with the highest level of applied ammonium sulphate. Nitrate accumulated to the greatest extent where 100 ppm urea-N was added; with the other two levels of urea nitrate which accumulated early in incubation disappeared by 6 weeks. Where 50 and 200 ppm ammonium sulphate-N was applied nitrate disappeared up to 3 weeks but increased during the 3 - 6 week period.

Experiment 10

When the HIGH FLAT (HF) soil was treated with 1% compost or 1% straw+urea (100 ppm N) and kept moist for 6 months at 18 - 22°C, about 80% of the compost-carbon and 97% of the straw-carbon disappeared.

These soils, including the control, were treated with 0.2% calcium carbonate and 100 ppm urea-N, alone or in combination and incubated (30°C, 50% mwhc) for 3 weeks. Where no calcium carbonate was added the mineralization rate of urea was little affected by the pre-incubation treatments. Where calcium carbonate was added (to raise soil pH to about 6) both pre-incubation treatments tended

to decrease the extent of mineralization of urea to a limited extent. The pre-incubation straw treatment prevented nitrate accumulation during incubation, whilst the compost treatment increased nitrification slightly.

CHAPTER 8

MINERAL-N LEVELS DURING INCUBATION OF HIGH FLAT (HF) SOIL TREATED WITH NITROGEN AS UREA, OXAMIDE AND PEA SEED WITHOUT AND WITH PRE-INCUBATION TREATMENT WITH STRAW+N OR COMPOST

Experiment 11

Mineral-N levels during incubation (30°C, 50% mwhc) for 1, 3 and 6 weeks of HIGH FLAT soil treated with 100 ppm N as urea, oxamide and pea seed

Experiment 12

Effect of previous treatment* of HIGH FLAT (HF) soil with straw+N or compost on mineral-N levels during incubation (6 weeks, 30°C & 50% mwhc) immediately after treatment with 100 ppm N as urea, oxamide or pea seed

*(The previous treatment involved holding in a moist condition control, straw+N and compost treated soils for 6 months)

CHAPTER 8

MINERAL-N LEVELS DURING INCUBATION OF HIGH FLAT (HF) SOIL TREATED WITH NITROGEN AS UREA, OXAMIDE AND PEA SEED WITHOUT AND WITH PRE-INCUBATION TREATMENT WITH STRAW+N OR COMPOST

Experiment 11

Mineral-N levels during incubation (30°C, 50% mwhc) for 1, 3 and 6 weeks of HIGH FLAT soil treated with 100 ppm N as urea, oxamide and pea seed

In this experiment two simple organic sources (urea and oxamide) and a vegetable source (pea seed) of nitrogen were applied to the HIGH FLAT (HF) soil at the same rate of nitrogen and mineral-N levels were determined during subsequent aerobic incubation.

METHODS

The materials used were reagent grade urea (47%N), oxamide (32%N) and ground dried pea seed (3.4%N). Bulk samples of the orginal HF soil (1 mm sieved)were mixed with these materials in finely ground form to supply 100 ppm N on dry soil basis. 10g portions of treated and control

soils were weighed into incubation tubes and wetted to 50% mwhc. Sufficient replicates of each were prepared to allow for analysis in duplicate initially and after 1, 3 and 6 weeks of incubation at 30°C. pH was determined after each period and the Morgan reagent was used for extraction of mineral-N.

RESULTS

Table 26 shows mineral-N levels initially and after each incubation period. Table 27 A shows mineral-N accumulation values due to soil and added organic materials and Table 27 B shows accumulation values due to added organic materials.

Maximum mineral-N accumulation from urea occurred after 3 weeks of incubation, from oxamide after 1 week and from pea seed only after 6 weeks. There was little accumulation of mineral-N from pea seed up to 3 weeks. Mineral-N accumulation from urea was somewhat greater than from oxamide, whilst that from pea seed was relatively low.

Nitrate accumulation from urea and oxamide increased with incubation up to 3 weeks and remain approximately the same up to 6 weeks. There was no significant accumulation of nitrate from pea seed up to 1 week, and nitrate disappeared up to 3 and 6 weeks.

TABLE 26

MINERAL-N LEVELS DURING INCUBATION (30°C, 50% mwhc) FOR 1, 3 & 6 WEEKS OF HIGH FLAT SOIL TREATED WITH 100 ppm N AS UREA, OXAMIDE AND PEA SEED

(Results are in ppm N on dry soil basis)

Treatment	NH_4-N	NO_3-N	min-N	pH
		Initial Values		
Control	9.8	8.4	18.2	4.2
Urea	9.8	11.2	21.0	4.2
Oxamide	26.6	8.4	35.0	4.2
Pea seed	9.5	8.7	18.2	4.2
		After 1 week incubation		
Control	19.6	10.8	30.4	4.2
Urea	86.1	16.8	102.9	4.8
Oxamide	80.5	14.0	94.5	4.8
Pea seed	22.4	10.5	32.9	4.6
		After 3 weeks incubation		
Control	22.4	11.2	33.6	4.2
Urea	86.8	25.2	112.0	4.8
Oxamide	77.0	20.3	97.3	4.7
Pea seed	28.0	6.3	34.3	5.2
		After 6 weeks incubation		
Control	23.8	15.4	39.2	4.4
Urea	80.5	30.1	110.6	4.8
Oxamide	74.2	22.4	96.6	4.8
Pea seed	53.9	2.1	56.0	5.2

L.S.D. $P < 0.05$ NH_4-N 3.2, NO_3-N 1.4, min-N 2.1 ppm

TABLE 27 A

MINERAL-N ACCUMULATION VALUES FOR EXPERIMENT 11 AFTER EACH INCUBATION PERIOD (See Table 26)

(Results are in ppm N on dry soil basis)

After 1 week incubation

Treatment	NH_4-N	NO_3-N	min-N	pH
Control	9.8	2.4	12.2	4.2
Urea	76.3	5.6	81.9	4.8
Oxamide	53.9	5.6	59.5	4.8
Pea seed	12.9	1.5	24.4	4.6
After 3 weeks incubation				
Control	12.6	2.8	15.4	4.2
Urea	77.0	14.0	91.0	4.8
Oxamide	50.4	11.9	62.3	4.7
Pea seed	18.5	-2.4	16.1	5.2
After 6 weeks incubation				
Control	14.0	7.0	21.0	4.4
Urea	70.7	18.9	89.6	4.8
Oxamide	47.6	14.0	61.6	4.8
Pea seed	44.4	-6.6	37.8	5.2

L.S.D. $P < 0.05$ NH_4-N 3.2, NO_3-N 1.4, min-N 2.1 ppm

TABLE 27 B

MINERAL-N ACCUMULATION VALUES DUE TO ADDED N SOURCE FOR EXPT.11 AFTER EACH INCUBATION PERIOD (See Table 27 A)

(Results are in ppm N on dry soil basis)

After 1 week incubation

Treatment	NH_4-N	NO_3-N	min-N	pH
Urea	66.5	3.2	69.7	4.8
Oxamide	44.1	3.2	47.3	4.8
Pea seed	3.1	-0.9	2.2	4.6
After 3 weeks incubation				
Urea	64.4	11.2	75.6	4.8
Oxamide	37.8	9.1	46.9	4.7
Pea seed	5.9	-5.2	0.7	5.2
After 6 weeks incubation				
Urea	56.7	11.9	68.6	4.8
Oxamide	33.6	7.0	40.6	4.8
Pea seed	30.4	-13.6	16.8	5.2

L.S.D. at $P < 0.05$ NH_4-N 3.2, NO_3-N 1.4, min-N 2.1 ppm

Mineral-N accumulated mainly as ammonium from urea and oxamide and entirely as ammonium from pea seed.

DISCUSSION

It is seen that most of the urea and oxamide which was mineralized did so within the 1st week of incubation. The small decreases in mineral-N which occurred during the last 3 weeks was probably due to immobilization. The lower apparent recovery of oxamide-N as mineral-N compared with that of urea-N may be due to lower mineralization of oxamide-N than of urea-N. Alternatively, immobilization of oxamide-N may be higher than that of urea-N after all periods of incubation. Both these materials rely on enzyme activity to convert the nitrogen to ammonium form. The extent of increase in pH due to both of these materials was virtually the same after all periods of incubation so that the lower recovery as mineral-N of oxamide-N than of urea-N is not due to pH but either to a lower mineralization rate or immobilization rate of oxamide-N. This immobilization is probably due to decomposition of native organic compounds of wide C/N ratio.

Pea seed contains proteins and carbohydrates. The lack of any mineral-N accumulation due to pea seed up to 3 weeks is probably due to the relatively slow rate of

mineralization of the proteins of pea seed. The decomposing carbohydrates in pea seed appeared to prefer immobilization of nitrate over ammonium, as indicated by the gradual decrease or even disappearance of nitrate with time of incubation. It is possible that the nitrate accumulation from urea and oxamide may have been due to heterotrophic nitrification of these materials in view of the fact that pH during incubation did not exceed 4.8, which pH is probably too low for autotrophic nitrification.

Experiment 12

Effect of previous treatment* of HIGH FLAT (HF) soil with straw+N or compost on mineral-N levels during incubation (6 weeks, 30°C and 50% mwhc) immediately after treatment with 100 ppm N as urea, oxamide or pea seed

(*The previous treatment involved holding in a moist condition control, straw+N and compost treated soils for 6 months)

The previous experiment reported on mineral-N levels during incubation following addition of urea, oxamide and pea seed to the original HF soil. It was considered of interest to determine whether previous treatment of the soil with straw or compost, as described in chapter 7,

would influence the nitrogen mineralizing characteristics of the soil immediately following addition of the same three sources of nitrogen.

METHODS

The HF soil which had been pretreated with compost or straw+urea-N as described in chapter 7 was used in this experiment. The treatments of the pre-incubation control and treated soils with urea, oxamide and pea seed were the same as described in the previous experiment (Experiment 11). Sufficient replicates were set up to allow for analysis in duplicate initially and after 6 weeks of incubation (30°C, 50% mwhc).

RESULTS

Table 28 shows mineral-N levels initially and after 6 weeks of incubation, and Table 29 A shows corresponding accumulation values after 6 weeks of incubation. Table 29 B shows accumulation values due to the added sources of N (urea, oxamide and pea seed).

In the pre-incubation control soil mineral-N accumulation over 6 weeks due to added nitrogen source decreased in the order urea, oxamide and pea seed. Mineral-N accumulated

TABLE 28

EFFECT OF PREVIOUS TREATMENT OF HIGH FLAT (HF) SOIL WITH STRAW + N OR COMPOST ON MINERAL-N LEVELS DURING INCUBATION (6 weeks 30°C & 50% mwhc) IMMEDIATELY AFTER TREATMENT WITH 100 ppm N AS UREA, OXAMIDE OR PEA SEED

(The previous treatment involved holding in moist condition control, straw+N and compost treated soils for 6 months)

(Results are in ppm N on dry soil basis)

Initial Values

Pre-incubation control (No previous straw or compost treatment)

N source added before incubation	NH_4-N	NO_3-N	min-N	pH
0 (control)	13.3	2.8	16.1	4.1
Urea	14.7	6.3	21.0	4.1
Oxamide	21.2	5.1	26.3	4.1
Pea seed	9.5	6.1	15.6	4.1
Pre-incubation straw+N treatment				
0 (control)	46.4	27.1	73.5	4.1
Urea	47.6	25.9	73.5	4.1
Oxamide	87.5	28.0	115.5	4.0
Pea seed	47.6	30.1	77.7	4.1
Pre-incubation compost treatment				
0 (control)	35.7	31.5	67.2	4.1
Urea	35.0	34.3	69.3	4.1
Oxamide	77.7	35.0	112.7	4.0
Pea seed	49.0	32.2	81.2	4.0
After 6 weeks incubation				
Pre-incubation control				
0 (control)	21.0	15.4	36.4	4.2
Urea	82.6	29.4	112.0	4.8
Oxamide	70.0	21.7	91.7	4.8
Pea seed	54.6	2.8	57.4	5.3
Pre-incubation straw+N treatment				
0 (control)	64.4	26.6	91.0	4.4
Urea	113.4	43.4	156.8	4.9
Oxamide	126.0	25.9	151.9	5.0
Pea seed	77.0	4.2	81.2	5.0
Pre-incubation compost treatment				
0 (control)	50.4	33.6	84.0	4.3
Urea	126.0	43.4	169.4	4.8
Oxamide	113.4	29.4	142.8	4.8
Pea seed	65.8	2.8	68.6	5.1

L.S.D. at $P < 0.05$ NH_4- N 3.7, NO_3-N 3.5, min-N 4.3 ppm

TABLE 29 A

MINERAL-N ACCUMULATION VALUES FOR EXPERIMENT 12 AFTER 6 WEEKS OF INCUBATION (See Table 28)

(Results are in ppm N on dry soil basis)

N source added before incubation	NH_4-N	NO_3-N	min-N	pH
Pre-incubation control				
0 (control)	7.7	12.6	20.3	4.2
Urea	67.9	23.1	91.0	4.8
Oxamide	48.8	16.6	65.4	4.8
Pea seed	45.1	-3.3	41.8	5.3
Pre-incubation straw+N treatment				
0 (control)	18.0	-0.5	17.5	4.4
Urea	65.8	17.5	83.3	4.9
Oxamide	38.5	-2.1	36.4	5.0
Pea seed	29.4	-25.9	3.5	5.0
Pre-incubation compost treatment				
0 (control)	14.7	2.1	16.8	4.3
Urea	91.0	9.1	100.1	4.8
Oxamide	35.7	-5.6	30.1	4.8
Pea seed	16.8	-29.4	-12.6	5.1

L.S.D. at $P<0.05$ NH_4-N 3.7, NO_3-N 3.5, min-N 4.3 ppm

TABLE 29 B

MINERAL-N ACCUMULATION VALUES DUE TO ADDED N SOURCES FOR EXPT.12 AFTER 6 WEEKS OF INCUBATION (See Table 29 A)

(Results are in ppm N on dry soil basis)

Added N source	NH_4-N	NO_3-N	min-N	pH
Pre-incubation control				
Urea	60.2	10.5	70.7	4.8
Oxamide	41.1	4.0	45.1	4.8
Pea seed	37.4	-15.9	21.5	5.3
Pre-incubation straw+N treatment				
Urea	47.8	18.0	65.8	4.9
Oxamide	20.5	-1.6	18.9	5.0
Pea seed	11.4	-25.4	-14.0	5.0
Pre-incubation compost treatment				
Urea	76.3	7.0	83.3	4.8
Oxamide	21.0	-7.7	13.3	4.8
Pea seed	2.1	-31.5	-29.4	5.1

L.S.D. at $P<0.05$ NH_4-N 3.7, NO_3-N 3.5, min-N 4.3 ppm

mainly as ammonium where urea and oxamide were added and entirely as ammonium where pea seed was added. There was significant loss of nitrate due to pea seed.

The pre-incubation straw+N treatment slightly decreased mineral-N accumulation due to urea, but decreased it considerably due to oxamide and pea seed. The pre-incubation straw+N treatment decreased ammonium accumulation and increased nitrate accumulation where urea was added. Ammonium and nitrate accumulation were decreased by the pre-incubation treatment where oxamide and pea seed were added, and nitrate loss was increased by the pre-incubation treatment where pea seed was added.

The pre-incubation compost treatment increased mineral-N accumulation where urea was added but decreased it considerably where oxamide and pea seed were added. The pre-incubation compost treatment resulted in the greatest loss of mineral-N where pea seed was added. The pre-incubation compost treatment increased ammonium accumulation where urea was added, but decreased it where oxamide and, in particular, where pea seed was added. The pre-incubation compost treatment had little effect on nitrate accumulation where urea was added, but caused some loss of nitrate where oxamide and considerable loss of nitrate where pea seed was added.

DISCUSSION

The main purpose of this experiment was to see if pre-treatment of the soil with organic materials (straw+N or compost allowed to react with the moist soil for 6 months) would have any effect on nitrogen changes during subsequent incubation of the soils when treated with urea, oxamide and pea seed.

The pre-incubation treatments had considerably different effects depending on the source of nitrogen added before incubation. The effects of the pre-incubation treatments on urea changes were generally small, the straw+N resulting in a slight decrease and the compost treatment resulting in a moderate increase in mineral-N from urea. Both pre-incubation treatments decreased mineral-N accumulation from oxamide and, in particular, from pea seed. In fact, where pea seed was added there was a net loss of mineral-N during incubation and this loss was accounted for mainly as ammonium. The loss was higher with the pre-incubation compost than with the pre-incubation straw+N treatment. Thus it appears that the extent of immobilization of nitrogen, which presumably caused loss of mineral-N, was greater due to the compost residues than to the straw residues. The organic carbon contents of the control, straw and compost treated soils

were 0.92%, 0.93% and 0.99% respectively after the pre-incubation period. It is probable that the residues of compost and straw decomposed further during the incubation period. Since there was more residue of compost than of straw this explains the greater loss of mineral-N due to compost than to straw residues which occurred with the oxamide and pea seed treatments. It is difficult to explain why there was no comparable loss of mineral-N with the urea treatment, particularly, in view of the similarity of urea and oxamide. It may be that the microbiological changes involving nitrogen are rather different between urea and oxamide. It is seen that oxamide-N mineralized less readily than urea-N even without pre-incubation treatment and that this difference increased with both pre-incubation treatments and, particularly, with the compost treatments.

SUMMARY

Mineral-N levels were determined during incubation ($30^{o}C$, 50% mwhc) immediately following addition of 100 ppm N as urea, oxamide, or pea seed to original HIGH FLAT (HF) soil and HF soil which had been allowed to react with straw+urea-N or compost for 6 months.

Mineral-N accumulation due to urea was increased

somewhat by the pre-incubation compost treatment, and was slightly reduced by the pre-incubation straw+N treatment. Both pre-incubation treatments decreased mineral-N accumulation due to oxamide and pea seed, and there was a net loss of mineral-N due to pea seed.

The proportion of mineral-N which accumulated as ammonium-N was decreased to a greater extent by pea seed than by oxamide due to both pre-incubation treatments; the proportion due to urea treatment was decreased only by the pre-incubation straw+N treatment.

Nitrate accumulated in significant amounts only where urea was added and was increased by the pre-incubation straw+N treatment.

CHAPTER 9

EFFECTS OF VARYING CONSTANT AND CHANGING MOISTURE CONTENTS ON MINERAL-N LEVELS DURING INCUBATION

Experiment 13

Effects of constant 50% and 100% mwhc and changing (50% or 100% mwhc to air-dried) moisture contents during incubation for 11 weeks on mineral-N levels of LOW FLAT (LF) and HIGH FLAT (HF) soil without and with added urea (50 ppm N)

CHAPTER 9

EFFECTS OF VARYING CONSTANT AND CHANGING MOISTURE CONTENTS ON MINERAL-N LEVELS DURING INCUBATION

Field soils are usually subjected to variation in moisture content due to evapo-transporation and downward movement of rainfall and irrigation water. The extent to which soil moisture content can vary during the period of loss of moisture by evapo-transporation could range from saturation down to air-dried condition. Since mineral-N changes due to changing moisture content may be different from those occurring at static moisture contents, an experiment was set up to compare nitrogen changes during incubation of HF and LF soils held at 50% or 100% mwhc for the whole period compared with allowing the soils to dry to the air dried condition from 50% or 100% mwhc over 2 cycles.

Experiment 13

Effects of constant 50% and 100% mwhc and changing (50% or 100% mwhc to air-dried) moisture contents during incubation for 11 weeks on mineral-N levels of LOW FLAT (LF) and HIGH FLAT (HF) soil without and with added urea (50 ppm N)

METHODS

The original LF and HF soils were used and with each

soil the experiment was done without and with addition of 50 ppm urea-N initially. Sufficient replicates were set up to allow for analysis in duplicate after 11 weeks of incubation using the following treatments : (i) constant 50% mwhc, (ii) constant 100% mwhc, (iii) 50% mwhc allowed to dry during incubation to the air dried state (2 cycles) and (iv) 100% mwhc allowed to dry during incubation to the air dried state (2 cycles). With treatments (i) and (ii) the incubation tubes were closed with rubber bungs and the barium peroxide method (226) was used for aeration and absorption of carbon dioxide. With treatments (iii) and (iv) the tubes were left open during incubation to allow water to evaporate. All tubes were held in an incubator at 30°C. It was found that it required 11 weeks of incubation to cover 2 cycles of wetting and drying with the alternating 100% mwhc——→air dried treatment. Because of this all treatments were incubated for 11 weeks. One series of each soil was set up without and another series with addition of 50 ppm urea-N (mixed in finely ground form with a bulk quantity of each soil). At the end of incubation pH was determined and mineral-N was extracted with 1N-sodium acetate pH 7.0.

RESULTS

Table 30 A shows ammonium, nitrate and mineral-N levels and pH, Table 30 B shows corresponding accumulation

values for soil and urea-N and Table 30 C shows accumulation values due to urea-N for the LF soil. Tables 31 A, 31 B and 31 C present the results in a similar way for the HF soil.

Mineral-N

Mineral-N accumulation was always greater at constant 50% or 100% mwhc than with the corresponding changing moisture contents in both the LF and HF soils. However, the differences between the constant and changing moisture regimes were generally greater in the HF than in the LF soil whether or not urea was added. In the HF soil the mineralization of urea-N was somewhat greater with constant than with changing moisture regimes of both 50% and 100% mwhc. This also applied to the LF soil at 50% mwhc but at 100% mwhc the reverse occurred.

Nitrate-N

At 50% mwhc nitrate accumulated to a greater extent in both soils with constant than with changing moisture regimes. However, the differences between the two regimes were greater in the HF than in the LF soil, particularly where urea was applied. At 100% mwhc there was significant but slight accumulation of nitrate in the HF soil under both moisture regimes except the changing regime without

EFFECTS OF CONST.50% AND 100% mwhc AND CHANGING (50% or 100%mwhc to air-dried) MOISTURE CONTENTS DURING INCUBATION FOR 11 WEEKS ON min-N LEVELS OF LF SOIL WITHOUT AND WITH ADDED UREA(50 ppm N)

TABLE 30 A MINERAL-N LEVELS

(Results are in ppm N on dry soil basis)

	NH_4-N	NO_3-N	min-N	pH
Initial values	8.8	7.4	16.2	4.1
Moisture treatment	50% maximum water holding capacity			
Const. (nil N)	24.9	14.3	39.2	4.4
Const. (Urea-N	62.3	21.0	83.3	4.7
Changing*(nil N)	13.3	11.9	25.2	4.3
Changing*(urea-N)	53.2	10.5	63.7	4.6
	100% maximum water holding capacity			
Const. (nil N)	34.7	6.6	41.3	6.0
Const. (urea-N)	70.7	6.7	77.4	6.3
Changing**(nil N)	17.5	5.3	22.8	4.4
Changing**(urea-N)	58.5	3.8	62.3	4.7

L.S.D. at P<0.05 NH_4-N 0.7, NO_3-N 1.4, min-N 1.1 ppm

TABLE 30 B MINERAL-N ACCUMULATION VALUES

50% maximum water holding capacity

Moisture treatment	NH_4-N	NO_3-N	min-N	pH
Const. (nil N)	16.1	6.9	23.0	4.4
Const. (urea-N)	53.5	13.6	67.1	4.7
Changing*(nil N)	4.5	4.5	9.0	4.3
Changing*(urea-N)	44.4	3.1	47.5	4.6
	100% maximum water holding capacity			
Const. (nil N)	25.9	-0.8	25.1	6.0
Const. (urea-N	61.9	-0.7	61.2	6.3
Changing**(nil N)	8.7	-2.1	6.6	4.4
Changing**(urea-N)	49.7	-3.6	46.1	4.7

L.S.D. at P<0.05 NH_4-N 0.7, NO_3-N 1.4, min-N 1.1 ppm

TABLE 30 C MINERAL-N ACCUMULATION VALUES DUE TO ADDED UREA-N

50% maximum water holding capacity

Moisture treatment	NH_4-N	NO_3-N	min-N	pH
Const. (urea-N)	37.4	6.7	44.1	4.7
Changing* (urea-N)	39.9	-1.4	38.5	4.6
	100% maximum water holding capacity			
Const. (urea-N)	36.0	0.1	36.1	6.3
Changing**(urea-N)	41.0	--1.5	39.5	4.7

* Changing 50% mwhc- air dried
** Changing 100% mwhc- air dried

EFFECTS OF CONST.50% AND 100% mwhc AND CHANGING (50% or 100% mwhc to air-dried) MOISTURE CONTENTS DURING INCUBATION FOR 11 WEEKS ON min-N LEVELS OF HF SOIL WITHOUT AND WITH ADDED UREA (50 ppm N)

TABLE 31 A MINERAL-N LEVELS

(Results are in ppm N on dry soil basis)

	NH_4-N	NO_3-N	min-N	pH
Initial values	9.8	8.4	18.2	4.2
50% maximum water holding capacity				
Const. (nil N)	28.0	18.9	46.9	4.4
Const. (urea-N)	60.9	33.6	94.5	4.6
Changing* (nil N)	15.0	12.3	27.3	4.4
Changing* (urea-N)	56.0	13.7	69.7	4.7
100% maximum water holding capacity				
Const. (nil N)	34.0	10.1	44.1	6.1
Const. (urea-N)	81.6	10.5	92.1	6.4
Changing** (nil N)	16.5	8.0	24.5	4.5
Changing** (urea-N)	53.6	11.5	65.1	4.7

L.S.D. at $P < 0.05$ NH_4-N 1.4, NO_3-N 1.1, min-N 2.4 ppm

TABLE 31 B MINERAL-N ACCUMULATION VALUES

50% maximum water holding capacity

	NH_4-N	NO_3-N	min-N	pH
Const. (nil N)	18.2	10.5	28.7	4.4
Const. (urea-N)	51.1	25.2	76.3	4.6
Changing* (nil N)	5.2	3.9	9.1	4.4
Changing* (urea-N)	46.2	5.3	51.5	4.7
100% maximum water holding capacity				
Const. (nil N)	24.2	1.7	25.9	6.1
Const. (urea-N)	71.8	2.1	73.9	6.4
Changing** (nil N)	6.7	-0.4	6.3	4.5
Changing** (urea-N)	43.8	3.1	46.9	4.7

L.S.D. at $P < 0.05$ NH_4-N 1.4, NO_3-N 1.1, min-N 2.4 ppm

TABLE 31 C MINERAL-N ACCUMULATION VALUES DUE TO ADDED UREA-N

50% maximum water holding capacity

	NH_4-N	NO_3-N	min-N	pH
Const. (urea-N)	32.9	14.7	47.6	4.6
Changing* (urea-N)	41.0	1.4	42.4	4.7
100% maximum water holding capacity				
Const. (urea-N)	47.6	0.4	48.0	6.4
Changing** (urea-N)	37.1	3.5	40.6	4.7

* Changing 50% mwhc - air dried
** Changing 100% mwhc - air dried

urea-N; in the LF soil there was significant but slight loss of nitrate under changing but not under constant moisture regimes. Nitrate accumulation due to urea-N was highest in both soils, particularly in the HF soil, under the constant 50% moisture regime. There was slight accumulation of nitrate from urea-N under both changing moisture regimes only in the HF soil.

At the end of incubation urea treatments increased soil pH by 0.2 - 0.3 units under all constant and changing moisture regimes. There was little difference in pH between constant and changing moisture regimes of 50% mwhc. However, at 100% mwhc the constant moisture regime resulted in a final pH of 1.6 to 1.7 units more than did the changing moisture regime. The differences in pH due to regime were similar without or with added urea in both soils.

DISCUSSION

The generally lower accumulation of mineral-N under changing than under constant moisture conditions with 50% mwhc is due to the decreased mineralization which occurred during the drier periods of changing moisture contents. The greater decrease due to soil+urea-N than due to urea-N alone indicates that mineralization of soil organic-N was decreased to a greater extent than that of urea-N during

the drier period. This is confirmed by the very considerable decrease in mineral-N accumulation due to changing moisture content where urea was not applied. The same effects were noted with 100% mwhc insofar as soil organic-N was concerned with both the soils. However, the effects of constant versus changing moisture contents of 100% mwhc on urea mineralization differed between LF and HF soils. Whereas the HF soil showed less mineralization of urea-N with changing than with constant moisture levels, the LF soil showed the reverse effect. This indicates differences in the extent of urease activity between the two soils at high moisture (near water-logging) content. The LF soil appears to have a lower urease activity than the HF soil at constant high moisture content, but the difference disappeared when moisture was allowed to vary between saturation and air drying.

At 50% mwhc less nitrate accumulated with changing than with constant moisture levels in both soils, but the extent of difference due to moisture regime was greater in the HF soil. If heterotrophic nitrification was occurring (and this seems possible in view of the fact that pH did not exceed 4.7) it appears that this process was less active during the dry periods of the changing moisture regime. This is in agreement with the results obtained with varying static moisture content where nitrate accumulation increased with moisture content from 20 - 60% mwhc (chapter 3).

A considerable increase in pH (1.6 - 1.7 units) in both soils due to constant 100% mwhc compared with the relatively small increase (0.2 - 0.3 units) under changing moisture contents is difficult to explain. Although ammonia accumulation was somewhat higher under constant than under changing moisture regimes, it was calculated that the increased ammonia accumulation between the two regimes could not have increased pH by more than 0.1 unit (the calculation was based on the buffer capacity of the soils as determined by cation exchange capacity). The increased pH due to constant 100% mwhc was probably due to decomposition of some of the soil minerals with the release of metallic cations. The increase in pH due to urea addition at constant 100% mwhc also could not be explained on the basis of increase in ammonia accumulation and indicates that the urea stimulated to a relatively small extent the decomposition of minerals resulting in the release of metallic cations.

SUMMARY

Mineral-N levels were determined after 11 weeks of incubation of HF and LF soils without and with 50 ppm urea-N comparing constant moisture contents of 50% and 100% mwhc changing moisture contents where the soils were allowed to to dry from 50% or 100% mwhc to the air dried state over 2 cycles.

Mineral-N accumulation and mineralization of urea-N were greater at constant 50% or 100% mwhc than under corresponding changing moisture contents in the HF soil; the same applied to LF soil except that mineralization of urea was greater with changing than with constant 100% mwhc.

There was no accumulation of nitrate under either constant or changing 100% mwhc with or without added urea. At 50% mwhc there was greater nitrate accumulation with constant than with changing moisture regime and the difference due to moisture regime was greatest with urea addition.

The greatest increase in pH due to incubation occurred at constant 100% mwhc with or without added urea.

CHAPTER 10

EFFECT OF PERIOD AND TEMPERATURE OF STORAGE OF AIR-DRY SOILS ON MINERAL-N LEVELS AND MINERALIZABLE-N DURING SUBSEQUENT MOIST INCUBATION

Experiment 14

Effect of period and temperature of storage of air-dried HIGH FLAT (HF) and LOW FLAT (LF) soils on mineral-N levels and mineralizable-N during subsequent moist incubation (50% mwhc, 30°C)

Experiment 15

Effect of air-dry storage at room temperature over 70 weeks on (1) mineral-N and nitrate levels and (2) mineralizable-N during subsequent incubation (12-13 weeks at 80% mwhc and 30°C) of HIGH FLAT (HF) soil

CHAPTER 10

EFFECT OF PERIOD AND TEMPERATURE OF STORAGE OF AIR-DRY SOILS ON MINERAL-N LEVELS AND MINERALIZABLE-N DURING SUBSEQUENT MOIST INCUBATION

The advantages of storing soil samples in air-dry condition over storing them in moist condition are obvious. If an experiment has to be repeated with a certain soil sample it might be thought that no changes would occur due to the period of air-dry storage, whereas if the sample had been stored in moist condition this would not be the case. Harpstead and Brage (179), however, found that in American soils the period of air-dry storage had quite a profound effect on the extent to which nitrate is produced following subsequent wetting and incubation under standard conditions. They found, on an average for many soils, that the nitrate produced during incubation decreased as a result of the first three weeks of storage but thereafter increased with period of air-dry storage up to 50 weeks. Birch (234), working with tropical soils, found a significant positive linear correlation between the NH_4^- + NO_3-N mineralized on subsequent wetting and incubation and the logarithm of the period of air-dry storage up to 15 weeks.

Gasser (180), on the other hand, found that in English

soils the period of air-dry storage in general had little effect on the amounts of mineral-N produced during subsequent incubation for air-dry storage periods up to 12 to 16 weeks, although for longer periods of storage mineral-N production tended to increase on subsequent incubation. Cornfield (181) found that mineralizable-N during air-dry storage of 9 English soils at room temperature for 11 weeks showed decreases due to air-dry storage in 3 of the soils, increases in 2 of the soils and no effect in the other 4 soils. In a later study Chaudhury and Cornfield (235), using 8 English soils, found a general increase in mineralizable-N after air-dry storage at room temperature for up to 12 weeks. However, when the air-dried soils were stored at 2°C there were no significant increases in mineralizable-N over 12 weeks of storage.

Experiment 14

Effect of period and temperature of storage of air-dried HIGH FLAT (HF) and LOW FLAT (LF) soils on mineral-N levels and mineralizable-N during subsequent moist incubation (50% mwhc, 30°C)

The HF and LF soils were tested to see whether there were any changes in mineral-N and mineralizable-N due to air-dry storage at room temperature (approximately 20°C) and at 3°C.

METHODS

Since both soils were sent to United Kingdom in air-dried (1mm sieved) condition, 500g. portions of each sample were placed in pots and wetted to 50% mwhc. The soils were held at room temperature for one month, with addition of water when necessary. It was considered that during this period microbiological equilibrium had become more or less established. The soils were then spread on brown paper to air-dry (10 days). Both soils were then immediately analysed for mineral-N. In addition samples of both soils were set up for incubation (50% mwhc, 30°C) for 3 weeks and mineral-N then determined. The results obtained will be referred to as "no-storage" results. The remainder of both soils was divided into two portions and placed in screw top bottles. One bottle of each soil type was held at room temperature (18 - 22°C; this will be referred to as "20°C storage") and the other bottle of each was held in a refrigerator at 3°C. After 4, 8, 12 and 16 weeks of storage samples from both soils held at each temperature were analysed for mineral-N and mineralizable-N as described above.

For each analysis and incubation 10g. portions of soil were used and Morgan reagent was used for extraction.

RESULTS

Table 32 shows results for mineral-N and nitrate and mineralizable-N and nitrate initially and after each storage period. Table 33 shows corresponding accumulation values.

HIGH FLAT soil

With 20°C air-dry storage mineral-N increased up to 8 to 12 weeks and decreased slightly up to 16 weeks. With 3°C storage mineral-N decreased up to 4 weeks and increased up to 12 to 16 weeks. With 20°C storage mineralizable-N was unaffected up to 4 weeks, increased to a maximum with up to 8 weeks of storage and declined somewhat with further storage. With 3°C storage mineralizable-N increased up to 4 weeks and was at a maximum after 8 weeks but declined up to 16 weeks.

The nitrate content during storage of the air-dried HF soil at 20°C increased up to 8 weeks, decreased to its initial value up to 12 weeks and increased somewhat again after 12 weeks. With 3°C storage nitrate content increased up to 16 weeks. Nitrate accumulation due to incubation decreased after 4 weeks of air-dry storage at both temperatures. Storage for 8 to 16 weeks at 20°C had no effect on nitrate accumulation due to incubation. However, storage at 3°C beyond 4 weeks resulted in increased nitrate accumulation during incubation, but only after 8 and 12 weeks of storage.

TABLE 32

MINERAL-N AND NITRATE-N LEVELS AND MINERALIZABLE-N IN HIGH FLAT (HF) AND LOW FLAT (LF) SOILS AS AFFECTED BY PERIOD AND TEMPERATURE OF AIR-DRY STORAGE

(Results are in ppm N on dry soil basis)

No Storage

Soil used	Temp. (°C)	Before incubation		After incubation	
		min-N	NO_3-N	min-N	NO_3-N
HF	20	23.5	9.1	35.0	12.6
HF	3	23.5	9.1	35.0	12.6
LF	20	18.6	7.0	30.5	10.5
LF	3	18.6	7.0	30.5	10.5

After 4 weeks storage / After 8 weeks storage

Soil Used	Temp. (°C)	Before incub. min-N	Before incub. NO_3-N	After incub. min-N	After incub. NO_3-N	Before incub. min-N	Before incub. NO_3-N	After incub. min-N	After incub. NO_3-N
HF	20	25.9	10.5	35.0	9.8	27.7	11.6	41.0	12.6
HF	3	22.4	8.7	36.4	10.1	26.9	11.9	41.0	14.0
LF	20	20.0	10.2	27.7	6.3	21.4	7.0	34.7	11.9
LF	3	19.3	9.1	32.9	9.8	21.0	9.5	39.2	13.3

After 12 weeks storage / After 16 weeks storage

HF	20	27.3	9.1	39.9	13.3	25.9	10.5	37.5	13.0
HF	3	28.0	11.9	39.6	16.5	27.3	13.3	35.7	12.6
LF	20	27.3	11.5	36.8	12.3	23.1	10.5	37.8	16.1
LF	3	24.2	10.5	37.1	15.4	26.6	14.0	33.6	14.0

L.S.D. at $P < 0.05$ min-N 0.7, NO_3-N 1.1.

TABLE 33

MINERAL-N AND NITRATE-N ACCUMULATION VALUES FOR EXPT.14

(See Table 32)

(Results are in ppm N on dry soil basis)

Soil Used	Temp. (°C)	Before incubation		After incubation	
		min-N	NO_3-N	min-N	NO_3-N
After 4 weeks storage					
HF	20	2.4	1.4	0.0	-2.8
HF	3	-1.4	-0.4	1.4	-2.5
LF	20	1.4	3.2	-2.8	-4.2
LF	3	0.7	2.1	2.4	-0.7
After 8 weeks storage					
HF	20	4.2	2.5	6.0	0.0
HF	3	3.4	2.8	6.0	1.4
LF	20	2.8	0.0	4.2	1.4
LF	3	2.4	2.5	8.7	2.8
After 12 weeks storage					
HF	20	3.8	0.0	4.9	0.7
HF	3	4.5	2.8	4.6	3.9
LF	20	8.7	4.5	6.3	1.8
LF	3	5.6	3.5	6.6	4.9
After 16 weeks storage					
HF	20	2.4	1.4	2.5	0.4
HF	3	3.8	4.2	0.7	0.0
LF	20	4.5	3.5	7.3	5.6
LF	3	8.0	7.0	3.1	3.5

L.S.D. at $P < 0.05$ min-N 0.7, NO_3-N 1.1

LOW FLAT soil

The mineral-N levels increased with time of storage at both temperatures; the level was at a maximum after 12 weeks with 20°C storage and after 16 weeks with 3°C storage. With 20°C storage mineralizable-N decreased after 4 weeks, but increased with further storage up to 16 weeks. With 3°C storage mineralizable-N increased with storage up to 8 weeks; there was less increase in mineralizable-N with further storage.

With 20°C storage the nitrate content of the air-dried soil was not affected by 8 weeks of storage but was increased by the other periods of storage. With 3°C storage nitrate increased with storage up to 16 weeks. Nitrate accumulation due to incubation decreased after 4 weeks at 20°C, but increased with further storage up to 16 weeks. Nitrate accumulation during incubation of soil stored at 3°C was not affected by 4 weeks storage; with storage beyond 4 weeks it increased, with a maximum increase occurring after 12 weeks.

DISCUSSION

It will be noted that there were general increases in mineral-N and nitrate in both soils during air-dried storage. It is particularly interesting to note that these

increases after 16 weeks of storage were greater at 3°C than at 20°C and that the increased mineral-N was accounted for mainly as nitrate. Thus it appears that the very low moisture content of the air-dried soil did not prevent the activity of N-mineralizing and nitrifying organisms. This mineral-N and nitrate accumulation during air-dry storage was greater at 3°C than at 20°C in both soils. It appears, therefore, that at the very low air-dry moisture content some organism(s) is present which functions better at the low than at the high temperature in causing mineralization of nitrogen and nitrification. The fact that virtually all the mineral-N increase was accounted for as nitrate, particularly at 3°C in both soils, again suggest the probability of heterotrophic nitrification. Alexander (1) has indicated that some fungal species are operative at low moisture content and temperature.

Changes in mineralizable-N due to air-dry storage are due to the effects of storage on the activity of the organisms responsible for mineralization after moisture content and temperature were increased to 50% mwhc and 30°C respectively. It is seen that in the case of HF soil maximum mineralizable-N occurred after 8 weeks of storage at both temperatures. This indicates that no stimulating effect on N-mineralizing organisms occurred until after 8 weeks of storage at both temperatures and that the stimulating effect due to storage declined with

storage beyond 8 weeks. This also applied to the LF soil when stored at 3°C, but when stored at 20°C maximum mineralizable-N occurred after 16 weeks of air-dry storage. Thus there appears to be a difference due to storage temperature for the LF soil but not the HF soil. This difference may be due to the types of microorganisms present in the two soils. There was no correlation between mineral-N changes and mineralizable-N changes due to storage except for the HF soil stored at 20°C. It is likely that the organisms responsible for mineralization during the air-dry period are different from those responsible for mineralization during the incubation period.

The HF and LF soils behaved similarly with respect to nitrate accumulation during air-dry storage at 3°C; nitrate increased with storage up to 16 weeks. At 20°C inconsistent results were obtained for nitrate changes in both soils in that nitrate increased up to 4 or 8 weeks, then declined and again increased with further storage.

Nitrified-N (mineral-N which accumulated as nitrate during incubation), in general, decreased after 4 weeks storage in both soils at both temperatures. With further storage nitrified-N was unaffected in the HF soil stored at 20°C. In the HF soil stored at 3°C and the LF soil stored at both temperatures nitrified-N increased with storage beyond 4 weeks with maximum effects occurring after 12

or 16 weeks of storage. There was no correlation between changes in nitrate due to air-dry storage and nitrified-N. This indicates, as for mineralization, a difference in the nature of the organisms responsible for nitrification during air-dry storage and during incubation.

The main purpose of this experiment was to compare the effect of temperature of storage of air-dry soil on subsequent mineralization of nitrogen during moist incubation. The effect of storage temperature on mineralizable-N was affected by time of storage. With up to 8 weeks storage mineralizable-N was either greater or the same at 3°C than at 20°C. With up to 12 weeks of storage there were no differences due to temperature in mineralizable-N. With 16 weeks of storage mineralizable-N was greater at 20°C than at 3°C in both soils. These results differ from those obtained by Chaudhury and Cornfield (235) using English soils, who found that there was a consistent increase in mineralizable-N with storage for up to 12 weeks at 20°C, but no increase with storage at 2°C.

A practical implication of these results may be discussed. Since nitrate is able to accumulate even during the period when soils are very dry, this indicates that if heavy rainfall then occurs the nitrate may be leached out of the top soil. Thus nitrate formation during a prolonged dry spell followed by rainfall (or irrigation) may contribute to an overall loss of nitrogen from the soil.

Experiment 15

Effect of air-dry storage at room temperature over 70 weeks on (1) mineral-N and nitrate levels and (2) mineralizable-N during subsequent incubation (12 - 13 weeks at 80% mwhc and 30°C) of HIGH FLAT (HF) soil

The results for this experiment were obtained from Experiments 2, 3 and 20 (chapters 3 and 13) in which the HF soil was set up for incubation after varying periods of air-dry storage at room temperature (18 - 22°C). The soil was sampled in East Pakistan in mid May 1969, air-dried and ground to pass a 1 mm sieve and sent to United Kingdom in polythene bags. On arrival the main sample (10 kg.) was divided into four portions and stored in polythene bags. Incubation experiments 2, 3 and 20 were started on 16th June, 1969, 7th October, 1969 and 14th October, 1970 respectively. These dates represent approximately 4, 16 and 70 weeks of air-dry storage. Moisture content was 80% mwhc and temperature was 30°C during incubation. The period of incubation for Experiment 2 was 12 weeks and for Experimnents 3 and 20 was 13 weeks. N-sodium acetate or N-potassium chloride were used as the the extractants.

RESULTS AND DISCUSSION

Results are presented in Table 34 where min-N and nitrate levels before and after incubation and accumulation values due to incubation are shown. Mineral-N and nitrate levels in the dry soils were not significantly affected by storage for 16 and 70 weeks compared with 4 weeks. This agrees with the results obtained in Experiment 14 (chapter 10) when comparing the 4 and 16 weeks storage periods. In experiment 14 slight but significant changes in min-N occurred after 8 and 12 weeks of storage, but the overall effect between 4 and 16 weeks indicated no change. Analysis of soils before incubation carried out in several experiments which were started at varying periods between 16 and 70 weeks of air-dry storage showed that there were negligible changes (not greater than ± 1 ppm) in mineral-N and nitrate during intermediate storage period. The conclusion that can be made is that when the HF soil was stored at room temperature there were negligible changes in mineral-N and nitrate levels.

Mineralizable-N (the accumulation values shown in Table 34) increased significantly with period of air-dry storage. Nitrified-N decreased slightly between 4 and 16 weeks but increased to a fair extent between 16 and 70 weeks of air-dry storage. In every case mineralized-N

TABLE 34

EFFECTS OF AIR-DRY STORAGE AT ROOM TEMPERATURE (18-22°C) OVER 70 WEEKS ON (1) MINERAL-N AND NITRATE LEVELS AND (2) MINERALIZABLE-N DURING SUBSEQUENT INCUBATION(12-13 weeks at 80% mwhc and 30°C) OF HIGH FLAT(HF) SOIL

(Results are in ppm N on dry soil basis)

Weeks of air-dry storage*		Before incub.	After incub.	Accumulation.
4	min-N	18.2	49.6	31.4
	NO_3-N	8.4	39.1	30.7
16	min-N	17.7	52.5	34.8
	NO_3-N	8.4	35.0	26.6
70	min-N	17.7	61.6	43.9
	NO_3-N	8.4	50.4	42.0

L.S.D. at $P < 0.05$ min-N 1.4, NO_3-N 1.7

* Storage polythene bags

accumulated almost entirely as nitrate. Thus, although air-dry storage had no effect on mineral-N and nitrate levels in the soil, the 70-week period of storage appeared to have stimulated activity of those microorganisms responsible for N-mineralization and nitrification during subsequent incubation.

SUMMARY

Experiment 14

The effects of air-dry storage of HF and LF soils at room temperature (18 - 22°C) and 3°C on mineral-N and nitrate levels and on mineralizable-N and nitrate accumulation (nitrified-N) during subsequent incubation (50% mwhc, 30°C for 3 weeks) were studied. Mineral-N due to storage of the HF soil increased slightly but significantly to a maximum after 8 weeks with 20°C and after 12 weeks with 3°C storage; nitrate was at a maximum after 8 weeks at 20°C and after 16 weeks at 3°C. In the LF soil maximum mineral-N and nitrate occurred after 12 weeks at 20°C and after 16 weeks at 3°C. In the HF soil maximum mineralizable-N occurred after 8 weeks and maximum nitrified-N after 12 weeks at both temperatures. In the LF soil mineralizable-N was at a maximum after 8 weeks at 3°C and

after 16 weeks at 20°C; nitrified-N was at a maximum after 12 weeks at 3°C and after 16 weeks at 20°C.

Experiment 15

Mineral-N and nitrate, and mineralizable-N and nitrified-N (incubation at 80% mwhc and 30°C for 12 - 13 weeks) were determined after 4, 16 and 70 weeks of air-dry storage of HF soil at room temperature (18 - 22°C). There were no significant differences in mineral-N and nitrate after any storage period. Mineralizable-N increased with air-dry storage up to 70 weeks. Nitrified-N decreased slightly with storage up to 16 weeks but increased to a fair extent with storage up to 70 weeks.

CHAPTER 11

NITRIFICATION OF FIXED AMMONIUM IN THE CLAY FRACTION OF THE HIGH FLAT SOIL AND IN BENTONITE

Experiment 16

Nitrification of fixed ammonium in the clay fraction of HIGH FLAT (HF) soil

Experiment 17

Nitrification of fixed ammonium in bentonite clay inoculated with HIGH FLAT (HF) soil

CHAPTER 11

NITRIFICATION OF FIXED AMMONIUM IN THE CLAY FRACTION OF THE HIGH FLAT SOIL AND IN BENTONITE

Experiment 16

Nitrification of fixed ammonium in the clay fraction of HIGH FLAT (HF) soil

METHODS

Separation of the clay (<2 μ) fraction

200g. of air-dried 1 mm sieved soil were shaken with 2 litres of 0.1N-sodium hydroxide for 24 hours. The dispersed soil was set aside for 5½h., and then the top 10 cm. of suspension were transferred by suction to a beaker and just neutralised with hydrochloric acid. Approximately 100g. of calcium chloride was added and dissolved by stirring and the pH again adjusted to 7.0. After the coagulated clay had settled the supernatant liquid was decanted off and the suspension (about 50 ml) brought to the boil each time after the addition of 3 separate 20 ml portions of 30% H_2O_2. The purpose of this was to

destroy organic matter. The liquid was evaporated on the steam bath and the dried clay was then ground to pass a 1 mm sieve.

Induced fixation of added ammonium

The method used was similar to that described by Blasco and Cornfield (219). The 15g. of clay obtained from the above treatment was leached with 300 ml of N-ammonium acetate pH 7.0, the excess ammonium acetate washed out with 70% aqueous ethyl alcohol and finally washing with 50 ml of water. The ammonium saturated clay was dried at 100°C and then subjected to 3 more cycles of rewetting (with water) and drying at 100°C. The clay was then leached with 300 ml of N-calcium chloride (pH 7.0) and 100 ml of water to convert it into calcium-clay and then air dried. Fixed ammonium in the clay was determined by the N-hydroflouric-N-hydrochloric acid method (236).

Nitrification of fixed ammonium

1g. portions of the calcium-clay (containing fixed ammonium) were mixed with 1g. of the original HF soil (the pH of which had been ajusted to 7.0 with calcium carbonate; chapter 4) as inoculum and 8g. of acid-washed dried silver sand. Sufficient replicates were prepared to allow for analysis in duplicate after 3 and 6 weeks of incubation (30°C) at moisture contents of 33% and 100% mwhc.

To correct for nitrate production from the 1g. soil inoculum, blanks containing 1g. of soil and 9g. of sand were also set up for incubation. Aeration was done by the barium peroxide method (226). The soils were extracted with Morgan reagent and the extracts analysed for NH_4- and NO_3-N.

RESULTS AND DISCUSSION

Table 35 A shows levels of NH_4-, NO_3- and min-N after 3 and 6 weeks of incubation at both moisture contents in the inoculated clay-sand mixtures (levels of min-N produced in the blanks have been allowed for). Table 35 B shows results, on the clay basis, for exchangeable NH_4- and NO_3- and min-N. It is seen that 21 - 29% of the original fixed ammonium in the clay was converted to exchangeable ammonium during incubation, whilst 0 - 17% was converted to nitrate. At 33% mwhc exchangeable ammonium was little different between 3 and 6 weeks incubation, whilst at 100% mwhc exchangeable ammonium was somewhat less after 6 than after 3 weeks. Significant amounts of nitrate accumulated only at 100% mwhc and the amount increased from 3 to 6 weeks.

It is impossible to say whether fixed ammonium is converted directly to nitrate. It seems more likely that fixed ammonium is first converted to exchangeable ammonium

TABLE 35 A

LEVELS OF NH_4-, NO_3- AND min-N AFTER 3 AND 6 WEEKS OF INCUBATION (30°C) AT 33% AND 100% mwhc OF INOCULATED CLAY-SAND MIXTURES (LEVELS OF min-N FORMS IN THE BLANKS HAVE BEEN ALLOWED FOR)

(Results are in ppm on clay+sand+inoculum basis)

	3 weeks				6 weeks			
Treatment	NH_4-N	NO_3-N	min-N	pH	NH_4-N	NO_3-N	min-N	pH
Clay-sand 33% mwhc	20.3	1.4	21.7	7.7	19.6	0.0	19.6	8.0
Clay-sand 100% mwhc	18.9	4.9	23.8	7.9	14.7	11.9	26.6	8.0

L.S.D. at $P < 0.05$ NH_4-N 2.2 ppm, NO_3-N 3.4 ppm, min-N 3.5 ppm

TABLE 35 B

(See also Table 35 A)

EXTRACTABLE NH_4-, NO_3- AND min-N AND PERCENTAGE OF FIXED AMMONIUM (values in brackets) CONVERTED TO EXCHANGEABLE NH_4-, NO_3- AND min-N DUE TO INCUBATION(30°C) FOR 3 AND 6 WEEKS AT 33% mwhc AND 100% mwhc

(Results are in ppm on clay basis)

Initial fixed NH_4-N = 686 ppm (4.9 m. equ. per 100 g.)

	3 weeks		6 weeks	
	33% mwhc	100% mwhc	33% mwhc	100% mwhc
Exchangeable NH_4-N	203(29%)	189(27%)	196(28%)	147(21%)
NO_3-N	14(2%)	49(7%)	0(0%)	119(17%)
min-N	217(31%)	238(34%)	196(28%)	266(38%)

and this in turn is converted to nitrate. This is possible since with all treatments there was always a residue of exchangeable ammonium after both incubation periods. The occurrence of nitrification at 100% mwhc and the negligible occurrence at 33% mwhc is in agreement with the results obtained (chapter 3) when the original HF soil was incubated. This indicates the possibility that the same types of microorganisms are responsible for nitrification of organic N in the original soil and of fixed ammonium in the separated clay. The fact that significant nitrification of the fixed ammonium in the clay occurred only at 100% mwhc suggest that the process of nitrification is likely to be heterotrophic. In a similar study by Blasco and Cornfied (236) using 8 soils from Colombia, South America, it was found that nitrification of fixed ammonium occurred at 33% mwhc in all the soil clays, but did not occur at 100% mwhc in any of them. With these soils it appears that autotrophic nitrification, which can only occur under aerobic conditions, was responsible.

If it is assumed that the heterotrophic process is operative in nitrifying fixed ammonium in the clay fraction of the HF soil, then this cannot be a direct process, since heterotrophic organisms require an organic source of nitrogen. It is suggested that the following indirect process occurs:

$$\text{Fixed } NH_4 \xrightarrow{\text{chem.}} \text{exchangeable } NH_4 \xrightarrow{\text{immob. of exch. } NH_4} \text{org.N} \xrightarrow[\text{nitrifi.}]{\text{hetero}} NO_3$$

The carbonaceous material present in the soil inoculum would be able to immobilize ammonium.

Experiment 17

Nitrification of fixed ammonium in bentonite clay inoculated with HIGH FLAT (HF) soil

METHODS

Na-bentonite was converted to ammonium-bentonite by leaching with N-ammonium acetate pH 7.0 and water. Fixation was induced by 3 cycles of drying (100°C) and wetting. Exchangeable ammonium was then leached out with N-calcium chloride and water to give calcium-clay containing fixed ammonium, which was determined by the N-hydroflouric-N-hydrochloric acid method (236). Nitrification of fixed ammonium was done as described in previous experiment except that 0.5g. portions of clay and HF soil (pH 7.0), as inoculum, were used.

RESULTS AND DISCUSSION

Table 36 A shows NH_4-, NO_3- and min-N levels after 3 and 6 weeks of incubation at both moisture contents of the inoculated bentonite clay-sand mixtures (levels of min-N forms in the blanks have been allowed for). Table 36 B shows results for exchangeable NH_4- and NO_3- and min-N expressed on the dry clay basis.

It is seen that no nitrate accumulated at 33% mwhc after 3 or 6 weeks of incubation. At 100% mwhc 11% and 7% of the initial fixed ammonium was converted to nitrate after 3 and 6 weeks respectively. After 3 weeks exchangeable ammonia which accumulated accounted for 83% to 87% of the

TABLE 36 A

LEVELS OF NH_4-, NO_3- AND min-N AFTER 3 AND 6 WEEKS OF INCUBATION (30^oC) AT 33% mwhc AND 100% mwhc OF INOCULATED BENTONITE CLAY-SAND MIXTURES (LEVELS OF min-N FORMS IN THE BLANKS HAVE BEEN ALLOWED FOR)

(Results are in ppm on clay+sand+inoculum basis)

	3 weeks				6 weeks			
Treatment	NH_4-N	NO_3-N	min-N	pH	NH_4-N	NO_3-N	min-N	pH
Clay-sand 33% mwhc	32.2	0.0	32.2	7.7	22.4	0.0	22.4	7.8
Clay-sand 100% mwhc	33.6	4.2	37.8	7.8	2.8	2.8	5.6	7.9

L.S.D. at $P<0.05$ NH_4-N 3.0, NO_3-N 2.2, min-N 2.5 ppm

TABLE 36 B

(See also Table 36 A)

EXTRACTABLE NH_4-, NO_3- AND min-N AND PERCENTAGE OF FIXED AMMONIUM (values in brackets) CONVERTED TO EXCHANGEABLE NH_4-, NO_3- AND min-N DUE TO INCUBATION (30^oC) FOR 3 AND 6 WEEKS AT 33% mwhc AND 100% mwhc

(Results are in ppm on clay basis)

Initial fixed NH_4-N = 387 ppm (2.7 m.eq. per 100g.)

	3 weeks		6 weeks	
	33% mwhc	100% mwhc	33% mwhc	100% mwhc
Exchangeable NH_4-N	322(83%)	336(87%)	224(58%)	28(7%)
NO_3-N	0(0%)	42(11%)	0(0%)	28(7%)
min-N	322(83%)	378(98%)	224(58%)	56(14%)

initial fixed ammonium. After 6 weeks exchangeable ammonia decreased at both moisture contents, and to a considerable extent at 100% mwhc. This was probably due to loss of ammonia by volatilization or to refixation during the 3 - 6 week period.

It is seen that the results obtained for nitrification of fixed ammonium in bentonite clay inoculated with HF soil were similar to those obtained for fixed ammonium in HF soil clay. The main point of interest in both experiments is that the organisms responsible for nitrification were operative at the virtually anaerobic moisture content of 100% mwhc but not at the aerobic moisture content of 33% mwhc. This suggests that heterotrophic rather than autotrophic processes are responsible for nitrification.

S U M M A R Y

The clay fraction of the HF soils and bentonite were subjected to treatments to induce ammonium fixation. After removal of exchangeable ammonium and conversion of both clays to calcium-saturated forms of pH 7 approx., the clays (containing only fixed ammonium) were set up for incubation at 33% and 100% mwhc after mixing with sand and inoculation with HF soil (pH 7.0). At 33% mwhc there was no or negligible nitrate accumulation from the HF soil clay or bentonite. At 100% mwhc a maximum of 17% (6 weeks incubation) of the original fixed ammonium-N accumulated as nitrate-N from the HF soil clay. A maximum of 11% (3 weeks incubation) of the original fixed ammonium-N accumulated as nitrate-N from the bentonite.

CHAPTER 12

NITROGEN MINERALIZATION AND NITRIFICATION DURING INCUBATION OF PAKISTAN HIGH FLAT SOIL AND AN ENGLISH SOIL ALONE AND MIXED

Experiment 18

NH_4-, NO_3- and min-N levels during incubation (30°C, 50% mwhc) for 0, 3 and 6 weeks of NEUTRAL Pakistan HIGH FLAT (HF) soil and English soil alone and in mixtures

Experiment 19

NH_4-, NO_3- and min-N levels during incubation (30°C) for 13 weeks at 40% and 80% mwhc of ACID Pakistan HIGH FLAT (HF) soil and English soil alone and mixed

CHAPTER 12

NITROGEN MINERALIZATION AND NITRIFICATION DURING INCUBATION OF PAKISTAN HIGH FLAT SOIL AND AN ENGLISH SOIL ALONE AND MIXED

Results obtained so far with Pakistan HF soil have shown that nitrification during incubation has occurred at low pH (4.2) at high moisture contents (80% and 100% mwhc) and at a high temperature (50°and 60°C). In typical English soils nitrification does not occur under these conditions. It has already been suggested that nitrification in the Pakistan HF soil under these unusual conditions may be due to the presence of microorganisms (probably heterotrophic fungi) which are different from the traditional autotrophic bacteria responsible for nitrification in temperate region soils.

It was considered that a comparison of nitrification in the Pakistan HF soil and in English soil of similar texture when incubated by themselves and in mixtures would give some indication (i) of the relative nitrifying capacities of the two soils and (ii) whether or not the type of micro-organism(s) present in either soils would be able to operate in the other soil.

The English soil selected for this study was a sandy loam of approximately the same texture as the Pakistan HF

soil but had a higher organic carbon (2.1%) and nitrogen (0.21%) content. Ekpete (232) and Enwezor (237) found that this soil produced very little nitrate when incubated at moisture contents exceeding 60% mwhc, at temperatures exceeding 40°C and at pH <5.0 .

Experiment 18

NH_4-, NO_3- and min-N levels during incubation (30°C, 50% mwhc) for 0, 3 and 6 weeks of NEUTRAL Pakistan HIGH FLAT (HF) soil and English soil alone and in mixtures

METHODS

Since the original HF soil and English soil had pH values 4.2 and 6.0 respectively, bulk samples of each soil were treated with sufficient calcium carbonate (in amounts based on lime requirement determinations) to bring their pH values to approximately 7. The soils were then placed in pots, wetted to 40%to 50% mwhc and held for 4 weeks at room temperature in order to allow complete reaction between the soil and added calcium carbonate. The soils were then air-dried and ground to pass a 1 mm sieve. The HF soil was then found to have pH 7.2 and the English soil pH 6.7. These pH values were considered to be near enough each other to be used for the experiment. The "treatments" used for incubation were (i) 10g. HF soil alone, (ii) 10g.

English soil alone, (iii) 5g. HF soil mixed with 5g. English soil and (iv) 9.9g. HF soil mixed with 0.1g. English soil. Sufficient incubation tubes were set up to allow for analysis in duplicate initially and after 3 and 6 weeks of incubation (30°C) at 50% mwhc. The barium peroxide method(226) was used for aeration and absorption of carbon dioxide. The Morgan reagent was used for extraction of min-N forms.

RESULTS

Table 37 A shows levels of NH_4-, NO_3- and min-N initially and after each incubation period, and Table 37 B shows corresponding accumulation values after each incubation period.

When incubated alone both the HF and English soils showed maximum min-N accumulation after 3 weeks followed by small decreases after 6 weeks. Mineral-N accumulation in the HF soil containing 1% English soil was similar to that occurring in the HF soil alone. The sample consisting of equal amounts of HF and English soil behaved very similarly to the English soil with respect to min-N accumulation.

When incubated alone nitrate accumulation in both soils was similar after 3 weeks but was considerably greater in the English than in the HF soil after 6 weeks. The HF soil treated with 1% English soil behaved similarly to the English soil alone with respect to nitrate accumulation,

TABLE 37 A

NH_4-, NO_3- AND min-N LEVELS DURING INCUBATION (30°C, 50% mwhc) FOR 0, 3 AND 6 WEEKS OF NEUTRAL PAKISTAN HIGH FLAT (HF) SOIL AND ENGLISH SOIL ALONE AND IN MIXTURE

(Results are in ppm N on dry soil basis)

Soil used	NH_4-N	NO_3-N	min-N	pH	NH_4-N	NO_3-N	min-N	pH
	After 0 week incubation				After 3 weeks incubation			
HF (10g.)	35.0	12.6	47.6	7.2	42.0	18.6	60.6	7.3
Eng.(10g.)	25.2	0.7	25.9	6.7	55.3	7.0	62.3	7.2
HF+Eng. (5g.+5g.)	23.8	10.5	34.3	6.9	18.2	54.6	72.8	7.0
HF (9.9g.)+ Eng.(0.1g.)	37.1	9.8	46.9	7.2	49.0	11.2	60.2	7.3
	After 6 weeks incubation							
HF (10g.)	32.6	22.0	54.6	7.1	L.S.D. at P<0.05 NH_4-N 3.5, NO_3-N 3.4, min-N 4.0 ppm			
Eng.(10g.)	10.5	41.3	51.8	7.2				
HF+Eng. (5g.+5g)	0.0	64.8	64.8	7.0				
HF (9.9g)+ Eng.(0.1g.)	16.1	41.7	57.8	7.0				

TABLE 37 B

MINERAL-N ACCUMULATION VALUES FOR EXPT.18 (See Table 37 A) AFTER EACH PERIOD OF INCUBATION

(Results are in ppm N on dry soil basis)

Soil used	NH_4-N	NO_3-N	min-N	pH	NH_4-N	NO_3-N	min-N	pH
	After 3 weeks incubation				After 6 weeks incubation			
HF (10g.)	7.0	6.0	13.0	7.3	-2.4	9.4	7.0	7.1
Eng.(10g.)	30.1	6.3	36.4	7.2	-14.7	40.6	25.9	7.2
HF+Eng. (5g.+5g.)	-5.6	44.1	38.5	7.0	-23.8	54.3	30.5	7.0
HF(9.9g.)+ Eng.(0.1g.)	11.9	1.4	13.3	7.3	-21.0	31.9	10.9	7.0

L.S.D. at P<0.05 NH_4-N 3.5, NO_3-N 3.4, min-N 4.0 ppm

except that the extent of nitrate accumulation was somewhat lower with the former treatment. The treatment consisting of equal parts of HF and English soil showed higher nitrate accumulation, particularly after 3 weeks, than did all other treatments. This was the only treatment where NH_4-N disappeared after 6 weeks of incubation.

Experiment 19

NH_4-, NO_3- and min-N levels during incubation (30°C) for 13 weeks at 40% and 80% mwhc of ACID Pakistan HIGH FLAT (HF) soil and English soil alone and mixed.

METHODS

In this experiment the Pakistan HF soil was used at its original pH (4.2). The sample of English soil used was one which had previously had its pH reduced to 4.1 by treatment with ferrous sulphate (233). The treatments for incubation were (i) 10g. HF soil alone, (ii) 10g. English soil alone and (iii) a mixture of 5g. each of English soil and HF soil. Sufficient tubes of each treatment were set up for incubation (30°C, 13 weeks) at moisture contents of 40% and 80% mwhc. The barium peroxide method (226) was used for aeration and absorption of carbon dioxide. Morgan reagent was used for extraction of min-N forms.

RESULTS

Table 38 A shows results for min-N levels initially and after 13 weeks of incubation and Table 38 B shows corresponding accumulation values.

At both 40% and 80% mwhc min-N accumulation was greater in the English than in the Pakistan soil when these were incubated by themselves. When incubated in 1:1 mixture min-N accumulation at 40% mwhc was approximately half way between the values obtained when the soils were incubated alone. At 80% mwhc mixed incubation accumulated an amount of min-N somewhat less than half way between the amounts produced by separate incubation.

Nitrate accumulation in the Pakistan soil incubated alone was greater than in the English soil incubated alone at both 40% mwhc and, in particular, at 80% mwhc. The extent of nitrate accumulation in the 1:1 mixture at both 40% and 80% mwhc was similar to nitrate accumulation occurring in the English soil.

After 13 weeks incubation the lowest NH_4-N accumulation occurred in the Pakistan soil incubated alone at 80% mwhc and the highest accumulation in the English soil incubated alone at 80% mwhc. For the treatments as a whole pH after incubation was roughly correlated with extent of NH_4-N accumulation.

TABLE 38 A

NH_4-, NO_3-, AND min-N LEVELS DURING INCUBATION (30°C) FOR 13 WEEKS AT 40% AND 80% mwhc OF ACID PAKISTAN HIGH FLAT (HF) SOIL AND ENGLISH SOIL ALONE AND MIXED

(Results are in ppm N on dry soil basis)

	After 0 week incubation				After 13 weeks incubation			
Treatment	NH_4-N	NO_3-N	min-N	pH	NH_4-N	NO_3-N	min-N	pH
HF soil 40% mwhc	9.1	9.1	18.2	4.2	23.8	23.1	46.9	4.4
English soil 40% mwhc	25.6	1.1	26.7	4.1	71.4	8.4	79.8	4.6
HF+English soil(1:1) 40% mwhc	18.2	4.9	23.1	4.1	51.1	11.9	63.0	4.5
HF soil 80% mwhc					11.4	37.6	49.0	4.0
English soil 80% mwhc					95.6	5.6	101.2	4.9
HF+English soil(1:1) 80% mwhc					54.3	11.2	65.5	4.4

L.S.D. at $P<0.05$ NH_4-N 3.0, NO_3-N 2.2, min-N 2.5 ppm

TABLE 38 B

MINERAL-N ACCUMULATION VALUES FOR EXPT. 19 (See Table 38 A) AFTER 13 WEEKS INCUBATION

(Results are in ppm N on dry soil basis)

Treatment	NH_4-N	NO_3-N	min-N	pH
HF soil 40% mwhc	14.7	14.0	28.7	4.4
English soil 40% mwhc	45.8	7.3	53.1	4.6
HF+Eng.(1:1) 40% mwhc	32.9	7.0	39.9	4.5
HF soil 80% mwhc	2.3	28.5	30.8	4.0
English soil 80% mwhc	70.0	4.5	74.5	4.9
HF+Eng.(1:1) 80% mwhc	36.1	6.3	42.4	4.4

L.S.D. at $P<0.05$ NH_4-N 3.0, NO_3-N 2.2, min-N 2.5 ppm

DISCUSSION OF EXPERIMENTS 18 AND 19

During aerobic incubation (50% mwhc) of the neutral soils and their 1:1 mixture (Expt.18) N-mineralization was greater in the mixture than would be expected if there was no interaction between the two types of soils (the expected N-mineralization values were 19.7 and 16.4 ppm after 3 and 6 weeks respectively, but the values obtained were 38.5 and 30.5 ppm respectively). This indicates mutual beneficial effects of N-mineralizing organisms present in both soils. Odum (238) defines this as protocooperation: "an association of mutual benefit to two species but without the cooperation being obligatory for their existence or for their performance of some reaction". Although this definition strictly applies only to two species of organisms, it is suggested that it may be extended to include two groups of organisms, namely, the N-mineralizing organisms present in the English and Pakistan soils. The fact that protocooperation occurred in the mixture of the two neutral soils indicates that the N-mineralizing organisms in the two soils differed.

In the acid 1:1 soil mixture during aerobic incubation (40% mwhc) the expected N-mineralization of 40.9 ppm was not significantly different from the value obtained (39.9 ppm). This indicates that no protocooperation was occurring. Odum (238) defines this as neutralism, in which two

microorganisms (or groups of microorganisms) behave entirely independently. Thus it appears that the two types of acid soils contained similar types of N-mineralizing organisms which did not mutually interact.

With regard to nitrification under aerobic conditions, in the neutral soils 1:1 mixture there was considerable protocooperation (25.0 ppm NO_3-N expected but 54.3 ppm NO_3-N obtained after 6 weeks of incubation). In the acid soils mixture, on the other hand, nitrification under aerobic conditions was low and very similar to that occurring in the English soil incubated by itself, in contrast to the significantly greater nitrification in the Pakistan soil incubated by itself. This indicates that under acid aerobic conditions the activity of the nitrifying organisms present in the Pakistan soil were suppressed by some factor(s) present in the English soil. In the acid soils mixture with near-anaerobic (80% mwhc) incubation nitrification was low and similar to that occurring in the English soil alone, in contrast to the Pakistan soil alone where considerable nitrification occurred. Here again, some factor(s) was present in the English soil which suppressed nitrification in the Pakistan soil.

In the treatment where 0.1g. neutral English soil was mixed with 9.9g. of neutral Pakistan soil and incubated at an aerobic moisture content, the considerable increase

in nitrification over that obtained in the Pakistan soil alone indicates that substrate conditions in the neutralised Pakistan soil were satisfactory for proliferation of the autotrophic nitrifying organisms introduced by the English soil.

The efficiency of N-mineralization from the organic nitrogenous materials present in the Pakistan and English soils may be defined as the amount of min-N which accumulated per unit of organic-N (which is usually the same of total N) present in the two soils. It was found that in the aerobic neutral soils efficiency of N-mineralization was 19% less in the Pakistan than in the English soil. In the acid soils under aerobic conditions N-mineralization was 62% more efficient in the Pakistan than in the English soil, whilst in the near-anaerobic (80% mwhc) conditions the Pakistan soil was 24% more efficient than the English soil.

S U M M A R Y

Nitrogen mineralization and nitrification were determined during incubation of Pakistan HF soil and an English soil alone and in 1:1 mixtures. In neutral soils at 50% mwhc N-mineralization and nitrification were higher in the English than in the Pakistan soils, whilst the mixed soils gave results similar to English soil. In acid soils at 40% mwhc N-mineralization was higher but nitrification

was lower in the English than in the Pakistan soil; in the mixed soils N-mineralization lay between the values obtained with the separate soils, but nitrification was similar to that which occurred in the English soils. In acid soils at 80% mwhc N-mineralization in the English soil was greater than in the Pakistan soil, whilst the mixed soils behaved more like to Pakistan soil. Nitrification was higher in the Pakistan soil than in the English or mixed soils.

When 9.9g. neutral Pakistan soil was mixed with 0.1g. neutral English soil the pattern of N-mineralization and nitrification during aerobic incubation for the first 3 weeks was similar to that in the Pakistan soil alone but during the next 3 weeks of incubation was similar to that in the English soil.

CHAPTER 13

MISCELLANEOUS EXPERIMENTS

Experiment 20

Effect of varying doses of gamma irradiation of HIGH FLAT (HF) soil on mineral-N levels during subsequent incubation (30°C, 80% mwhc) for 13 weeks.

Experiment 21

Effect of addition of major and trace elements on N-mineralization and nitrification during incubation of LOW FLAT (LF) soil (pH 4.1) and HIGH FLAT (HF) soil (pH 5.6)

Experiment 22

Loss of nitrate by denitrification during water-logged incubation of HIGH FLAT (HF) and LOW FLAT (LF) soils

CHAPTER 13

MISCELLANEOUS EXPERIMENTS

Experiment 20

Effect of varying doses of gamma irradiation of HIGH FLAT (HF) soil on mineral-N levels during subsequent incubation (30°C, 80% mwhc) for 13 weeks

Previous experiments have shown that nitrate accumulated during incubation of the HF soil. Because of the low pH of this soil (4.2), which would generally not allow autotrophic nitrification, it was suggested that heterotrophic nitrification may be operative. Other workers (Schmidt(50) and Eylar and Schmidt(51)) found that several strains of Aspergillus flavus and one strain of Penicillium spp were able to convert some organic nitrogenous compounds to nitrate in culture tests in the absence of autotrophic nitrifying organisms. Fungi are generally more sensitive to gamma irradiation than bacteria (239). This suggested that treatment of the HF soil with varying doses of gamma irradiation before incubation might give some indication as to whether heterotrophic process (due to fungi) or

the autotrophic process (due to the bacteria Nitrosomonas and Nitrobacter) was operative.

METHODS

Fourteen 10g. portions of the air-dried soil were placed in separate glass vials (4 inch x 1 inch diameter) which were then closed by polyvinyl chloride stoppers. The tubes were irradiated at the Wantage Research Laboratory using a source of cobalt-60 (dose rate 0.556 Mrad per hour). The doses received by duplicate tubes were 0 (control), 0.05, 0.10, 0.25, 0.50, 1.00, and 2.00 Mrad. All the tubes were then treated with sufficient water to bring the moisture content of the soil to 80% mwhc. The stoppered tubes were incubated at 30°C for 13 weeks using the barium peroxide method (226) for aeration and absorption of carbon dioxide. After incubation pH was determined after addition of 10 ml of water and 0.5N-potassium chloride was used for extraction of mineral-N. The extracts were analysed for ammonium, nitrite, and nitrate and carbon dioxide release was determined during incubation.

RESULTS AND DISCUSSION

Results are shown in Table 39 for mineral-N levels and corresponding accumulation values. Carbon dioxide release values and pH after incubation are also shown.

TABLE 39

EFFECT OF VARYING DOSES OF GAMMA IRRADIATION* ON MINERAL-N LEVELS AND ACCUMULATION VALUES AND CARBON DIOXIDE RELEASE AFTER 13 WEEKS OF INCUBATION (30°C, 80% mwhc) OF HIGH FLAT (HF) SOIL

(Results are in ppm N on dry soil basis)

	NH_4-N	NO_2-N	NO_3-N	min-N	pH	
Initial values	9.3	0.0	8.4	17.7	4.2	
After incubation Mineral-N levels						
Treatment	NH_4-N	NO_2-N	NO_3-N	min-N		
Control	11.2	0.05	50.4	61.6		
0.05	7.0	0.06	50.4	57.4		
0.10	14.0	0.06	44.8	58.8		
0.25	30.8	0.06	22.4	53.2		
0.50	49.0	0.05	9.8	58.8		
1.00	60.2	0.08	1.4	61.6		
2.00	56.0	0.06	2.8	58.8		
Mineral-N accumulation values						
Treatment	NH_4-N	NO_2-N	NO_3-N	min-N	CO_2-rel.	pH
Control	1.9	0.05	42.0	43.9	4726	3.6
0.05	-2.3	0.06	42.0	39.7	5052	3.6
0.10	4.7	0.06	36.4	41.1	4602	3.8
0.25	21.5	0.06	14.0	35.5	4739	4.0
0.50	39.7	0.05	1.4	41.1	4428	4.4
1.00	50.9	0.08	-7.0	43.9	4819	4.4
2.00	46.7	0.06	-5.6	41.1	4339	4.4

L.S.D. at $P < 0.05$ NH_4-N 2.8, NO_2-N 0.01, NO_3-N 3.0, min-N 3.0 ppm

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* The dose rate was 0.556 Mrad per hr.

All doses of gamma irradiation generally had little effect on mineralization of nitrogen except with 0.25 Mrad, where mineralization was decreased somewhat. Nitrification was unaffected by 0.05 Mrad but decreased with increasing dose and was completely inhibited at 0.5 Mrad. With 1.00 - 2.00 Mrad there was some disappearance of nitrate present initially, probably due to reduction of nitrate by the high irradiation dose (240). There was an inverse correlation between ammonium-N and nitrate-N accumulation, and very small but significant amounts of nitrite-N (0.05 to 0.08 ppm) accumulated in all incubated soils including the control. Final soil pH decreased with doses of 0.25 Mrad or less and increased slightly with higher doses and was correlated with extent of accumulation of ammonium-N.

The accumulation of nitrate in the control soil and in those receiving 0.05 - 0.25 Mrad is in sharp contrast to a woodland soil of similar pH from Southern England where no nitrification occurred with any including the control treatment over 7 days of incubation (241). In five English soils with pH 6.4 - 7.6 (241), doses of irradiation up to 2.5 Mrad stimulated nitrification during subsequent incubation, indicating that the nitrifying organisms in these soils are much more numerous or less sensitive to irradiation than those in the HF soil; in fact, they were still capable of oxidising large amounts of irradiation released ammonia. Because treatment with up to 2.00 Mrad gave no significant increase in total

mineral-N in the HF soil, it appears that there was a very low microbial population to undergo damage and lysis, and soil organic matter was not degraded.

Johnson and Osborne (242) reported the elimination of Aspergillus flavus in soil treated with 0.5 Mrad, but some survivors were detected after 0.25 Mrad. Such findings permit the suggestion that inhibition of nitrification in the HF soil by 0.5 Mrad is due to the death of fungi, whilst the occurrence of nitrification after 0.25 Mrad or lower doses indicates the possibility of heterotrophic nitrification. However, it is also possible that autotrophic nitrification is responsible for all nitrification or is occurring concurrently with heterotrophic nitrification. On the other hand, the accumulation of nitrate during incubation of this HF soil at 80% mwhc even without irradiation treatement is all the more unusual since in English soils nitrification is usually negligible at this near-anaerobic moisture content even when pH is high enough to permit autotrophic nitrification (64). Since fungi can operate at both low pH and anaerobic conditions (1) this supports the suggestion that heterotrophic nitrification may be the main mechanism responsible for nitrate formation in the HF soil.

SUMMARY

Ammonium-N and nitrate-N levels were determined after 13 weeks of incubation (30°C, 80% mwhc) of HIGH FLAT (HF) soil (pH 4.2) which had been subjected to varying doses

of gamma irradiation (0.05 to 2.0 Mrad) just before incubation. The extent of accumulation of mineral-N (NH_4- + NO_3-N) during incubation was little affected by any dose of gamma irradiation. Nitrate accumulation during incubation was not affected by 0.05 Mrad but decreased with increasing dose and was completely inhibited by doses of 0.5 Mrad or higher. The results are discussed in relation to the possible occurrence of heterotrophic nitrification in the acid HF soil.

Experiment 21

Effect of addition of major and trace elements on N-mineralization and nitrification during incubation of LOW FLAT (LF) soil (pH 4.1) and HIGH FLAT (HF) soil (pH 5.6)

The purpose of this experiment was to determine whether N-mineralization and nitrification in the two tea soils was being limited by major and trace elements. The total contents of major and trace elements in the two tea soils (Table 2, chapter 2) are seen to be low compared with values obtained in English soils. The LF soil was tested at its natural pH (4.1). The HF soil was tested at pH 5.6, since it was found (chapter 5) that over the pH range from 4.2 - 7.3 maximum nitrification occurred at pH 5.6.

METHODS

10g. portions of soil were treated with 100 ppm (dry soil basis) of potassium (K), calcium (Ca), and magnesium (Mg) as sulphates, phosphorus (P) as $Ca(H_2PO_4)_2$, 50 ppm copper (Cu) and Zinc (Zn) as sulphates and 20 ppm molybdenum (Mo) as sodium molybdate. All added elements were applied in solution in the water added to bring soil moisture content to 50% mwhc. Where ammonium sulphate was added (50 ppm N on dry soil basis), this was applied in the same way. The unstoppered tubes were then allowed to dry at 30°C for 1 week and the contents of each tube stirred with a glass rod in order to ensure of thorough mixing of the added compound with soil. Water was then added to 50% mwhc and the tubes incubated at 30°C for 4 weeks, using the barium peroxide method (226) for aeration and absorption of carbon dioxide. After incubation pH was determined after addition of water and mineral-N was then extracted with the Morgan reagent.

RESULTS

Table 40 and Table 41 show NH_4-, NO_3- and min-N accumulation values and soil pH after incubation for the LF and HF soils respectively.

LF soil (pH 4.1)

Where no ammonium sulphate was added all treatments

TABLE 40

EFFECT OF ADDITION OF MAJOR AND TRACE ELEMENTS ON ACCUMULATION VALUES OF NH_4-, NO_3- AND min-N AFTER 4 WEEKS OF INCUBATION (30°C, 50% mwhc) OF ORIGINAL LF SOIL pH 4.1 WITHOUT AND WITH ADDITION OF 50 ppm N AS AMMONIUM SULPHATE

(Results are in ppm N on dry soil basis)

Without added N

Treatment	ppm	NH_4-N	NO_3-N	min-N	pH
Control	Nil	5.3	3.2	8.5	4.1
Potassium (K)	100	12.2	1.0	13.2	4.2
Calcium (Ca)	100	9.5	1.8	11.3	4.1
Magnesium (Mg)	100	10.9	2.9	13.8	4.0
Phosphate (P)	100	10.2	1.4	11.6	4.3
Copper (Cu)	50	8.4	4.5	12.9	4.1
Zinc (Zn)	50	7.4	4.9	12.3	4.2
Molybdenum (Mo)	20	9.8	2.5	12.3	4.3
With added N					
Control	Nil	6.8	2.5	9.3	4.2
Potassium (K)	100	5.3	0.4	5.7	4.2
Calcium (Ca)	100	1.8	0.9	2.7	4.1
Magnesium (Mg)	100	3.2	2.6	5.8	4.1
Phosphate (P)	100	4.0	2.4	6.4	4.2
Copper (Cu)	50	-0.4	3.4	3.0	4.1
Zinc (Zn)	50	4.4	0.0	4.0	4.2
Molybdenum (Mo)	20	1.4	4.1	5.5	4.3

L.S.D. at $P < 0.05$ NH_4-N 1.4, NO_3-N 1.9, min-N 2.0 ppm

TABLE 41

EFFECT OF ADDITION OF MAJOR AND TRACE ELEMENTS ON ACCUMULATION VALUES OF NH_4-, NO_3- AND min-N AFTER 4 WEEKS OF INCUBATION (30°C, 50% mwhc) OF HIGH FLAT (HF) SOIL pH 5.6 WITHOUT AND WITH ADDITION OF 50 ppm N AS AMMONIUM SULPHATE

(Results are in ppm N on dry soil basis)

Treatment	ppm	NH_4-N	NO_3-N	min-N	pH
		Without added N			
Control	Nil	7.0	11.2	18.2	5.7
Potassium (K)	100	8.2	3.7	11.9	5.5
Calcium (Ca)	100	9.0	9.9	18.9	5.6
Magnesium (Mg)	100	1.4	13.3	14.7	5.5
Phosphate (P)	100	5.6	11.2	16.8	5.6
Copper (Cu)	50	9.8	1.4	11.2	5.5
Zinc (Zn)	50	8.9	8.6	17.5	5.5
Molybdenum (Mo)	20	7.7	0.0	7.7	5.6
		With added N			
Control	Nil	-5.6	13.3	7.7	5.7
Potassium (K)	100	-4.2	15.4	11.2	5.6
Calcium (Ca)	100	-4.2	12.6	8.4	5.6
Magnesium (Mg)	100	-10.5	11.2	0.7	5.6
Phosphate (P)	100	-0.7	14.0	13.3	5.6
Copper (Cu)	50	1.4	4.9	6.3	5.5
Zinc (Zn)	50	-1.4	7.0	5.6	5.5
Molybdenum (Mo)	20	-5.6	12.6	7.0	5.6

L.S.D. at $P < 0.05$ NH_4-N 2.1, NO_3-N 1.5, min-N 2.8 ppm

had a significant though small effects in increasing mineral-N accumulation. Nitrate accumulation was significantly decreased by 100 ppm K but was not significantly effected by the other treatments.

Where ammonium sulphate was added all treatments, particularly 100 ppm Ca and 50 ppm Cu and Zn, decreased min-N accumulation. Nitrate accumulation was significantly decreased by 100 ppm K and 50 ppm Zn, but was not significantly affected by the other treatments.

HF soil (pH 5.6)

Where no ammonium sulphate was applied min-N accumulation was significantly decreased by 100 ppm K and Mg and 50 ppm Cu and, in particular, 20 ppm Mo. Nitrate accumulation was decreased by 100 ppm K and 50 ppm Zn and, in particular, 50 ppm Cu and 20 ppm Mo.

Where ammonium sulphate was applied min-N accumulation was significantly increased by 100 ppm K and P and significantly decreased by 100 ppm Mg. Nitrate accumulation was significantly, though slightly, increased by 100 ppm K and decreased by 100 ppm Mg and 50 ppm Cu and Zn.

For both soils without and with ammonium sulphate addition pH of treated soils after incubation did not differ by more than 0.2 units from comparable control soils.

DISCUSSION

In the LF soil (pH 4.1) the increase in N-mineralization which occurred with all treatments indicates that all the elements tested were limiting this process. However, this occurred only where no ammonium sulphate was applied and where it was applied the reverse effect occurred, with all added elements decreasing N-mineralization. The generally lower N-mineralization with than without added ammonium sulphate indicates that ammonium sulphate depressed N-mineralization in the LF soil irrespective of treatments with major and trace elements. Similar trends occurred in the HF soil (pH 5.6) except that K, P and Mo additions prevented the decrease in N-mineralization which occurred when ammonium sulphate was added.

Nitrification in the LF soil was very low and the treatments had no or very small effects on nitrification without or with addition of ammonium sulphate. In the HF soil the treatments had either little effect on or decreased nitrification, with K, Cu, and Mo having the greatest effects. The presence of ammonium sulphate prevented the decrease in nitrification due to K and Mo treatments.

SUMMARY

The effects of addition of 100 ppm K, Ca, Mg and P (PO_4^{3-}), 50 ppm Cu and Zn and 20 ppm Mo (MoO_4^{2-}) on N-mineralization and nitrification during incubation (30°C, 50% mwhc) for 4 weeks of LF soil (pH 4.1) and HF soil (pH 5.6) were studied without and with addition of 50 ppm N as ammonium sulphate.

All treatments slightly stimulated N-mineralization in the LF soil but only where no ammonium sulphate was added, but did not increase nitrification whether or not ammonium sulphate was applied.

In the HF soil N-mineralization was not increased by any treatment where no ammonium sulphate was added; nitrification was increased slightly only by Mg, and was decreased by K, Cu, Zn and Mo. Where ammonium sulphate was applied N-mineralization was increased by K and P and decreased by Mg; ammonium sulphate prevented the decrease in nitrification due to K and Mo applications.

There was negligible nitrification of ammonium sulphate in either soil and none of the treatments increased this nitrification.

Experiment 22

Loss of nitrate by denitrification during water-logged incubation of HIGH FLAT (HF) and LOW FLAT (LF) soil

The purpose of this experiment was to determine the denitrifying capacity of the original HF and LF soils. The experimental conditions used were such as to give optimum conditions for denitrification, i.e., water-logged conditions and addition of easily decomposable organic material (straw).

METHODS

10g. portions of air-dry HF and LF soil were mixed with finely ground KNO_3 (to supply 600 ppm N on the soil basis) and 2% finely ground oat straw. The treated soils were placed in 150 ml Kjeldhal flasks and 20 ml of water was added to each flask. Sufficient replicates of each soil were prepared to allow for analysis of total nitrogen initially and after 12 weeks of incubation at 30°C. During incubation the flasks were closed with rubber bungs and the contents were shaken gently once in a week.

Total N (initially and after 12 weeks of incubation) was determined by heating after addition of 2g. iron powder and 4 ml conc. H_2SO_4 until the reaction was complete (this reduces nitrate to ammonium). 30 ml. conc. H_2SO_4, 0.1g. $CuSO_4$, $5H_2O$ and 10g. K_2SO_4 were then added and the

digestion was continued until organic material had been destroyed and for a further 2 hrs. NH_4-N in the digest was then determined by distillation, after addition of excess NaOH solution, into boric acid and titration with standard H_2SO_4.

RESULTS AND DISCUSSION

Results are shown in Table 42. The NO_3-N lost during incubation of each soil was the difference between the total N content before and after incubation. The results are also expressed as a percentage of the 600 ppm NO_3-N added initially.

The considerably greater loss of NO_3-N from the HF than from the LF soil indicate a higher denitrifying capacity in the former soil.

These results may be compared with an English soil (pH 4.7) of similar texture incubated under the same conditions as described here (243). 94.9% of the added NO_3-N was lost from this soil. It is seen therefore that the denitrifying capacity of the LF soil was considerably lower and of the HF soil somewhat lower than that of the English soil.

Alexander (1) states that bacterial denitrification under acid conditions (pH <5) is slow but chemical

TABLE 42

NITRATE-N LOSS (ppm AND PERCENTAGE OF ADDED 600 ppm NO_3-N) DURING 12 WEEKS OF WATER-LOGGED INCUBATION OF HF AND LF SOILS TREATED WITH 2% STRAW

(ppm on air-dry basis)

	HF	LF
Total N* before incubation (ppm)	1280	1172
Total N* after incubation (ppm)	798	1008
NO_3-N lost (ppm)	482	164
NO_3-N lost as % of NO_3-N added	80.4	27.4

L.S.D. at $P < 0.05$ for NO_3-N loss = 58.4 ppm)

* including 600 ppm added NO_3-N

denitrification can arise. This can occur by reaction of NO_2^-, formed by reduction of NO_3^-, with NH_4^+ to give N_2, or by spontaneous decomposition of NO_2^- to NO (nitric oxide).

In the field both the Pakistan tea soils are subjected to temporary water-logging during the monsoon period. This experiment has shown that denitrification is operative in both soils, particularly the HF soil, under water-logged conditions and in the presence of easily decomposable organic materials. The extent to which such losses would be of agronomic or economic significance can only be determined by field trials with lysimeters.

SUMMARY

The extent of loss of 600 ppm added NO_3-N during 12 weeks of water-logged incubation of HF and LF soils treated with 2% straw was determined. The HF soil lost 80.4% and the LF soil 27.4% of the applied NO_3-N.

CHAPTER 14

GENERAL DISCUSSION AND SUMMARY

CHAPTER 14

GENERAL DISCUSSION AND SUMMARY

NITRIFICATION

In view of the occurrence of nitrification in both the tea soils under conditions (low pH, high moisture content and high temperatures) which usually prevent autotrophic nitrification, samples of both soils were sent to Dr. N. Walker of Rothamsted Experimental Station for counts of autotrophic nitrifying bacteria. Using plate counts on silica gel medium Dr. Walker reported that there was no sign of either ammonia- or nitrite-oxidising bacteria after 3 weeks of incubation. The dilutions used indicated that the numbers of nitrifiers in either soil sample was either zero or less than 40 per gram of soil. This is in contrast to soils containing these autotrophs which at pH > 6.0 and in the presence of ammonium salts give numbers ranging up to 10^6 or 10^7 per gram of soil (1). Dr. Walker also noted fair numbers of fungal colonies growing on the silica gel plates.

Effect of pH.

The HF and LF soils behaved rather differently with respect to changes in nitrification rate due to increasing pH through addition of calcium carbonate (chapter 4, Expt.4). Over the pH range of approximately 4 to 8 maximum nitrification occurred at pH 4.9 to 5.0 in the HF soil, but at pH 5.0 to 5.8 in the LF soil early in incubation. With extended incubation the LF soil showed maximum nitrification at pH 5.0 and the HF soil at pH 7.3. This indicates that in the LF soil heterotrophic nitrification was mainly operative over the whole period. In the HF soil, on the other hand, heterotrophic nitrification was probably more important in early incubation and autotrophic nitrification in the later stages. It is possible that a small number of autotrophs were present in the HF soil and the increase in numbers due to increasing pH occurred only after a prolonged period of incubation. Alternatively, increasing nitrification with pH may be due to the fact that the heterotrophic organism(s) concerned also becomes more active with increasing pH.

Effect of moisture content

The most significant evidence for heterotrophic nitrification arose from the results obtained when the original HF soil (pH 4.2) was incubated at various moisture

contents ranging from 20% to 133% mwhc after 12 and 13 weeks of incubation (chapter 3, Expts. 2 & 3). Nitrate accumulation was greatest at 80% mwhc, and significant accumulation occurred even at 100% mwhc, whereas accumulation at 20% to 60% mwhc was relatively small. These results differ considerably from the pattern of nitrification obtained in relation to moisture contents in English soils (e.g. 64) of approximately neutral pH. These soils show maximum nitrification at 40% to 60% mwhc, whilst at 80% to 100% mwhc there is negligible nitrification and usually some loss of nitrate, either by denitrification or immobilization. The high nitrification in the HF soil at the high moisture contents and at low pH suggests that the organism(s) responsible is probably a fungus, since fungi can operate under these conditions. The traditional autotrophic nitrifiers, on the other hand, usually show low activity under these conditions. The occurrence of higher nitrification at 100% mwhc, where conditions were almost anaerobic, than at aerobic moisture contents of 20% to 60% mwhc supports the suggestion that the organism(s) responsible for nitrification in the HF soil requires very little oxygen, and is therefore probably not the traditional autotrophic system (Nitrosomonas - Nitrobacter) which requires oxygen.

Effect of temperature

In the HF and LF soils significant nitrate accumulation occurred with incubation at 50°C, although the extent of accumulation was somewhat less than 30°C (chapter 5, Expts. 6 & 7). In addition nitrification in the HF soil occurred even at 60°C. The autotrophic nitrifying organisms are mesophilic and many investigations have shown that autotrophic nitrification is negligible above 40°C (1). This indicates that either (i) the autotrophs in both tea soils have adapted themselves to operate at high temperatures or (ii) heterotrophic nitrifiers are responsible. The latter seems more likely since heterotrophic organisms, particularly fungi, are not inhibited by high temperature. The optimum temperature for ammonifying organisms is usually between 40° and 60°C (1) and it may be that the heterotrophic nitrifiers suggested to be present in the tea soils are of the thermophilic type. The fact that in both tea soils significant nitrification occurred at high temperatures only after fairly long incubation (6 to 8 weeks) indicates that there was a fair lag phase during which microbial numbers increased to an extent where significant nitrification occurred. Finally the occurrence of significant, though small, nitrification in water-logged HF soil at 50°C indicates that the organisms concerned is capable of functioning under three combined conditions, namely, low pH, high temperature and high moisture content, any one of which is able to prevent autotrophic nitrification.

Nitrification of added nitrogen sources

Nitrification in the tea soils were determined during incubation after addition of ammonium sulphate, urea and oxamide.

In the two experiments (chapter 4, Expt. 5 and chapter 7, Expt.9) where ammonium sulphate was applied before incubation, not only was there no nitrification of ammonium sulphate but the presence of ammonium sulphate completely inhibited nitrification in both the soils. The absence of nitrification of ammonium sulphate even after the soil (LF) had its pH raised to 7.3 suggests that autotrophic nitrifiers were absent. It also appears that the activity of the heterotrophic nitrifier(s) present in the original soil was suppressed by the presence of ammonium sulphate.

There was significant nitrification of urea in all the four experiments (chapter 4, Expt.5; chapter 7, Expt.9 and chapter 8, Expts. 11 & 12) where this material was added before incubation. However, the extent of nitrate accumulation was small compared with extent of conversion of urea into NH_4-N. The results indicate that urea may be a suitable substrate for the proliferation of the suspected heterotrophic nitrifiers and could be useful in isolating the organism(s) in pure culture studies.

In the experiment (chapter 8, Expt. 11) where oxamide

was added to the original HF soil significant nitrification of this nitrogen source occurred, although most of the oxamide-N was converted to NH_4-N. Thus oxamide may also be a useful substrate for isolating the suspected heterotrophic nitrifiers. Oxamide differed from urea however, in that only the latter material was nitrified when residues of added organic materials were present in the soil (chapter 8, Expt. 12).

Other factors influencing nitrification

The experiment (chapter 13, Expt. 20) in which the HF soil was subjected to varying levels of gamma irradiation (0.05 - 2.00 Mrad) before incubation at 80% mwhc also suggest that heterotrophic nitrification may be the significant mechanism responsible for nitrification in this soil.

Where the HF and LF soils were stored air-dried at 20°C and 3°C for varying periods (chapter 10, Expt. 14) there were significant trends for min-N to accumulate as nitrate even in this air-dried condition, but usually only after 12-16 weeks of storage. Storage at even 3°C did not prevent nitrification. During incubation following the various periods of air-dry storage there was also usually increased nitrate accumulation after 8 weeks of air-dry storage, with the LF soil usually showing the greater changes than the HF soil at both storage temperatures. When the air-dried HF soil (chapter 10, Expt. 15) was stored

for 70 weeks at room temperature nitrate accumulation during subsequent incubation was greater than after 4 weeks of air-dry storage. Thus the activity of the organism(s) responsible for nitrification in this soil appeared to have been increased by the prolonged period of air-dry storage.

In the studies (chapter 12, Expt. 19) where the HF soils and an English soil (both at pH 4.2 approximately) of similar texture were incubated alone or in mixtures (the purpose of this was to see how the organisms in either soil would effect nitrification in the other soil), a mixture of equal parts of the two soils gave a nitrification pattern similar to that of the English soil, namely, low nitrate accumulation, compared with the high nitrification in the HF soil alone. This indicates that the substrate condition of the English soil was toxic to the nitrifying organisms in the HF soil. The other result of interest was where the HF soil (chapter 12, Expt. 18) after adjustment of pH 7.2 was inoculated with 1% of English soil of the same pH. It was found that nitrification in this inoculated soil was almost as high as in the English soil alone, but all the nitrate which accumulated did so during the last 3 weeks, and none during the first 3 weeks of incubation. This indicates that providing the pH of the HF soil is raised to near neutrality it is a suitable substrate for autotrophic nitrification. When the HF soil of pH 7.2 was incubated without inoculation relatively little nitrate accumulated.

NITROGEN MINERALIZATION

Effect of pH

Where aerobic incubation (30°C, 50% mwhc) was started just after addition of varying levels of calcium carbonate to give pH levels ranging from 4.2 to about 7.5 N-mineralization in both tea soils increased with pH (chapter 4, Expt. 4). Where the LF soil (chapter 4, Expt.5) had its pH increased by previous moist reaction with calcium carbonate at various levels before incubation was started, there was little net mineralization of nitrogen. The decrease in NH_4-N which occurred with increasing pH was counter-balanced by the increase in nitrate.

Mineralization of urea-N was rapid in the LF soil at all pH levels although there was a tendency for it to be somewhat less rapid at pH 7.3 than at lower pH levels (chapter 4, Expt. 5).

Effect of moisture content

Over the soil moisture content range of 20% to 100% mwhc N-mineralization in the LF soil (chapter 3, Expt. 1) was at a maximum at 40% to 60% mwhc. In the HF soil (chapter 3, Expt. 2) N-mineralization was at a maximum at 60% mwhc early in incubation but at 100% mwhc in the later stages.

Nitrogen mineralization of added dried blood was at a maximum at 60% mwhc in both the soils at all moisture contents.

Effect of temperature

With either aerobic (50% mwhc) or anaerobic (133% mwhc) incubation N-mineralization in the HF soil (chapter 5, Expt.7) increased with temperature over the range 30° to 60°C. A comparison of incubation at 30°and 50°C of the LF soil at various pH levels (chapter 5, Expt. 6) showed that there was little net N-mineralization at 30°C,while at 50°C there was greater N-mineralization at pH levels of 4.1, 4.7, and 5.7 and then at pH 7.2.

Efficiency of N-mineralization

The efficiency of N-mineralization from the organic nitrogenous materials present in the Pakistan and English soils may be defined as the amount of min-N which accumulated per unit of organic-N (which is virtually the same of total N) present in the two soils. It was found (chapter 12) that in aerobic neutral soils efficiency of N-mineralization was 19% less in the Pakistan than in the English soil. In the acid soils under aerobic conditions N-mineralization was 62% more efficient in the Pakistan than in the English soil, whilst in the near-anaerobic (80% mwhc) conditions the Pakistan soil was 24% more efficient than the English soil.

EFFECT OF ADDITION OF ORGANIC MATERIALS

During incubation of the HF soil (chapter 6, Expt. 8) after addition of 0.5% levels of various organic materials (plus 200 ppm N as ammonium nitrate to ensure no lack of N for decomposition), sucrose caused the greatest decrease whilst grass and straw caused the least decrease in min-N during the early (immobilization) stage of incubation. With further incubation, up to 13 weeks there was relatively small mineralisation of nitrogen with any of the treatments, and there was no accumulation of nitrate. Where the immobilization of N occurred there was a marked preference for nitrate over ammonium by the immobilizing organisms. In another experiment (chapter 8, Expt. 11), where ground pea seed was added (simulating a source fairly high in organic nitrogen) there was no immobilization of N early in incubation and relatively little mineralization of pea seed-N later in incubation. There was however, a tendency for nitrate to disappear, presumably by immobilization, but this was counter-balanced by increased ammonification.

When 1% w/w ground straw or compost was allowed to decompose for 6 months in mixtures with HF soil (chapter 8, Expt. 12), the straw residues prevented nitrification whilst the compost residues slightly increased nitrification

during subsequent incubation. Nitrification of urea added just before incubation was slightly decreased by the straw residues and not affected by the compost residues.

MISCELLANEOUS EXPERIMENTS

Experiments (chapter 11, Expts. 16 & 17) in which nitrification of artificially fixed ammonium by the HF soil clay fraction and bentonite clay (both inoculated with HF soil) showed that significant though small nitrification occurred during incubation at 100% mwhc but not at 33% mwhc.

Where a comparison was made between a constant moisture content of 50% mwhc during incubation and 2 cycles of drying from 50% to air-dry during incubation it was found in both LF and HF soils (chapter 9, Expt. 13) that N-mineralization and nitrification of both native organic N and added urea-N were greater under constant than under changing moisture contents. A comparison of constant and changing moisture contents of 100% mwhc showed little differences in mineralization and nitrification of either native N or urea-N.

Where a number of major and trace elements were added to LF and HF soils before incubation (chapter 13, Expt.21) there were sometimes significant, but slight, changes in N-mineralization and nitrification.

SUGGESTIONS FOR FURTHER STUDY

The isolation and identification of the heterotrophic nitrifying microorganism(s), which this study has indicated may be present in the tea soils, is desirable. Pure culture studies using suitable substrates will be necessary for this. Using these techniques Doxtader and Alexander (15) reported that a number of heterotrophic bacteria, actinomycetes and fungi have the capacity for forming nitrite from many types of relatively simple organic nitrogenous compounds as substrates. However, none of the microorganisms formed nitrate from the substrates tested. These workers used culture solutions at pH 7.0. It is suggested that when studying the tea soils in this way a culture solution of the same pH of the tea soil should be used since this may be the most suitable pH for proliferation of the suspected heterotrophic nitrifying organisms. At this low pH there would be little point in analysing for nitrite because of its ability to rapidly decompose chemically and also to be lost as nitrogen through reaction with amino groups or ammonium. The latter two reactions result in complete loss of nitrite and nitrogen while the former reaction can result in conversion of some or all of the nitrite to nitrate. Thus in an acid medium

the formation of nitrate should be used as a criterion for heterotrophic nitrification.

With regard to possible substrates, the present study has indicated that urea and oxamide may be suitable for isolating the heterotrophic nitrifiers which may be present in the tea soils. In addition a wide range of organic nitrogenous compounds should be examined with a view to finding which is the most efficient. Doxtader and Alexander (15) found that acetaldoxime was the most effective substrate for heterotrophic nitrification at pH 7.0.

It is suggested that field studies should be made in acid East Pakistan tea soils to determine what levels of nitrate accumulate and the factors affecting this accumulation. If it is found that significant amounts of nitrate accumulate under field conditions it would then be useful to carry out further studies of the performance of tea bushes comparing nitrate sources with other sources of nitrogen.

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